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# Hematopoietic Stem Cell Transplantation for Treatment of Multiple Sclerosis in Sweden

CHRISTINA ZJUKOVSKAJA



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### **Abstract**

Zjukovskaja, C. 2023. Hematopoietic Stem Cell Transplantation for Treatment of Multiple Sclerosis in Sweden. (Трансплантация гемопоэтических стволовых клеток для лечения рассеянного склероза в Швеции). *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1895. 64 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1688-8.

This thesis investigated the efficacy and safety of autologous hematopoietic stem cell transplantation (AHSCT) as a treatment of multiple sclerosis.

This thesis demonstrates that when compared to one of the most powerful disease-modifying drugs (DMDs), specifically alemtuzumab (ALZ), AHSCT is able to hold its ground. Patients that were treated with AHSCT were more likely to maintain NEDA-3 or freedom from disease in three parameters (no confirmed disability worsening, clinical relapses or MRI events) versus those treated with ALZ. The three year post-treatment Kaplan-Meier (KM) estimates of NEDA-3 were 88 % for AHSCT and 37 % for ALZ treated patients. With AHSCT, adverse events were more common in the first three months, as is expected with an intense one-time treatment. ALZ, though powerful and with little early adverse effects, has more late adverse effects instead, namely thyroid disease. At 3 years post-treatment, the KM-estimates of thyroid disease were 21 % for AHSCT and 46 % for ALZ. We then sought to address potential concerns in using AHSCT on patients previously treated with potent DMDs like alemtuzumab, cladribine or rituximab. The concern lay in the fact that these powerful DMDs have long-lasting effects on the immune system. We found that the rates of treatment-related and long-term complications were the same for all patients regardless of previous use of DMDs. This finding therefore allays those concerns: previous treatment with a powerful DMDs need no longer be a possible deterrent to giving a patient AHSCT.

Using a series of CSF biomarker studies, we gauged treatment efficacy. We showed that CNS inflammation is measurably affected by treatment, especially when it comes to AHSCT. Post-AHSCT treatment, many of the proteins we studied decreased towards the direction of healthy individuals, as early as the first year after treatment and remained so during subsequent follow-ups. Additionally we saw that tissue-injuring disease processes, for example represented by biomarkers like NFL and MBP, normalized for the majority of patients. These promising results suggest that tissue-injury could possibly be stopped in its tracks with AHSCT. Finally, we observed that biomarkers of B- and T-cell activation also became normalized, showing that AHSCT treatment also shuts down acute inflammation. All of these factors together explain why patients treated with AHSCT are able to maintain NEDA-3 so effectively even when compared to one of the most potent drugs available. These results compellingly illustrate that AHSCT treatment yields quantifiably long-lasting and beneficial effects that are not commonly seen with other available treatments for MS. Therefore, use of AHSCT should be advocated, promoted and utilized more for the benefit of patients and society as a whole.

*Keywords:* AHSCT, HSCT, hematopoietic stem cell transplantation, multiple sclerosis, RRMS, inflammation, biomarkers

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*Вперёд, товарищи, к вершинам знаний!*



# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

## Clinical Studies:

- I. **Zhukovsky C\***, Sandgren S\*, Silfverberg T, et al. (2021) Autologous haematopoietic stem cell transplantation compared with alemtuzumab for relapsing-remitting multiple sclerosis: an observational study. *Journal of Neurology, Neurosurgery, and Psychiatry*. 92(2):189-194. doi:10.1136/jnnp-2020-323992
- II. Kvistad SAS, Burman J, Lehmann AK, Tolf A, **Zjukovskaja C**, Melve GK, Bø L, Torkildsen Ø. (2022) Impact of previous disease-modifying treatment on safety and efficacy in patients with MS treated with AHSCT. *Journal of Neurology, Neurosurgery, and Psychiatry*. 93(8):844-848. doi:10.1136/jnnp-2022-328797

## Biomarker studies:

- III. **Zhukovsky C**, Herman S, Wiberg A, Cunningham JL, Kultima K, Burman J. (2021) Urokinase, CX3CL1, CCL2, TRAIL and IL-18 induced by interferon- $\beta$  treatment. *Acta Neurologica Scandinavica*. 143(6):602-607. doi:10.1111/ane.13400
- IV. Burman J, **Zjukovskaja C**, Svenningsson A, Freyhult E, Wiberg A, Kultima K. (2022) Cerebrospinal fluid cytokines after autologous haematopoietic stem cell transplantation and intrathecal rituximab treatment for multiple sclerosis. *Brain Communications*. doi: 10.1093/braincomms/fcad011
- V. **Zjukovskaja C**, Larsson A, Cherif H, Kultima K, Burman J. (2022) Biomarkers of demyelination and axonal damage are decreased after autologous hematopoietic stem cell transplantation for multiple sclerosis. *Multiple Sclerosis and Related Disorders*. 68:104210. doi:10.1016/j.msard.2022.104210
- VI. Lundblad K, **Zjukovskaja C**, Larsson A, Cherif H, Kultia K, Burman J. (2023?) Cerebrospinal fluid concentrations of CXCL13 and sCD27 are decreased after autologous hematopoietic stem cell transplantation for multiple sclerosis. *Neurology*:

*Neuroimmunology & Neuroinflammation*, Manuscript submitted  
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\*Author with equal contribution

Related papers not included in this thesis:

- I. Herman S, Arvidsson M S, **Zhukovsky C**, Emami Khoonsari P, Svenningsson A, Burman J, Spjuth O, Kultima K. (2020) Disease phenotype prediction in multiple sclerosis [Internet]. Available from: <http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-415212>
- II. **Zhukovsky C**, Bind MA, Boström I, Landblom AM. (2020) Air pollution as a contributing factor of relapses and cases of multiples sclerosis, *Health Risk Analysis*, 3(20), doi: 10.21668/health.risk/2020.3.20.eng
- III. Iacobaeus E, Boström I, **Zhukovsky C**, Berntsson SG, Landtblom AM (2021) The risk of COVID-19 severity in patients with ms appears to be associated with immunotherapy *Health Risk Analysis* 3(14), doi: 10.21668/health.risk/2021.3.14.eng
- IV. Sjölin K, Kultima K, Larsson A, Freyhult E, **Zjukovskaja C**, Alkass K, Burman J. (2022) Distribution of five clinically important neuroglial proteins in the human brain. *Molecular Brain*. 15(1):52. Published 2022 Jun 29. doi:10.1186/s13041-022-00935-6



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# Abbreviations

ALZ	Alemtuzumab
CIS	Clinically Isolated Syndrome
CSF	Cerebrospinal Fluid
DMD	Disease-modifying Drug
DMT	Disease-modifying Treatment
EDA	Evidence of Disease Activity
EDSS	Expanded Disability Status Scale
HSC	Hematopoietic Stem Cells
HSCT	Hematopoietic Stem Cell Transplantation
IFN- $\beta$	Interferon- $\beta$
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NEDA	No Evidence of Disease Activity
PMS	Progressive Multiple Sclerosis
PPMS	Primary Progressive Multiple Sclerosis
RTX	Rituximab
RRMS	Relapsing-remitting Multiple Sclerosis
SPMS	Secondary Progressive Multiple Sclerosis



# Chapter 1: Introduction

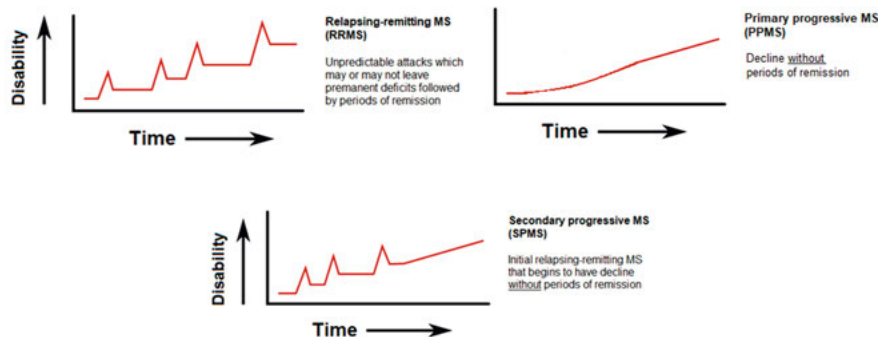
## What is multiple sclerosis (MS) and why is it important?

### Epidemiology of MS: Sufferers in the world and in Sweden

Multiple sclerosis (MS) is a demyelinating and neurodegenerative autoimmune disease of the central nervous system (CNS). MS is the most common inflammatory neurological disease of young adults with a mean diagnosis age of 30.<sup>1</sup> The highest age-standardized MS prevalence estimates per 100 000 people were in high-income North America, western Europe and Australasia while the lowest were observed in eastern and central sub-Saharan Africa as well as Oceania.<sup>1</sup> Previous studies have shown that prevalence of MS increases with higher latitudes in both hemispheres, though some discrepancies do appear.<sup>2,3</sup> In Sweden, the overall prevalence was 190/100 000 with 113 in men and 260 in women.<sup>4</sup> This in itself is one of the highest nationwide estimates when comparing with the rest of the world.<sup>4</sup> Sweden has seen an increase of cases from 96 (1988) to 189 (2008) per 100 000, though this can be partly explained by better case assessment, awareness and improved health service.<sup>5</sup> As the disease, generally, appears at the commencement of an adult's life and affects them indefinitely, it is imperative to be able to not only provide the right treatment, but to also assess the effectiveness of said treatment.

### Types of MS

There are three basic types of MS. Relapsing-remitting multiple sclerosis (RRMS) is characterized by relapses and remissions. The attacks or relapses are generally unpredictable and the deficits from them may or may not be permanent. Between relapses, patients experience periods of remission. Primary progressive multiple sclerosis (PPMS) is progressive in nature, which means that they have a steady decline and no periods of remission. Secondary progressive multiple sclerosis (SPMS) is the combination of the RRMS and PPMS where the initial RRMS form progresses into a steady decline with no remissions, fig 1.<sup>6</sup> Additionally, the progressive MS (PMS) is used to collectively represent SPMS and PPMS patients.



**Fig 1:** RRMS = relapsing-remitting multiple sclerosis; PPMS = primary progressive multiple sclerosis; SPMS = secondary progressive multiple sclerosis \*in some older articles, progressive-relapsing is also presented as a type of MS.

## Pathology

### CNS

Multiple sclerosis is restricted to the CNS, which makes it difficult to both treat and diagnose. The disease is predominantly represented by two pathological manifestations: 1. gliosis and neurodegeneration and 2. inflammation and demyelination. Two major measurable outcomes of MS used to describe the clinical course as well prognosis have been qualitative (disability, progression) and quantitative (lesions, relapses).

### Immunology

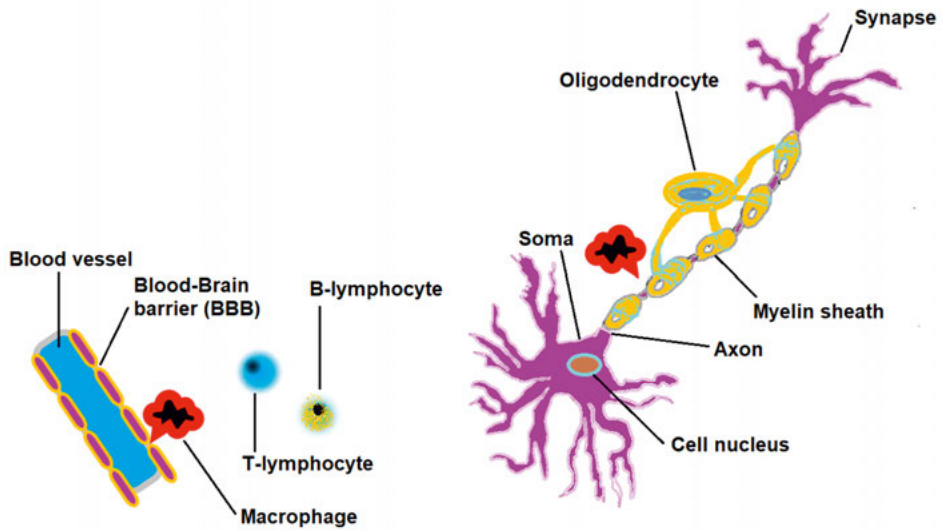
The pathology of MS is multifactorial and some of the risks and factors include genetic<sup>7</sup> and environmental,<sup>8</sup> such as obesity, smoking, vitamin D levels<sup>8,9</sup> and Epstein-Barr infection.<sup>8,10</sup> When looking at immunopathogenesis, which is likewise multifactorial and yet to be fully understood, it is believed that inflammation leading to destruction of myelin is due, at least in part, to infiltration of T- and B-cells.<sup>11,12</sup> It should be noted that regulatory B-cells, which are immature transitional B-cells, that protect from autoimmunity via IL-10 production are depleted in MS patients.<sup>13,14</sup>

There are two main cells of the adaptive immune system, the T- and B-cells. Both develop from common progenitors in the bone marrow but with different functions. The main roles of the B-lymphocytes are antigen presentation to T-lymphocytes and production of antibodies. Since B-cells are important antigen presenting cells and can produce an array of cytokines, their role as well as

interaction with T-cells has been more scrutinized lately. The heterogeneity of B-cells' role though has made them more difficult to categorize. Nonetheless, B-cell depleting therapy in MS and other autoantibody CNS diseases, has highlighted the importance of B-cells in these diseases.<sup>15,16</sup> Additionally it has been noted that B cells can be detected in CNS lesions from patients with early to late stages of MS and more abundantly in active lesions from RRMS in comparison to non-active lesions and lesions from patients with PMS overall.<sup>17</sup> However, B-cell depletion with CD20 antibodies can cause disease exacerbation, which is associated with an increased production of pro-inflammatory TNF since regulatory B-cells control other inflammatory cells and processes.<sup>18</sup>

T helper cells (CD4<sup>+</sup> T-cells) play a major role through the release of cytokine and immune mediators. In order to be activated, they require an interaction with major histocompatibility complex class II expressing cells, such as in macrophages, dendritic cells or B-cells. Cytokine and mediator release also leads to an influx of macrophages to the CNS and additional proinflammatory cytokine release. Cytotoxic T cells (CD8<sup>+</sup> T-cells) are prominent in the inflammatory infiltrate in MS lesions and involved in the process by directly interacting with major histocompatibility complex I in antigen expressing cells like neurons and oligodendrocytes.<sup>11,16,19</sup> The constant release of proinflammatory cytokines and the addition of cellular damage leads to monocyte and macrophage recruitment as well as microglia activation, leading to further inflammation.

Active lesions contain a large number of inflammatory cells, mainly macrophages and T-lymphocytes, as well as to a lesser extent B- and plasma cells (Fig 2). Their activation and migration across the blood-brain barrier (BBB) is what leads to the characteristic demyelination seen in MS.



**Fig 2:** MS plaques are distinguished by inflammation, myelin loss, axonal damage and gliosis, with the lesions varying in size from less than a millimeter to several centimeters.

In general, the immune response to a pathogen can be described as follows. A macrophage first engulfs a pathogen, digests it and presents antigens. T-helper cells bind to the macrophage and activates. These activated T-helper cells bind to B-cells and in turn activates them. Activated B-cells either turn into plasma cells and are released in blood or they become memory B-cells. The former then secrete antibodies, which bind to antigens to fight the invading pathogen. This binding is to physically stop the function of the pathogen and attract macrophages, for example, to attack and phagocytose.

In MS the identified pathogen is in fact no foreign pathogen at all but rather integral components of the CNS that make it functional. During the disease, macrophages show signs of myelin phagocytosis while T cells express cytokines and recruit other inflammatory cells. Oligodendrocytes are responsible for myelin production in the CNS and produce the insulating sheath of axons. Therefore, the targeting of oligodendrocytes, and the myelin sheath they produce, leads to demyelination, decreased axon density and eventually neuronal death. Some of the post-demyelination components that can be found in CSF include myelin basic proteins (MBPs) and oligodendrocyte glycoproteins.

## Clinical Presentation and Definitions

One of the most common definitions of a *relapse* is a period of acute worsening of neurological function, which lasts  $\geq 24$  hrs.<sup>20</sup> In order to substantiate

and confirm relapses, often magnetic resonance imaging (MRI) is used as an evaluation tool. If gadolinium enhanced lesions are present, then they are usually considered to be an affirmation of an ongoing relapse. Since MS affects the brain, neurological impairment is another characteristic clinical presentation of the disease. The Expanded Disability Status Scale (EDSS) is used to evaluate disability and focuses on frequent symptoms of multiple sclerosis. Seven functional systems are tested: visual, brainstem, pyramidal, cerebellar, sensory, bowel-bladder and cerebral functions.<sup>21,22</sup> This allows for a somewhat clearer parameters while tracking disease progression as well as treatment efficacy in the long run.

## Diagnosis

The principles of dissemination in time and space are used to determine whether or not an individual is suffering from multiple sclerosis rather than clinically isolated syndrome (CIS) or other neurological diagnoses. Dissemination in time means that neurological damage is occurring at more than one time point, while dissemination in space means that multiple parts of the CNS have neurological damage.<sup>6</sup> The most up-to-date protocol for diagnosing MS is grounded in both the 2017 revised McDonald criteria, as well as clinical, radiological (specifically MRI), and cerebral spinal fluid (CSF) findings.<sup>23,24</sup>

A contrast agent containing gadolinium can be used during an MRI to better distinguish between active and inactive lesions. Gadolinium-enhancing lesions represent areas of active inflammation. T1-weighted MRI sequences can reveal breakdown in the BBB through intravenous gadolinium leakage as well as lesions. The T2-weighted MRI sequences with FLAIR will demonstrate MS lesions. Analysis of the CSF can reveal oligoclonal IgG bands and increased levels of IgG (IgG index) in more than 90 % of patients.<sup>23,25,26</sup>

## Therapies – Disease-Modifying Drugs

Since there is currently no definitive cure for MS, disease-modifying drugs (DMDs), alternatively referred to as disease-modifying treatments (DMTs), are most commonly used to modify the disease course through moderation of immune function.<sup>27,28</sup> With administration, there is a reduction in the relapse rate and accumulation of MRI lesions.<sup>23</sup> In some cases there are stabilization, delay of disability and modest improvement in disability as well.<sup>23</sup>

Overall, DMDs cover varying stages of the immune response in an effort to stop the chain of events that lead to CNS inflammation. Table 1, is a summary of DMDs from first to second-line treatment and mechanisms of action (if known).

Any first-generation or second-generation DMD exposure is associated with a lower hazard of mortality, by 26 % – 33 % respectively, when compared to no exposure. Although, DMD exposure does not reduce physician visits significantly, it does lower hospitalization.<sup>29,30</sup>

**Table 1:** Combined table based off Garg, Hauser, Rafiee and Williams et al.<sup>12,23,31,32</sup>

<b>First-line treatment</b>	<b>Mechanism</b>
Teriflunomide	Dihydroorotate dehydrogenase inhibitor
Glatiramer acetate	Downregulation of autoreactive T-cells,
IFN-b-1a (Rebif)	Inhibition of CD4+ T-cells
IFN-b-1a (Avonex)	Inhibition of CD4+ T-cells
PegIFN-b-1a (Plegridy)	Inhibition of CD4+ T-cells
IFN-b-1b (Betaseron)	Inhibition of CD4+ T-cells
Dimethyl fumarate and diroximel fumarate	Nuclear factor (erythroidderived 2)-like 2 pathway inhibitor

<b>Second-line treatment</b>	<b>Mechanism</b>
Fingolimod	Sphingosine-1-phosphate inhibitor
Siponimod	Sphingosine 1-phosphate receptor modulator
Ozanimod	Sphingosine 1-phosphate receptor modulator
Cladribine	Apoptosis of immune cells
Ocrelizumab	B cell depletion
Ofatumumab	B cell depletion
Rituximab	B cell depletion
Natalizumab	Inhibits extravasation of immune cells
Alemtuzumab	Depletion of T- and B-cells
Mitoxantrone	Inhibits B-, T-cell and macrophage proliferation

## Chapter 2: Aims

The aim of this doctoral thesis is to describe the efficacy and safety of hematopoietic stem cell transplantation (HSCT) as a treatment of multiple sclerosis.

The projects can be divided into two parts: clinical and biomarker studies.

The clinical studies (I – II) aimed to show how HSCT compares to DMDs in terms of efficacy and adverse events.

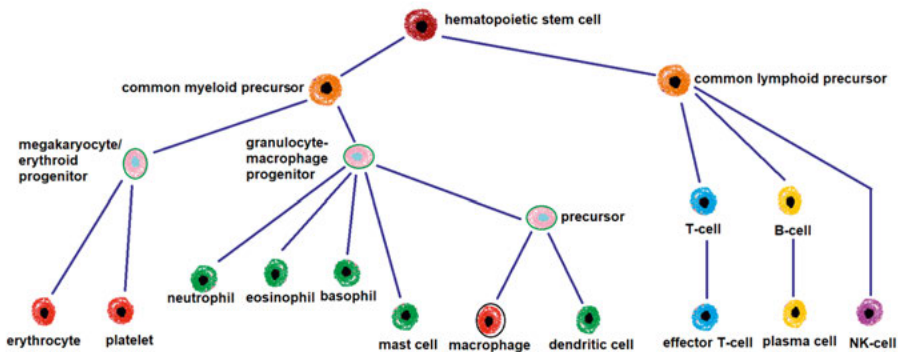
The biomarker studies (III – VI) aimed to show how HSCT for MS impacts the levels of novel and established biomarkers of MS over time.

# Chapter 3: The Nitty-Gritty

## Hematopoietic Stem Cells (HSCs)

### What are HSCs?

Hematopoietic stem cells (HSCs) are located in the bone marrow and give rise to other blood cells through the process of haematopoiesis.<sup>33</sup> There are two main cell lines: myeloid and lymphoid. The myeloid cells include macrophages, neutrophils, basophils and erythrocytes. Lymphoid cells include T- and B-cells and NK-cells, fig 3. Cell exposure to cytokines at different stages determines the type of cell that will be eventually created. Some of the cytokines are IL-1 to -8, IL-12 and stem cell factor (SCF), which enhances cytokine stimulated proliferation.<sup>34</sup>



**Fig 3:** Illustrates why HSCs are so significant. These cells are the undifferentiated progenitors of a multitude of cell including that of the immunity as well as blood. This diagram shows the advanced web leading from HSC to the final and mature forms. Different cytokines at different stages act as important growth factors and ultimately determine the final cellular products of the HSCs.

### AHSCT – Why it is important?

Hematopoietic stem cell transplantation can be divided into two types: autologous and allogenic. In autologous HSCT (AHSCT), patients receive their own hematopoietic stem cells, while in allogenic HSCT the patient receives a stem cell graft from a healthy donor.<sup>35</sup> The vast majority of MS patients have

been treated with AHSCT. Originally, stem cell transplantation was performed in order to treat patients in the chronic phase of leukemia and later other cancers.<sup>36 37</sup> The first non-cancer related successful human-to-human bone-marrow transplant was performed in 1968 to a 5-month-old child suffering from sex-linked lymphopenic immunological deficiency.<sup>38</sup>

For over two decades, AHSCT has been used as a therapeutic intervention for MS, based on data from uncontrolled clinical trials and case series.<sup>39</sup> The first randomized controlled trial was conducted in 2015: the phase II trial (ASTIMS) of AHSCT vs mitoxantrone concluded that AHSCT treatment led to a lower number of MRI lesions.<sup>40</sup> A Phase III study (MIST) from 2019 showed that AHSCT both increased the likelihood of disability improvement and prolonged the time to disease progression in RRMS<sup>41</sup>. In 2016, the Swedish Board of Health and Welfare approved AHSCT as a therapeutic option for RRMS.<sup>42</sup>

## Performing AHSCT and MS

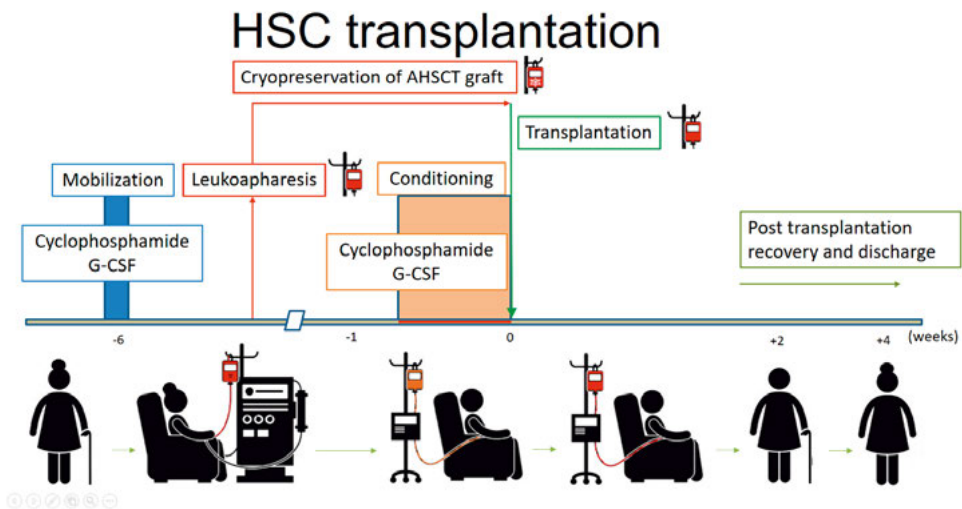
The AHSCT procedure is comprised of the following steps: mobilization, hematopoietic stem cell collection, conditioning and stem cell reinfusion. Treatment of MS with AHSCT started in 1995 in cases where DMDs were ineffective i.e. in progressive cases. Although AHSCT in patients with progressive disease and high pre-transplantation disability scores was not effective<sup>43</sup>, an early observation was that the few patients with RRMS who were treated, displayed long-term remission.<sup>44-46</sup>

The Swedish implementation of and experience with AHSCT<sup>47</sup> started in 2004 at Uppsala University Hospital and, after its subsequent success, led to an increase in use. Ten years later in 2014, in a summary of 48 patients (of which 38 were RRMS), 68 % had no evidence of disease activity using the outcome measure of NEDA-3 (no confirmed disability worsening, clinical relapses or MRI events).<sup>47</sup>

AHSCT causes the profound destruction, followed by reconstitution, of the immune system through hematopoietic stem cells, not merely just long-lasting suppression.<sup>46</sup>

The four main steps of AHSCT are pictured below in fig. 4 and include:

1. HSC Mobilization
  - a. Includes granulocyte colony-stimulating factor (G-CSF) administration with cytotoxic chemotherapy, e.g. cyclophosphamide.
2. HSC Harvesting and Cryopreservation
  - a. Harvesting is usually performed with peripheral blood (rather than bone marrow) and thereafter cryopreserved until transplantation.
3. Conditioning
  - a. Ablation of the immune system is achieved through conditioning with high-dose chemotherapy. (In Sweden, from 2013<sup>41,48</sup>, a low-intensity cyclophosphamide-based conditioning regimen is used instead of BEAM<sup>47</sup>.)
4. Transplantation
  - a. Infusion with HSCs facilitates recovery by assisting in marrow repopulation and immune system reconstitution.<sup>46</sup>



**Fig 4:** A visual depiction of hematopoietic stem cell transplantation divided into 4 distinct stages: mobilization, HSC harvesting and cryopreservation, conditioning and transplantation. The time line represented in the picture is in weeks.

# Biomarkers

## What are biomarkers?

There are four types of biomarkers or biological markers: molecular, histological, radiographic and physiological.<sup>49</sup> Various molecules can serve as biomarkers since they all indicate something about the health status and past or ongoing processes in the body. Biomarkers are therefore a valuable source of information pre- and post-treatment to help determine if, and to what extent, one or another treatment has been effective over time.

## Choosing Biomarkers

Choosing which biomarkers to examine is rooted in drawing a link between the biomarker and a specific disease condition or pathogenic process. Effective biomarker identification occurs as a product of comprehensive literature reviews and as well as data analysis and assay validation. To the utmost maximum, these combined processes should eliminate any obvious confounders.<sup>50</sup> Broad biomarker panels compiled using these insights then facilitate a broad-band perspective on where study participants stand vis-à-vis themselves as well as each. The types of measurements that can be taken are direct and indirect measurements. A direct measurement would involve biological matter like blood and CSF. An indirect measurement would be something like an fMRI scan where changes in function and composition can be observed without need for tissue sampling.

## Biomarkers of Interest

Biomarkers are an indication of an ongoing or past biological process and can reflect the disease spectrum from beginning to terminal stages.<sup>49</sup> Some biomarkers have long been established and their relationship with a disease well characterized, as in the case with MS and NFL.<sup>51,52</sup> Since MS is an autoimmune disease, biomarkers highlighter the effects of the disease, such as inflammation and cellular injury, make for excellent targets.

## Blood Biomarkers of Inflammation

### **uPA** <sup>III</sup>

Urokinase-type plasminogen activator (urokinase, uPA) is involved in vascular disease and cancer progression as well as several inflammation-related diseases such as rheumatoid arthritis and allergic asthma. uPA cleaves plasminogen and eventually activates the extracellular proteolytic enzymes matrix

metalloproteases (MMPs). MMPs are thought to be an essential step for activated T-cell migration into the CNS. MMP-8 and MMP-9 levels are increased in serum of MS patients, but after 6 months of IFN- $\beta$ -treatment are corrected to normal levels.<sup>53</sup>

### **CX3CL1<sup>III</sup>**

CX3CL1 (or fractalkine) is a chemokine and a mediator of several aspects of the immune response. It can both function as a proinflammatory chemoattractant and anti-inflammatory neuroprotective agent.<sup>54</sup> It is reported that CX3CL1 is increased in serum from MS patients.<sup>55</sup>

## **CSF Biomarkers of Inflammation**

### **CCL3<sup>IV</sup>**

CCL3 or chemokine ligand 3 is involved in the acute inflammatory state as well in the recruitment of leukocytes and leukemia stem cell population maintenance.<sup>56</sup> This macrophage inflammatory protein is has also become a prognostic biomarker in hematological malignancies.<sup>57</sup>

### **IL-12B<sup>IV</sup>**

IL-12B or subunit beta of interleukin-12 (IL-12p40) is associated with over-expression in patients with MS.<sup>58</sup> As a cytokine, it acts on T- and NK-cells, is expressed in macrophages along as well as dendritic cells and affects memory Th1 cells.<sup>59</sup> When faced with a pathogen, dendritic cells and phagocytes produce IL-12.<sup>60</sup>

### **CXCL10<sup>IV</sup>**

CXCL10 is a chemokine which is secreted by multiples sources like endothelial cells, fibroblasts etc. It is involved in the attraction of monocytes/macrophages, NK-, T- and dendritic cells.<sup>61</sup> This makes CXCL10 a marker of host immune response and integral in the creation of a feedback loop, which further perpetuates the autoimmune process.<sup>62</sup>

### **IL-8<sup>IV</sup>**

IL-8 is an inflammatory chemokine that is produced by macrophages, endothelial cells and other cells.<sup>63,64</sup> Any cells involved in the innate immune response with toll-like receptors (TLRs) can secrete IL-8 but macrophages tend to be the first and use it for the recruitment of other cells.<sup>65</sup> This chemokine is an important parameter of localized inflammation as well. IL-8 concentrations have been found to be elevated in MS patients when compared with individuals not suffering from a neuroinflammatory condition.<sup>66</sup> Additionally it has been observed that, in MS patients, higher levels of IL-8 are associated with clinical disability, disease reactivation and treatment switch.<sup>67</sup>

### **CXCL13**<sup>VI</sup>

CXCL13 or chemokine ligand 13 regulates B-cell migration in lymphoid tissue and can be found in active MS lesions.<sup>68</sup> This is of course important since some treatments like rituximab (RTX) are B cell – depleting monoclonal antibody medications. CXCL13 also correlates with immune activation markers, and with disease activity, in RRMS patients.<sup>69</sup>

### **sCD27**<sup>VI</sup>

Soluble form of CD27 (sCD27) is a transmembrane glycoprotein found specifically on T- and B-cells. It belongs to a recently identified receptor family, whose members are involved in cell differentiation and survival. When T-cells are activated, sCD27 is released into the supernatant and can therefore be detected in fluids such as serum and urine.<sup>70</sup> Since the 1990's, sCD27 has been investigated as a potential biomarker and increased levels of sCD27 are in fact indicative of an inflammatory neurological condition.<sup>71</sup>

## CSF Biomarkers of Tissue Injury

### **NFL**<sup>V</sup>

Neurofilament light (NFL) is part of the cytoskeletal structure of myelinated axons. When axon destruction occurs, it leads to measurable increases in CSF concentration of NFL.<sup>51</sup> Elevated levels of NFL in CSF were reported in amyotrophic lateral sclerosis as well as Alzheimer's and multiple sclerosis more than two decades ago.<sup>72</sup> Since NFL is a main structural protein of axons it follows that NFL can be used to monitor neurodegeneration in general and furthermore be potentially used as an evaluation tool for treatment.<sup>73</sup>

### **MBP**<sup>V</sup>

Myelin basic protein (MBP) is located on the intracellular surface of myelin membranes and is involved with myelin structural integrity. MBP has also been shown to be associated with relapses and gadolinium-enhancing lesions as can be seen using an MRI.<sup>51</sup> As demyelization occurs, vMBP is released into CSF. Therefore, it may be possible to use levels of MBP in order to monitor disease progress pre- and post-treatment.<sup>74</sup>

### **GFAP**<sup>V</sup>

Glial fibrillary acidic protein (GFAP) is a major intermediate cytoskeletal protein and is an integral part of astrocyte motility and structural stability. Astrocytes are star shaped glial cells in the brain and spinal cord. They control endothelial cell in the blood brain barrier (BBB,) provide nutrients to the nervous tissue, maintain ion balance, regulate cerebral blood flow and repair/scar the brain following trauma.<sup>75</sup> In the spinal cord, activated astrocytes have the abil-

ity to respond to almost all neurotransmitters and release neuroactive molecules.<sup>76</sup> Since GFAP synthesis occurs as an astrocyte response following tissue damage, monitoring it could lend insight into disease activity and status.<sup>77</sup>

# Chapter 4: Studies I – VI

## Clinical Findings

Even a decade ago, efficacy and safety of AHSCT for MS was under question. With the therapy given to progressive and only sporadically to RRMS patients, the verdict was not initially crystal clear. Once the therapy became more accepted, still additional concerns were raised.

- ✓ How does AHSCT compare to the leading DMTs available?<sup>I</sup>
- ✓ Is the rate of treatment-related and long-term complications for patients previously treated with DMTs that are immunologically long-lasting (anti-CD20, ALZ, cladribine) different than for those that are not?<sup>II</sup>

## Clinical Parameters

When it comes to clinical studies, it is important to determine parameters by which assessments of efficacy can be made.

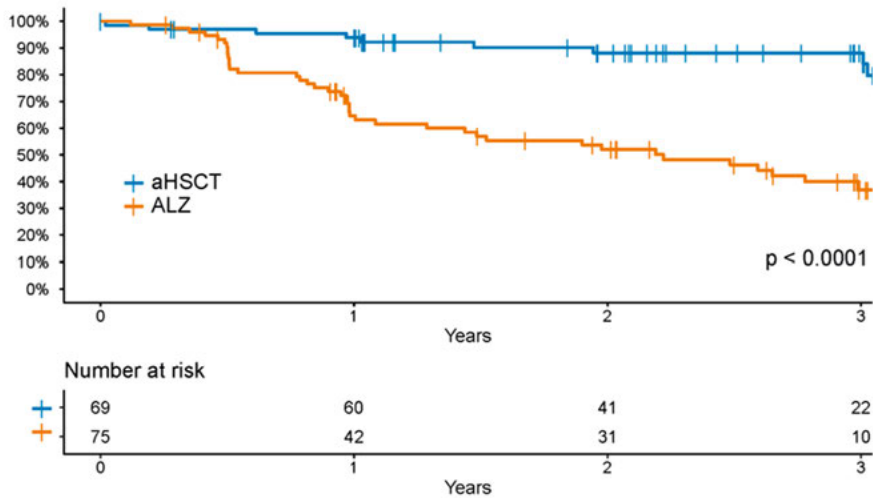
- ✓ EDSS
  - Tests seven functional systems: visual, brainstem, pyramidal, cerebellar, sensory, bowel-bladder and cerebral functions. Scoring ranges from 0 (no disability) to 10 (death)
- ✓ Clinical Relapse
  - Period of acute worsening of neurological function lasting  $\geq 24$  hours that is not due to an external cause
- ✓ Annualised Relapse Rate (ARR)
  - Number of relapses occurring during a time period divided by the number of years in that time period
- ✓ Confirmed Disability Improvement (CDI)
  - Decrease in EDSS score by at least one point from baseline sustained between two follow-up no less than 6 months apart (0.5 points if the baseline EDSS  $\geq 6$ )
- ✓ Confirmed Disability Worsening (CDW)
  - Increase in EDSS score by at least one point from baseline sustained between two follow-up visits no less than 6 months apart (1.5 point if EDSS at baseline was 0, 0.5 points if the baseline EDSS  $\geq 5.5$ )

- ✓ MRI Event
  - Appearance, in the brain or spinal cord, of any T2 lesion >3 mm or gadolinium enhancing lesion not present on the baseline scan
- ✓ No Evidence of Disease Activity (NEDA-3)
  - Absence of clinical relapses, CDW and MRI events.

## Study I. Different Treatments Compared

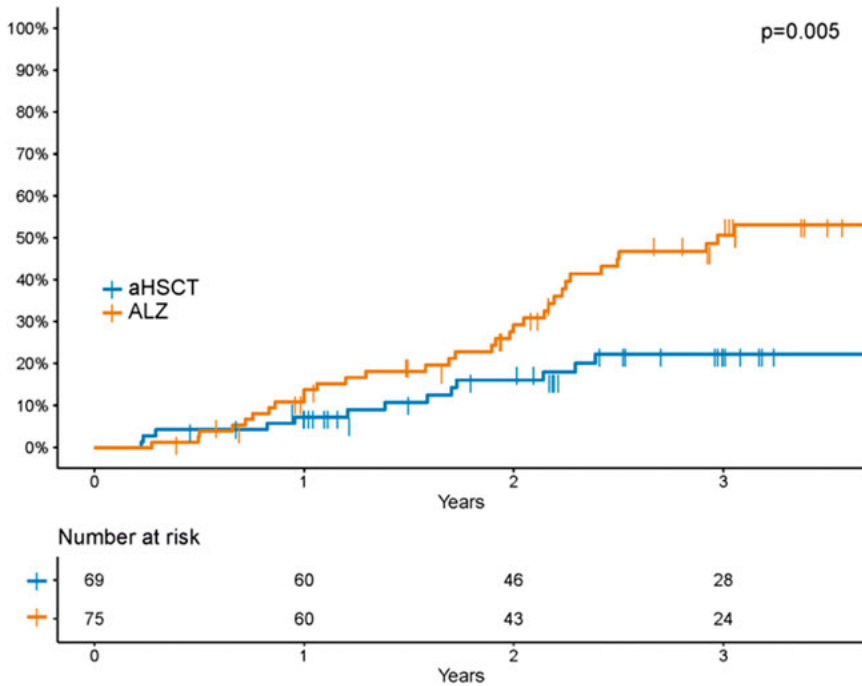
One of the most powerful drug treatments of late for RRMS has been alemtuzumab (ALZ). It was found to reduce clinical relapse, disability progression, lesion activity and brain volume loss.<sup>78</sup> This therapeutic drug also has long-lasting effects on immunity<sup>79,80</sup> and is therefore an excellent point of comparison for AHSCT when it comes to sustained effect and adverse events. In our 2020 observational cohort study, a total of 144 RRMS patients were included: 69 received AHSCT while 75 received ALZ as treatments. Yearly follow-up visits with assessment of EDSS, adverse events and MR investigations were conducted. The patients did not significantly differ in male to female proportions, number of previous treatments or disease duration. They did differ in a few parameters. ALZ patients had a lower baseline age related multiple sclerosis severity score (ARMSSS), baseline EDSS and annualized relapse rate (ARR) 1 year prior to treatment. These patients were as well a little bit older: ALZ 35 (IQR 30 – 41) vs AHSCT 30 (IQR 26 – 37).

When it came to efficacy, a Kaplan-Meier (KM) estimate of NEDA-3 at 3 years was 88 % (95 % CI 80 % – 97 %) for AHSCT and 37 % (95 % CI 26 % – 52 %) for ALZ,  $p < 0.0001$  (Fig. 5). This indicates that RRMS patients treated with AHSCT are more likely to achieve NEDA-3 than patients treated with ALZ.



**Fig 5:** NEDA-3 for AHSCT and ALZ up to 3 years. Those treated with AHSCT were more likely to achieve NEDA-3.

While looking at adverse effects, AHSCT is more invasive at administration and so in comparison to ALZ, more adverse events are expected during the first 100 days. These events through proved to be manageable and did not result in any recorded long-term morbidity. Neither group showed any trend of late adverse events that were grade 3 or higher. Grade 4 events are those, which are life threatening and require urgent intervention, while death is classified as a grade 5 event. When it came to autoimmune adverse events, 20% of AHSCT patients, compared with 47% of ALZ patients, experienced an event, with thyroid disease being the most common (fig 6).<sup>1</sup> This means that ALZ patients are more likely to develop thyroid disease than those treated with AHSCT.



**Fig 6:** Thyroid disease for AHSCT and ALZ. ALZ treated patients were much more likely to develop thyroid disease than those treated with AHSCT.

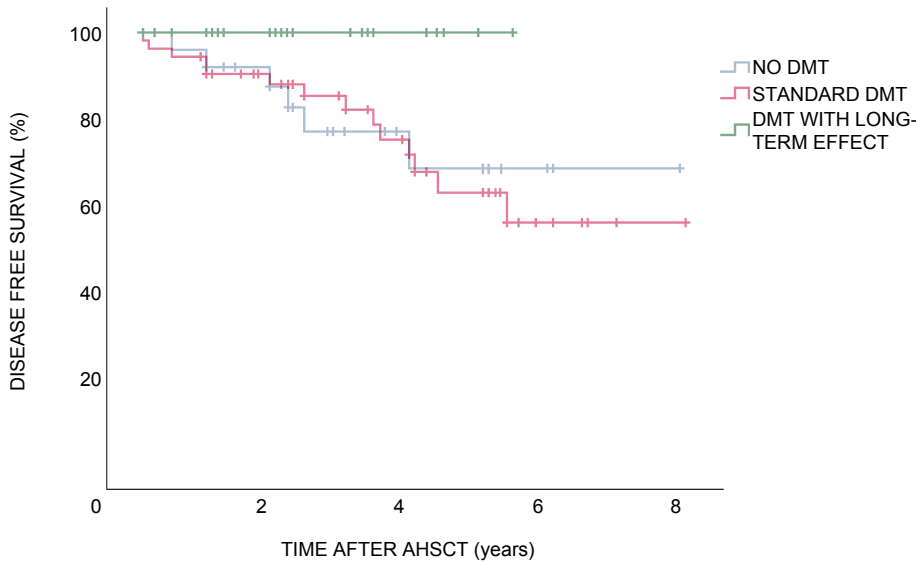
Based on this evidence, AHSCT appears to be more effective at maintaining RRMS patients in a state of NEDA and with less adverse events after 100 days post treatment initiation than ALZ.

## Study II. Powerful DMDs before AHSCT, yay or nay?

Now that we have established that AHSCT is a highly effective treatment, another concern needs to be addressed. Since some DMDs like ALZ, rituximab (RTX) and cladribine (CLD) have long-lasting effects, should patients who have taken them be included or excluded from having AHSCT as a treatment? For example, when it comes to ALZ, some centers chose to exclude AHSCT as a treatment.<sup>41</sup> A 2020 article by Boffa et al with a three person case shed light on safety and efficacy of AHSCT following ALZ treatment.<sup>73</sup>

Our study investigated the effects of AHSCT on 104 RRMS patients who were treatment naïve, had previously received a standard disease modifying treatment (DMT), or had previously received a DMT with long-lasting effect like ALZ, CLD or RTX.<sup>11</sup>

This retrospective study showed that patients treated with ALZ, CLD or RTX had the same frequency of both early and late adverse event when compared to those treated with other DMTs. Additionally all patients who had previously received long-term effect DMTs attained NEDA-3, fig 7.



**Fig 7:** All patient who had received long-term effect DMTs were able to achieve NEDA-3.

### Clinical Conclusions

The two clinical studies I and II presented evidence that AHST is a powerful treatment that is not inferior to the best DMD currently available<sup>I</sup> and the prior use of long-term effect DMDs should not exclude patients from receiving AHST as a treatment for RRMS<sup>II</sup>.

### Biomarker Findings

Biomarkers allow for a different picture to be painted. Since they are indicators of the internal ongoing processes in the body, they are a valuable source of information before and after treatments.

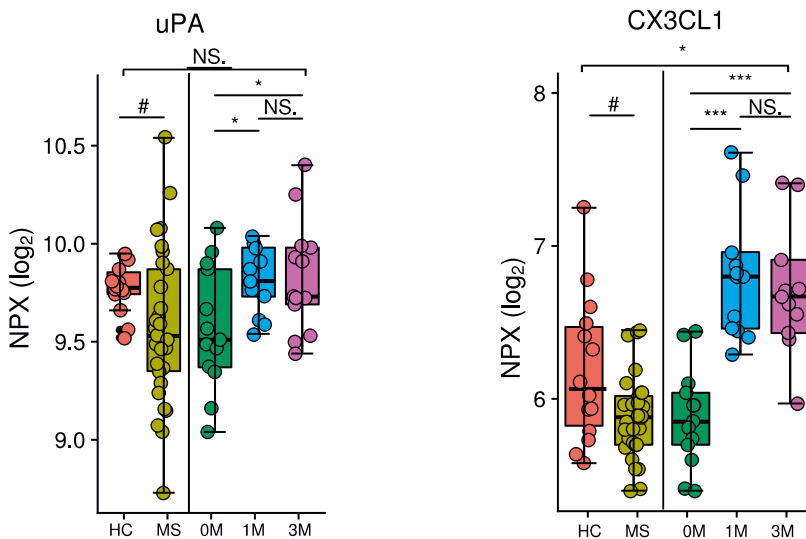
- ✓ Serum proteins pre-post interferon-β-1a treatment?<sup>III</sup>
- ✓ CSF PEA inflammatory panel looking at RRMS (AHST) and PMS (RTX) – are there differences/patterns?<sup>IV</sup>
- ✓ CSF NFL, MBP and GFAP – how does the tissue-injuring disease process look after AHST?<sup>V</sup>

- ✓ CSF CXCL13 and sCD27– how do the biomarkers of B- and T-cell activation fare after AHSCT?<sup>VI</sup>

### Study III. Pre–Post Treatment: Proteins Modulated with Interferon- $\beta$ -1a Treatment

An effective way of finding biomarkers of inflammation that are modulated pre- and post-treatment is through the use of a 92 protein inflammation panel, for example.

In study III, 29 RRMS patients and 15 healthy control (HC) subjects were investigated. For 13 of the RRMS patients, measurements were available at 1- and 3-months post treatment initiation. Biomarkers CCL2 and TRAIL were confirmed to be modulated with the treatment, showing that our findings correlated with established findings. Novel findings in this study though were that uPA and CX3CL1 were differentially expressed in MS, fig 8. Both uPA and CX3CL1 levels were significantly lower in MS patients than in controls and increased after IFN- $\beta$ -1a treatment.



**Fig 8:** Levels of uPA, CX3CL1 in HC, untreated MS patients and IFN-  $\beta$ -1a treated MS patients. # ( $p < 0.05$ ). 0 M (before treatment), 1 M (1 month post-treatment) and 3 M (3 months post-treatment); \* ( $q < 0.05$ ), \*\* ( $q < 0.01$ ), \*\*\* ( $q < 0.001$ ), N.S. (not significant)

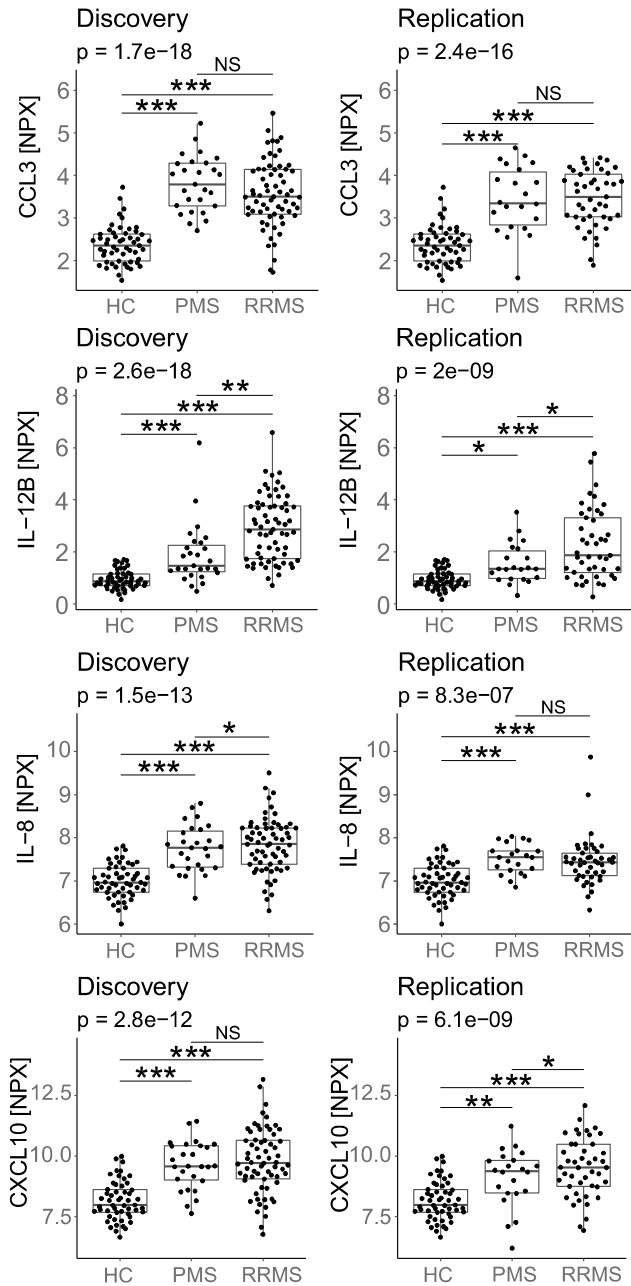
## Study IV. Pre–Post Treatment: Proteins Modulated with AHSCT and Intrathecal RTX

Now, using a 92 cytokine and other inflammation related protein panel one can go on to analyze in multiple directions:

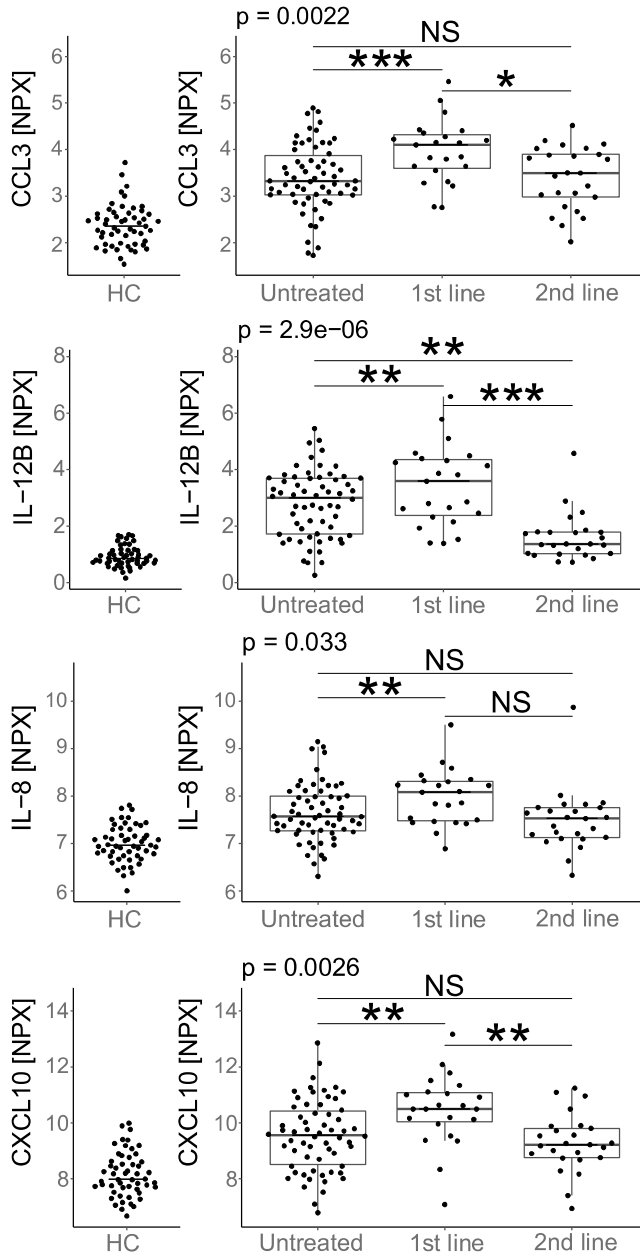
- ✓ Differences between individuals with MS and healthy controls (HC)
- ✓ Effects of treatment with AHSCT in RRMS patients or intrathecal RTX in PMS patients

This study comprised of 158 MS patients and with 53 HCs. Of the 158 MS patients: 64 RRMS patients and 27 PMS patients underwent a single occasion of lumbar puncture for CSF sample retrieval, 45 RRMS patients underwent AHSCT and follow-up lumbar punctures; 22 PMS patients underwent intrathecal RTX treatment and follow-up lumbar punctures. Upon analysis, 16 cytokines (proteins) were found to be altered after AHSCT, of which 11 proteins were decreased post-treatment. Of those cytokines that were affected, this study suggests that the inflammatory milieu of the CSF in multiple sclerosis is profoundly affected by AHSCT. As for intrathecal RTX, none of the studied cytokines were affected by treatment.

Since some proteins were highly associated with other, a cluster analysis ranked them. Between MS patients and controls, the highest ranked discriminating proteins were: CCL3, IL-12B, CXCL10 and IL-8. When it came to discriminating between patients with RRMS and PMS, only IL-12B differed, Fig 9. Impact of previous treatment is no less important to assess. In Fig 10, one can see a trend that concentrations of CCL3, IL-12B, and CXCL10 were elevated in patients with 1<sup>st</sup>-line treatment. After AHSCT, CSF concentrations of CCL3, IL-12B and CXCL10 were decreased, while those of IL-8 appeared to be unaffected by this therapy, Fig 11. Finally, high concentrations of IL-8 were associated with worse outcome in both treatment groups, Fig 11. Overall, the results suggest a profound effect of AHSCT on the inflammatory milieu of the CSF in RRMS.

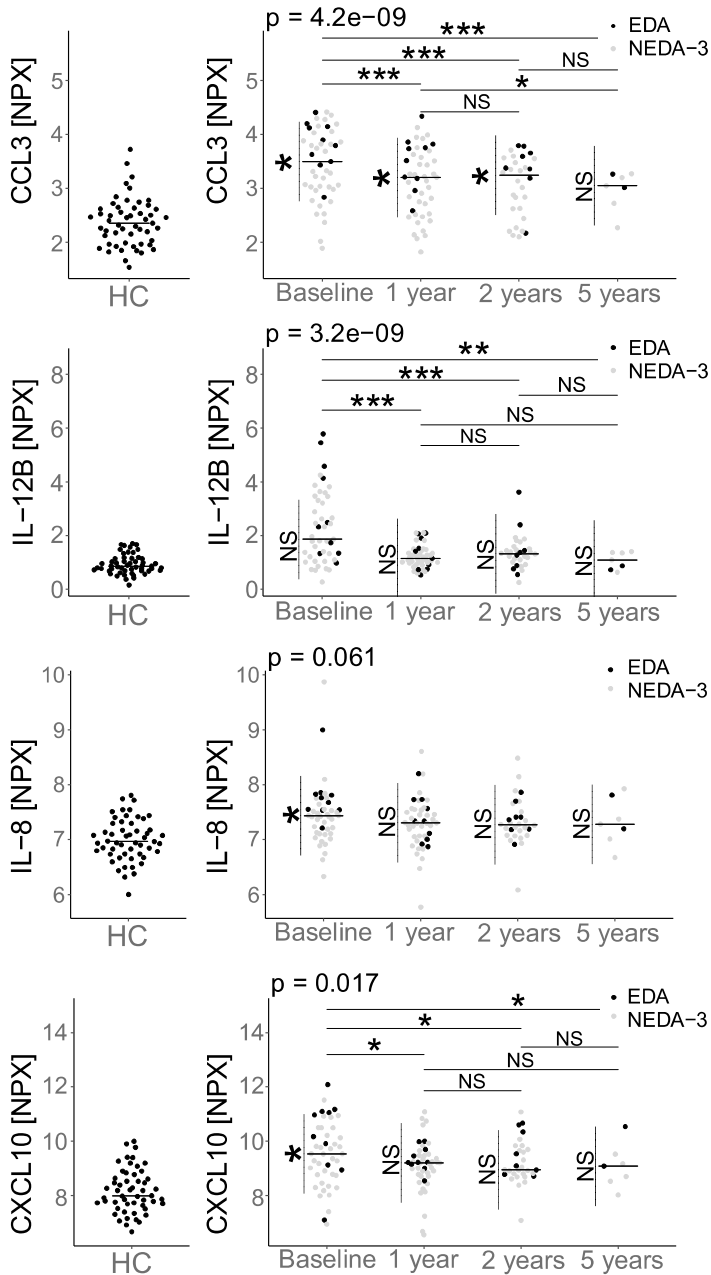


**Fig 9:** CSF of CCL3, IL-12B, IL-8 and CXCL10 in healthy controls (HC), patients with progressive MS (PMS) and patients with relapsing-remitting MS (RRMS). IL-12B, the only protein consistently higher in RRMS than in PMS patients. NS = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$



**Fig 10:** CSF concentrations of CCL3, IL-12B, IL-8 and CXCL10 in HC and RRMS patients who were untreated, treated with 1<sup>st</sup>- or 2<sup>nd</sup>-line treatment.

Patients with 1<sup>st</sup>-line treatment had higher CSF concentrations than in the other two categories. NS = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$



**Fig 11:** CSF concentrations of CCL3, IL-12B, IL-8 and CXCL10 in HC and AHSCT treated RRMS patients.

IL-12B rapidly decreased to around HC level. Though CCL3 and CXCL10 also decreased, they did so gradually and were not able to reach the levels of

HC. IL-8 was not affected by AHSCT did not affect levels of. Evidence of disease activity (EDA) was associated with higher post-treatment levels of CCL3, IL-8 and CXCL10. NS = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

## Study V. Biomarkers of Demyelination and Axonal Damage After AHSCT

This study took a closer look at whether the tissue-injuring process of RRMS is halted by AHSCT. Three commonly used biomarkers of tissue damage were chosen for the analysis: neurofilament light (NFL), myelin basic protein (MBP) and glial acidic fibrillary protein (GFAP). Forty-three RRMS patients with at least 1 follow-up after transplantation and 31 HC participated in the study. When examining the proportion of RRMS patients with values above the upper limit of normal at baseline vs 5-year follow up, the difference is astounding, Table 1. Baseline vs 5-year follow-up: 67 % vs 12 % for NFL, 63 % for MBP vs 12 % and 16 % vs 25 % for GFAP. The mean concentration of NFL and MBP decreased from baseline to 5-year follow-up, while GFAP remained unchanged, tables 2 – 3. NEDA-3 over the follow-up period was found in those patients that had lower baseline median CSF MBP concentrations, table 2.

**Table 2:** CSF concentrations of NFL, MBP and GFAP at baseline and follow-up

	n	NFL (pg/mL)		MBP (pg/mL)		GFAP (ng/mL)	
		Median (IQR)	Normal	Median (IQR)	Normal	Median (IQR)	Normal
Control	31	n/a	Normal	430 (290 – 730)	100%	18 (14 – 24)	100%
Baseline	43	920 (420 – 2700)	33%	1500 (630 – 3200)	37%	23 (18 – 30)	84%
1 year	43	440 (300 – 540)	65%	670 (520 – 1100)	67%	23 (18 – 31)	84%
2 years	26	350 (270 – 430)	81%	710 (490 – 1100)	69%	23 (18 – 34)	77%
5 years	8	270 (210 – 350)	88%	680 (600 – 760)	88%	27 (23 – 35)	75%

Median values with interquartile range (IQR) for neurofilament light (NFL), myelin basic protein (MBP) and glial acidic fibrillary protein (GFAP). Normal values of NFL had been established at the reference laboratory and the upper limit of normal was calculated as  $210.22 \times 1.031^{\text{age}}$  pg/mL. For MBP and GFAP the normal values were calculated as  $\pm 2SD$  from the mean of the controls.

**Table 3:** CSF concentrations of NFL, MBP and GFAP at baseline and follow-up divided into NEDA and EDA

	N NEDA/EDA	NFL (pg/mL)			MBP (pg/mL)			GFAP (ng/mL)		
		NEDA Median (IQR)	EDA Median (IQR)	P-value	NEDA Median (IQR)	EDA Median (IQR)	P-value	NEDA Median (IQR)	EDA Median (IQR)	P-value
B	34/9	720 (340 – 2300)	2100 (1400 – 4200)	ns	1200 (620 – 2700)	2400 (2400 – 9200)	0.03	23 (17 – 30)	23 (18 – 30)	ns
1	34/9	430 (290 – 540)	480 (370 – 600)	ns	630 (490 – 1000)	1100 (670 – 1500)	0.05	23 (18 – 32)	23 (19 – 25)	ns
2	19/7	330 (250 – 420)	540 (300 – 600)	ns	700 (460 – 1000)	970 (600 – 1100)	ns	25 (18 – 36)	21 (19 – 23)	ns
5	4/4	270 (200 – 350)	270 (220 – 390)	ns	590 (550 – 680)	710 (690 – 800)	ns	31 (27 – 35)	24 (23 – 33)	ns

\*: B – Baseline, 1 – 1 year, 2 – 2 years, 5 – 5 years

Median values with interquartile range (IQR) for neurofilament light (NFL), myelin basic protein (MBP) and glial acidic fibrillary protein (GFAP). Normal values of NFL had been established at the reference laboratory and the upper limit of normal was calculated as  $210.22 \times 1.031^{age}$  pg/mL. For MBP and GFAP the normal values were calculated as  $\pm 2SD$  from the mean of the controls.

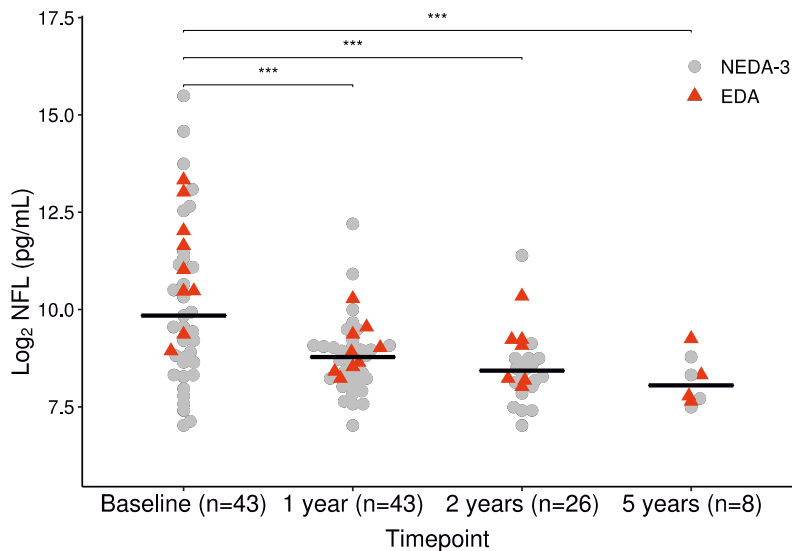
Higher baseline levels of NFL and MBP were observed in patients with confirmed disability worsening (CDW) as well as patients with confirmed disability improvement (CDI),  $p$ -value = 0.04, in comparison with patients who were EDSS stable post AHSCT, table 4.

**Table 4:** Biomarker levels at baseline based on whether EDSS status showed improvement, stability or worsening.

	Baseline NFL (pg/mL) Median (IQR)	Baseline MBP (pg/mL) Median (IQR)	Baseline GFAP (ng/mL) Median (IQR)
CDW ( $n = 5$ )	2100 (660 – 8300)	2400 (2400 – 9200)	30 (23 – 41)
Stable ( $n = 15$ )	450 (320 – 1400)	760 (480 – 1900)	18 (16 – 23)
CDI ( $n = 23$ )	1500 (540 – 4600)	1800 (810 – 4200)	25 (19 – 33)

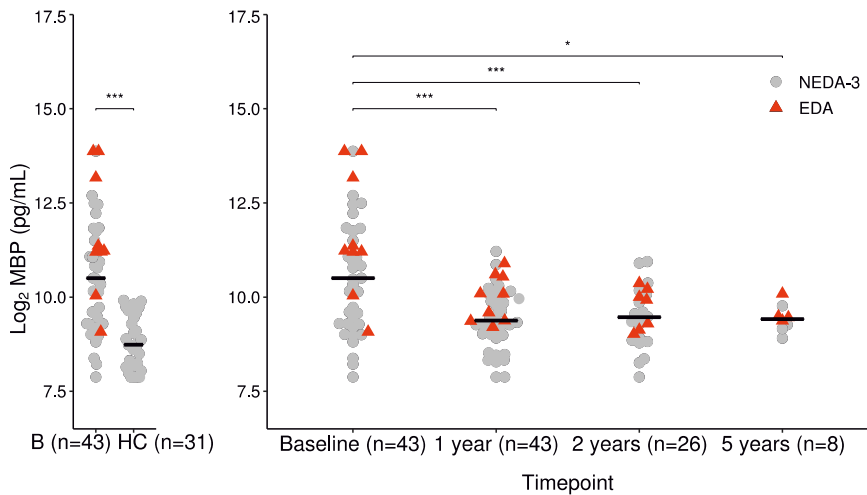
NFL, neurofilament light; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; EDSS, expanded disability status scale; CDW, confirmed disability worsening, CDI, confirmed disability improvement.

Finally, in a majority of patients, biomarkers of axonal damage (NFL), fig 12, and demyelination (MBP), fig 13, reached normal values within five years after AHSCT.



**Fig 12:** NFL levels before (baseline) and 1, 2, and 5 years after AHSCT.

NFL decreased after treatment with every year, approaching 88 % normal values at five years. Patients with EDA had numerically higher NFL levels at baseline (2100 pg/mL) than those with NEDA-3 (720 pg/ mL) - the difference statistically insignificant ( $p=0.13$ ). \*\*\*  $p$ -value < 0.0001

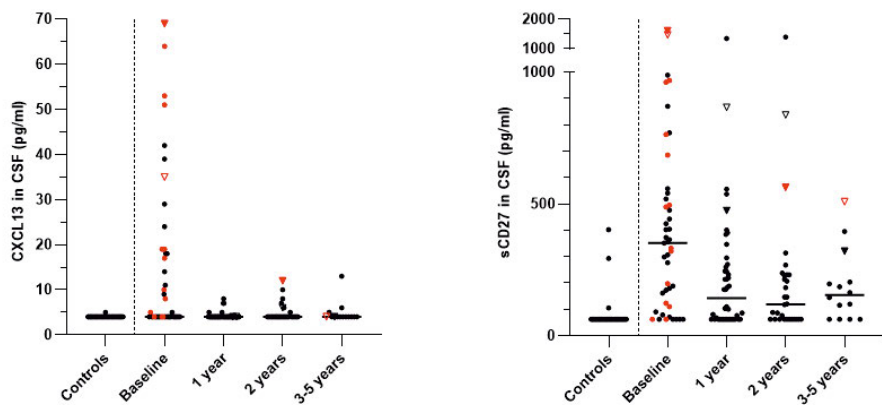


**Fig 13:** MBP levels before (baseline) and 1, 2, and 5 years after AHSCT.

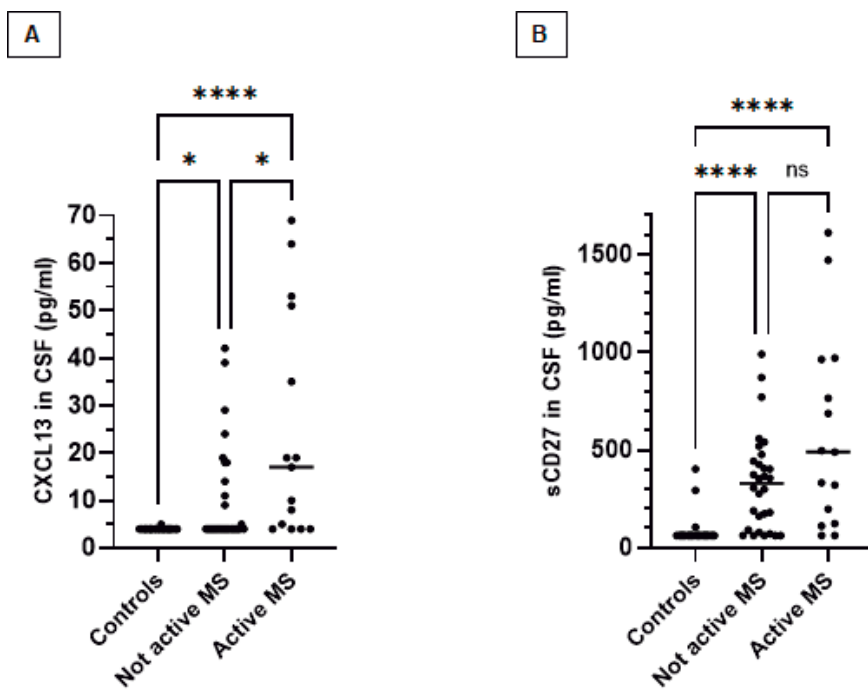
MBP decreased after AHSCT with every year, approaching 88 % normal at five years. Patients with EDA had higher MBP at baseline than those with NEDA-3 (2400 pg/mL vs 1200 pg/mL,  $p = 0.03$ ). \*  $p$ -value  $< 0.01$ , \*\*\*  $p$ -value  $< 0.0001$

## Study VI. Biomarkers of Immune Activation After AHSCT

This study with RRMS patients further delves into the aftereffects of AHSCT, specifically focusing on the biomarkers of B- and T-cell activation. This study had 32 HC and 45 RRMS patients that underwent AHSCT. Within one year of treatment, CXCL13 concentrations were normalized; within two years, sCD27 concentrations were normalized, Fig 14. When looking at CSF concentrations of CXCL13 and sCD27 and disease activity status, another picture emerges. Higher CXCL13 concentrations were found in patients with an active disease (relapse or MRI lesion) but for sCD27, disease activity did not give rise to a significant concentration difference, Fig 15. Ultimately, treatment with AHSCT for RRMS is associated with a definitive decrease in CSF concentrations of CXCL13 and sCD27.



**Fig 14:** Overview of CXCL13 and sCD27 concentrations in CSF. Overview of CXCL13 and sCD27 concentrations in HC and RRMS patients that underwent AHSCT. Horizontal lines = median values. Active disease (relapse or MRI lesion) at sampling = red coloring of data points. Two patients with clinical relapse at follow-up = triangles.



**Fig. 15:** Cerebrospinal fluid CXCL13 and sCD27 concentrations by disease activity status

CSF concentrations of CXCL13 and sCD27 in HC compared with baseline samples from RRMS patients. Patients with an active disease (relapse or MRI lesion) had higher CXCL13 concentrations. For sCD27, disease activity did not give rise to a significant concentration difference. Horizontal lines denote median values. ns = not significant \*  $p < 0.05$  \*\*\*\*  $p < 0.0001$

## Biomarker Conclusions

The four biomarker studies III to VI presented evidence that biomarkers are an effective tool to measure the impact of, and possibly provide outcome prediction of treatment. The first of the four biomarker studies<sup>III</sup>, acted as a proof-of-concept: the method was able to confirm known changes in biomarkers and provide novel findings. The second study<sup>IV</sup> revealed that a number of proteins are modulated with AHSCT, but not with intrathecal RTX. Previous treatment was not less important and allowed for a glimpse into how prior treatment can potentially affect future ones. Finally, one biomarker, IL-8, appeared to be a good candidate to monitor outcome in both treatment groups. The third study<sup>V</sup>, delved deeper into the biomarkers of tissue-damage and revealed that AHSCT in fact halts some of the degenerative processes, even at follow-up at 5 years. The final study<sup>VI</sup>, delved deeper into the possible immunological aftereffects of AHSCT by looking at biomarkers of B- and T-cell activation. Like some of the findings in the previous studies, AHSCT was able to not only decrease, but to also normalize CSF concentrations of these biomarkers. CSF inflammation is measurably affected by treatment and, as such, biomarkers are an excellent tool for viewing AHSCT outcome and efficacy.

# Chapter 5: Short Summaries of all Studies

## Clinical Findings

### Study I

In this study, we wanted to compare the outcomes after treatment with autologous hematopoietic stem cell transplantation (AHSCT) and the disease-modifying drug alemtuzumab (ALZ) in patients with relapsing-remitting multiple sclerosis (RRMS).

#### **Key results**

Our most important finding was that the estimates of the primary outcome ‘no evidence of disease activity’ in three parameters (NEDA-3) at 3 years post treatment was 88 % for AHSCT and 37 % for ALZ with a  $p < 0.0001$ . Other significant findings included the annualized relapse rate: 0.04 for AHSCT and 0.1 for ALZ,  $p = 0.03$ . At last follow-up with a  $p = 0.06$ : for AHSCT, 57 % of patients improved / 41 % of patents were stable / 1 % of patients worsened, for ALZ, 45 % of patients improved / 43 % of patents were stable / 12 % of patients worsened. In the first 100 days after treatment, grade three or higher adverse events were present in 48 out of 69 patients treated with AHSCT and 0 out of 75 patients treated with ALZ. With regard to long-term effects, according to Kaplan-Meier estimates at 3 years, rates of thyroid disease were 21 % for patients receiving AHSCT and 46 % for those receiving ALZ with  $p = 0.005$ .

#### **Limitations**

This study was limited by the fact that intervention was non-randomized. It would be best to confirm these studies in a randomized controlled trial. At least one is underway (ClinicalTrials.gov Id: NCT03477500) and has an estimated primary completion date of December 2025. With that said, in a true clinical setting, the choice of which treatment should be administered should be decided on an individual basis based on what side effects long- or short-term are acceptable for the patient.

#### **Conclusions**

This observational cohort study showed that higher likelihood of maintaining NEDA-3 was associated with AHSCT treatment. During the first 100 days

after treatment, adverse events were more frequent with AHSCT, after that period, adverse events were more common with ALZ.

## Study II

In this study, we wanted to evaluate the impact of previously administered long-lasting disease-modifying treatments (DMTs) for safety and efficacy on patients treated with AHSCT.

### **Key results**

Our most important finding was that for patients who had previously received alemtuzumab (ALZ), cladribine (CLD), or rituximab (RTX) did not have different rates of treatment-related or long-term complications; overall 81 % of patients achieved NEDA-3 status, including 100 % of the patients who had previously received RTX, ALZ or CLD. Other significant findings were that 66 % of patients had neutropenic fever, which was the most common AHSCT-related complication. Autoimmunity occurred in 19 % of patients during the follow-up period. Neither neutropenic fever, secondary autoimmunity or length of hospitalization differed based on DMT used prior to AHSCT.

### **Limitations**

This study was limited by the amounts of patients in each pre-AHSCT treatment group. Therefore, conclusions stemming from pre-AHSCT DMTs and NEDA-3 should be treated with caution.

### **Conclusions**

This study provides evidence that treatment with ALZ, RTX, or CLD prior to AHSCT is safe since it did not lead to increased rates of short or long-term complications vs other DMTs. Additionally, all of these patients treated with ALZ, RTX or CLD initially attained NEDA-3 status after AHSCT.

## Biomarker Findings

### Study III

In this study, looking at MS patients who were treated with interferon- $\beta$ -1a (IFN- $\beta$ -1a), we wanted to determine which serum proteins were associated with the disease and affected by the treatment.

### **Key results**

Our most important finding was that uPA and CX3CL1 were differentially expressed and increased after IFN- $\beta$ -1a treatment in MS patients. Other significant findings were that CCL2 and TRAIL were modulated, as confirmed

by other studies. Overall, 10 proteins were differentially expressed, five of which were due to IFN- $\beta$ -1a treatment.

### **Limitations**

This study was limited by the combination of low number of study subjects and high quantity of proteins examined. Ideally, these results should be confirmed with an additional independent cohort study.

### **Conclusions**

This study showed that uPA and CX3CL1, two novel proteins, were differentially expressed in baseline samples from MS patients when comparing to HCs and increased after IFN- $\beta$ -1a treatment. As validation of our method, CCL2 and TRAIL were shown to be modulated by IFN- $\beta$ -1a, consistent with available literature. IL-18 though provided a conflicting result as there is no current consensus in literature. From this exploration, it can be concluded that the study supports and provides evidence that IFN- $\beta$ -1a treatment affects several aspects of the immune system.

## **Study IV**

In this study, we used a 92 protein PEA inflammatory panel to determine which proteins were altered in RRMS or PMS patients, whether treatment would have an effect and patient disease activity post treatment.

### **Key results**

Our most important finding was that AHSCT had a profound effect on the inflammatory environment. Of the cytokines measured, 16 were found to be altered in MS and 11 of which decreased post-AHSCT in RRMS patients. Intrathecal RTX treatment in PMS patients was not reflected in the studied cytokines. There were 4 cytokines, CCL3, IL-12B, CXCL10 and IL-8, that discriminated between MS patients and HCs. After AHSCT treatment, CSF concentrations of CCL3, IL-12B and CXCL10 decreased. Among MS patients, IL-12B distinguished between RRMS and PMS.

### **Limitations**

This study was limited by a factor, which was both an advantage and a disadvantage. The fact that PEA panels have a fixed set of proteins in an area of interest, in this case inflammatory processes, allows for a large overview of the inflammation status of all the samples. At the same time, if a panel is fixed and validated, then there will be other markers that are not included though they could be a point of interest. Another limitation of PEA is that it is not able to yield absolute concentration values of the proteins that are measured, instead providing log<sub>2</sub> scaled normalized protein expression (NPX) values.

## **Conclusions**

In this study, the use of an inflammation panel of 92 proteins allowed us to determine four cytokines of limited association to each other that could distinguish between MS and HC: CCL3, IL-12B, CXCL10 and IL-8. When looking at effect of therapeutic intervention with AHSCT in RRMS or intrathecal RTX in PMS - CCL3, IL-12B, and CXCL10 all decreased in AHSCT, while IL-8, an important parameter of localized inflammation, was unaffected. In intrathecal RTX patients, none of the cytokines appeared to be altered. For AHSCT patients increased levels of IL-8 at baseline were associated with EDA while for intrathecal RTX patients, higher levels of IL-8 were associated with EDSS progression.

## **Study V**

In this study, we wanted to examine whether AHSCT has altered the tissue-injuring disease process in RRMS patients by studying three commonly used CSF biomarkers: NFL, MBP, GFAP.

### **Key results**

Our most important finding was that within five years after AHSCT, in the majority of patients, the biomarkers of demyelination and axonal damage reached normal values. At baseline versus at 5 years, values above the upper limit of normal were 67 % vs 12 % for NFL, 63 % vs 12 % for MBP, and 16 % vs 25 % for GFAP. Other significant findings included the observation that 93 % of patients with normal baseline NFL values remained in NEDA-3. Similarly, 94 % of patients with normal baseline MBP values remained in NEDA-3. For GFAP, 81 % of patients with normal baseline values remained in NEDA-3. When it came to CDW, CDI and stability post-AHSCT, higher baseline levels were observed in patients with CDW and CDI as compared to EDSS-stable patients in both NFL (CDW p-value = 0.049, CDI p-value = 0.04) and MBP (CDW p-value = 0.03, CDI p-value = 0.01). For GFAP, baseline levels were numerically higher in CDW and CDI vs EDSS stable patients at follow-up but not statistically significant.

### **Limitations**

This study was limited by the fact that lumbar punctures are not available for all patients treated with AHSCT since approximately 1/3 decline to undergo it. A minority of patients had a five year follow-up, therefore the long-term effects of AHSCT should be interpreted cautiously. Controls were ever so slightly mismatched for age and sex to the patients.

## **Conclusions**

Within five years after AHSCT, for the majority of patients, the biomarkers of demyelination and axonal damage had normalized. This consequential finding shows that some of the tissue-injuring process has been altered through treatment with AHSCT.

## **Study VI**

In this study, we wanted to investigate CSF concentrations of CXCL13 and sCD27, biomarkers of B- and T-cell activation, in RRMS patients pre- and post-AHSCT.

### **Key results**

Our most important finding was that at baseline concentrations of CXCL13 and sCD27 were higher than in HCs. After a year post-AHSCT, CXCL13 concentrations were normalized, whereas sCD27 concentrations took two years to completely lower. Other important results included that patients with active disease were found to have higher CXCL13 concentrations than patients without active disease while no such contrast could be found with sCD27. Also, for patients that were treated with second-line DMDs, their levels of CXCL13 were significantly lower at baseline when compared to treatment-naïve or first-line DMD treated patients. The relationship between CXCL13 and sCD27 concentrations and EDA could not be confirmed.

### **Limitations**

This study was limited by the low number of included patients and that not all patients were able to provide CSF samples at all time-points, though each had at least a baseline and follow-up sample. Additionally, controls were not perfectly matched to patients in age and sex.

### **Conclusions**

This study showed that after AHSCT, RRMS patient CSF concentrations of CXCL13 displayed a precipitous decrease and essentially normalized within just a year. For sCD27, the decrease was more gradual but concentrations nevertheless also decreased and stably remained so after two years. This study provided evidence that AHSCT treatment arrests acute inflammation.

## Chapter 6: Concluding Remarks

«Нет ни одного сколько-либо общего закона природы, который бы основался сразу; всегда его утверждению предшествует много предчувствий, а признание закона наступает не тогда, когда зародилась первая о нем мысль, даже не тогда, когда он вполне сознан во всем его значении, а лишь по утверждении его следствий - опытами, которые естествоиспытатели должны признавать высшей инстанцией своих соображений и мнений.»

*Менделеев Д. И. – «Выписки из 8-го издания Основ Химии»*

“No law of nature, however general, has been established all at once. Its recognition has always been preceded by many premonitions and the recognition of the law does not come when the first thought about it was born, not even when it is consciously and in all meaning explained. Rather it is after the approval of its consequences – by experiments that [scientists] must recognize as the highest authority of their considerations and opinions.”

*Mendeleev D.I. (creator of the Mendeleev Periodic Table of Elements) – «Extracts from the 8st edition of the Fundamentals of Chemistry»*

Multiple sclerosis is a ruthless disease that destroys people in the prime of their lives, slowly piece by piece making them not only live through it but their families too. This leaves long lasting impacts on family, society and even the healthcare system. Although there is no *magic* therapy, which can *cure* all MS patients who have all types of MS in all stages of their disease, there is hope in this journey. Better yet, there is proof that some therapies work better in some cases than others.

Over the decades, science and medicine have inched closer to being able to diagnose better and sooner, allowing patients to receive treatment without losing as much precious time as before. Now, have we more possibilities to analyze treatment response, and provide a one-time treatment rather than subscribe patients to a life-long pill prescription.

Our work here is not yet done! But this is a big step in the right direction – giving patients the possibility of long-lasting effects not commonly seen with other treatments as well as the choice of what therapy to pursue.

## Chapter 7: Abstract

This thesis investigated the efficacy and safety of autologous hematopoietic stem cell transplantation (AHSCT) as a treatment of multiple sclerosis.

This thesis demonstrates that when compared to one of the most powerful disease-modifying drugs (DMDs), specifically alemtuzumab (ALZ), AHSCT is able to hold its ground. Patients that were treated with AHSCT were more likely to maintain NEDA-3 or freedom from disease in three parameters (no confirmed disability worsening, clinical relapses or MRI events) versus those treated with ALZ. The three year post-treatment Kaplan-Meier (KM) estimates of NEDA-3 were 88 % for AHSCT and 37 % for ALZ treated patients. With AHSCT, adverse events were more common in the first three months, as is expected with an intense one-time treatment. ALZ, though powerful and with little early adverse effects, has more late adverse effects instead, namely thyroid disease. At 3 years post-treatment, the KM-estimates of thyroid disease were 21 % for AHSCT and 46 % for ALZ. We then sought to address potential concerns in using AHSCT on patients previously treated with potent DMDs like alemtuzumab, cladribine or rituximab. The concern lay in the fact that these powerful DMDs have long-lasting effects on the immune system. We found that the rates of treatment-related and long-term complications were the same for all patients regardless of previous use of DMDs. This finding therefore allays those concerns: previous treatment with a powerful DMDs need no longer be a possible deterrent to giving a patient AHSCT.

Using a series of CSF biomarker studies, we gauged treatment efficacy. We showed that CNS inflammation is measurably affected by treatment, especially when it comes to AHSCT. Post-AHSCT treatment, many of the proteins we studied decreased towards the direction of healthy individuals, as early as the first year after treatment and remained so during subsequent follow-ups. Additionally we saw that tissue-injuring disease processes, for example represented by biomarkers like NFL and MBP, normalized for the majority of patients. These promising results suggest that tissue-injury could possibly be stopped in its tracks with AHSCT. Finally, we observed that biomarkers of B- and T-cell activation also became normalized, showing that AHSCT treatment also shuts down acute inflammation. All of these factors together explain why patients treated with AHSCT are able to maintain NEDA-3 so effectively

even when compared to one of the most potent drugs available. These results compellingly illustrate that AHSCT treatment yields quantifiably long-lasting and beneficial effects that are not commonly seen with other available treatments for MS. Therefore, use of AHSCT should be advocated, promoted and utilized more for the benefit of patients and society as a whole.

## Chapter 8: Sammandrag

Denna avhandling undersökte effekten och säkerheten vid autolog hematopoetisk stamcellstransplantation (AHSCT) som behandling av multipel skleros.

Denna avhandling visar att AHSCT kan hålla sin mark jämfört med ett av de kraftfullaste sjukdomsmodifierande läkemedel (SML), därav alemtuzumab (ALZ). Patienter som behandlades med AHSCT var mer benägna att bibehålla sjukdomsfrihet i 3 parametrar (ingen bekräftad försämring av funktionshinder, kliniska återfall/skov eller nya lesioner, funna med magnetkameraundersökningar – vidare: NEDA-3) jämfört med behandling med ALZ. Efter påföljande 3 år var Kaplan-Meier-uppskattningar av NEDA-3 88 % för AHSCT och 37 % för ALZ-behandlade patienter. Rörande tillbud kan nämnas att biverkningarna är vanligare under de första 3 månaderna. Detta förväntas dock, ty AHSCT är en mer intensiv engångsbehandling. ALZ, som är kraftfull och med få tidiga biverkningar, har fler sena biverkningar istället, därav sköldkörtelsjukdomar. Vid 3 års efter behandling var KM-uppskattningarna av sköldkörtelsjukdomar 21 % för AHSCT och 46 % för ALZ. Dessutom var det möjligt att ta itu med oron över användningen av AHSCT hos patienter som tidigare har behandlats med starka SML, såsom cladribin eller rituximab och rituximab – utöver alemtuzumab. Farhågorna grundade sig på att dessa kraftfulla SML har långvariga effekter på immunsystemet. Det var möjligt att se att frekvensen av behandlingsrelaterade och långsiktiga komplikationer var desamma för alla patienter oavsett tidigare SML. Detta innebär att föregående behandling med en kraftfull andra-linjens SML inte längre behöver vara avskräckande faktor för att ge patienterna AHSCT.

Serie av CSF-biomarkörstudier har visat att CNS-inflammationen påverkas klart mätbart av behandlingen. Efter AHSCT har mängden hos flera av de undersökta proteinerna minskat och rört sig i riktningen mot mängden hos friska individer. Detta skedde så tidigt som under det första året efter behandlingens start och förblev så under uppföljningar. Dessutom kunde de vävnadsskadande sjukdomsprocesserna (därav representerade av biomarkörer som NFL och MBP) ses normaliseras för majoriteten av patienterna. Dessa lovande resultat tyder på att utvecklingen hos vävnadsskador kan stanna av tack vare AHSCT. Slutligen kunde biomarkörer av B- och T-cellaktivering också ses

normalisera sig, något som visade att behandlingen stänger ner akut inflammation. Alla dessa faktorer förklarar varför patienter som behandlas med AHSCT kan bibehålla NEDA-3 så pass effektivt – även i jämförelse med en av de främsta läkemedlen som förekommer. Dessa resultat illustrerar att AHSCT ger kvantifierbara långvariga positiva effekter som vanligtvis inte ses med andra tillgängliga behandlingar för multipel skleros. Därför bör användning av autolog hematopoetisk stamcellstransplantation förespråkas, främjas och användas mer till förmån för patienter och samhället som helhet.

## Chapter 9: Аннотация

В этой диссертации исследовалась эффективность и безопасность аутологичной трансплантации гемопоэтических стволовых клеток (АТГСК) как метод лечения рассеянного склероза.

Диссертация демонстрирует, что по сравнению с одним из самых мощных препаратов изменяющих течение РС (ПИТРС) – в частности алемтузумабом (АЛЗ), АТГСК способна устоять. Улучшение картины заболевания по трём параметрам, а именно отсутствие подтверждённого ухудшения инвалидности, клинических рецидивов либо выявления новых очагов при помощи магнитно-резонансного томографа (далее: состояние «нет данных об активности заболевания – 3») в большей степени замечено у тех пациентов, которые получали АТГСК по сравнению с получавшими АЛЗ. Каплан-Мейерные состояния оценки НДА3-3 через 3 года после начала лечения составили 88 % для АТГСК и 37 % для пациентов, получавших АЛЗ. С АТГСК замечены некоторые особенности. Например, побочные явления чаще встречаются у пациентов после трансплантации в первые три месяца, ввиду интенсивного единоразового лечения. АЛЗ, будучи мощнее, а также с небольшими ранними побочными эффектами, напротив вызывает больше поздних побочных эффектов, таких как заболевание щитовидной железы. Через 3 года после начала лечения, КМ-оценки заболевания щитовидной железы составили 21 % для АТГСК и 46 % для АЛЗ. Кроме того, удалось устранить опасения по поводу использования АТГСК у пациентов, ранее лечившихся сильнодействующими ПИТРС, такими как кладрибин и ритуксимаб – помимо алемтузумаба. Проблема заключалась в том, что эти мощные ПИТРС оказывают длительное воздействие на иммунную систему. Было показано, что частота связанных с лечением последующих осложнений является одинаковой у всех пациентов, независимо от предшествующих ПИТРС. Это означает, что предыдущее лечение мощными препаратами второй линии ПИТРС больше не должно быть сдерживающим фактором для проведения АТГСК.

В серии исследований биомаркеров ликвора показано, что лечение оказывает заметное влияние на воспаление в центральной нервной системе, особенно когда речь идёт об АТГСК. При изучении белков

воспаления в ликворе до и после лечения АТГСК, многие белки снизились в сторону уровней здоровых людей – в частности в течение первого года после лечения, и оставались такими при последующих наблюдениях. Кроме того, удалось показать, что патологические

процессы, повреждающие ткани (например, представленные уровнями тонкого полипептидного нейрофиламента и основного белка миелина) нормализовались у большинства пациентов. Таким образом процесс повреждения тканей может быть приостановлен при помощи АТГСК. Наконец, биомаркеры активации В- и Т-клеток также нормализовались и показали, что АТГСК останавливает острое воспаление. Все эти факторы объясняют, почему пациенты, получающие АТГСК, способны так эффективно поддерживать состояние НДА3-3 даже в сравнении с одним из наиболее мощных ПИТРС. Эти результаты уверенно указывают на существование длительных и полезных эффектов АТГСК, которые обычно не наблюдаются при других доступных методах лечения рассеянного склероза. Агитирование в пользу применения аутологичной трансплантации гемопоэтических стволовых клеток должно быть усилено, а использованию методики следует расширяться на благо пациентов и общества в целом.

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# Cover Art

First, fill in the blanks on the left and THEN fill them into the blank spaces in the story.  
DO NOT read the story ahead of time.

Life in research. (START HERE)

1  
(something you study in science)  
2  
(noun)  
3  
(noun)  
4  
(noun)  
5  
(noun)  
6  
(shape)  
7  
(name - possessive form and profession plural)  
8  
(liquid)  
9  
(number)  
10  
(liquid)  
11  
(amount of time)  
12  
(type of machine)  
13  
(verb)  
14  
(kitchen appliance)  
15  
(verb)  
16  
(bathroom appliance)  
17  
(adjective and plural noun)  
18  
(theory about something you study in science)

Life in research. (INSERT HERE)

Doing research to study 1 (something you study in science) keeps every day interesting. Today, I will use 2 (noun) with 3 (noun) to turn it into 4 (noun). Procedure must be “followed to a t” or the results will turn out to be 5 (noun). First, we open the 6 (shape) – shaped box from 7 (name - possessive form and profession plural). Next, the samples are defrosted from -80°C slowly with 8 (liquid) bath and we start mixing all of the chemicals in the kit. Once we pipette 9 (number) microliters of 10 (liquid) into each well, we just have to wait 11 (amount of time) before we can place the plate into 12 (type of machine) for the results. While we wait, I will give you a tour of the lab. When we need to 13 (verb) the samples, we use 14 (kitchen appliance). To 15 (verb) some of the equipment, we use 16 (bathroom appliance). Now it’s time to finish the experiment. Woah! Looking at the results, they are 17 (adjective and plural noun). This proves our theory that 18 (theory about something you study in science)! What a day of science!

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