**Discussion and summary**

**Clinical aspects**

In order for patients to be diagnosed in time for HCT, it is of great importance that the different possible presenting symptoms are recognized by physicians. We described in chapter 3 that in juvenile and adult onset patients, psychiatric symptoms can precede neurological signs. The combination of an initially normal child with a clear change of behavior together with mild cognitive deterioration should prompt diagnostic evaluation for neurometabolic disorders. Early and correct diagnosis is not only essential to allow for HCT, a possible life saving treatment, but also for appropriate palliative treatment and genetic counseling. In addition, only through correct (and timely) diagnosis, siblings can be diagnosed while they are still presymptomatic and thus ideal candidates for HCT.

**Treatment**

MLD patients not treated with HCT invariably develop spasticity and often also a dyskinetic movement disorder, which is painful in many patients and can hamper daily care. Baclofen is a GABA-agonist that inhibits neural transmission at the spinal cord and is therefore frequently used to improve spasticity. Intrathecal baclofen treatment (IBT) allows specific drug administration to tissues that are most responsible for spasticity with little exposure to the brain, thereby reducing side effects. In chapter 4 we studied ITB treatment in MLD patients to reduce spasticity and compared this to patients with spastic cerebral palsy (SCP). We showed that ITB is a safe and feasible therapy to improve comfort and daily care in MLD patients with both spastic and dyskinetic movement impairments. The treatment in MLD patients is comparable to SCP patients regarding baclofen dosage and complications. We recommend ITB early in the disease course for patients in whom oral baclofen no longer sufficiently reduces painful spasms and when spasticity hinders daily care.

In chapter 5, we compared our transplanted patients with patients no longer eligible for HCT, diagnosed in the same time period. We showed that HCT is a safe procedure, with no treatment related mortality (TRM) in this study. HCT has proven to be able to stop the disease once performed in pre- or early symptomatic patients with the juvenile or adult onset type. For more advanced and late-infantile patients, HCT at best delays disease progression. Together with our study, two other large studies compared the effect of HCT in MLD patients to non-transplanted patients. Boucher et al report a TRM of 23%, which they partially explain by the fact that part of their cohort was transplanted years ago when HCT was riskier due to less advanced protocols and techniques. In consensus with our results they report efficacy for early, pre-symptomatic transplantation in later onset MLD types, but their results also show benefit from HCT for long-term survival across all MLD subtypes. Gröschel et al report a TRM of 17% in a group of juvenile MLD patients. They recommend HCT in juvenile patients with an age of onset older than 4 years, pre- or early symptomatic (GMFCS-MLD 0 or 1 and IQ ≥ 85), MRI score less than 17 (with a temporal or parietooccipital white matter subscore ≤4) and no involvement of U-fibers.
The difference in TRM between the studies of Boucher and Gröschel and our own study without TRM is remarkable. Boucher et al already point out the influence of older, less advanced protocols. In line with this, different conditioning regimens used are likely of influence on TRM. Another difference is that our cohort consisted of a large percentage (46%) of adult patients, whilst the other studies did not include adult patients or a smaller number of patients. How the subtype would influence TRM however is not evident, since the same conditioning regimens are used for children and adults. Moreover, the incidence of GvHD tends to be lower in children than in adults.\(^6\) Due to the slower disease progression, adults are usually less affected at time of transplantation, which might make them less vulnerable to the intensive treatment.

Unfortunately, some of our patients did deteriorate cognitively after HCT, despite a pre- or early symptomatic clinical condition prior to HCT. The disease manifestations in these patients are not only evidently slower compared to the natural course, but also progress despite stable white matter changes on MRI. This suggests neuroaxonal involvement less amenable to HCT. Why this decline is seen in some, but not all patients after HCT remains unclear. Both late-infantile, juvenile and adult patients were affected, so the subtype and pace of disease progression are not the only explanation. Longer follow-up time also for adult patients in whom the disease evolves slower will clarify how the disease fully unfolds after HCT and how often this cognitive deterioration occurs.

Pre- and early symptomatic juvenile and adult MLD patients are good candidates for HCT. In these patients, HCT can result in disease stabilization or even some improvement. Patients that are no longer able to walk without support and whose cognitive function is clearly affected (IQ below 75) will have no benefit from HCT. However, for patients with early juvenile onset IQ should preferably be higher, whereas in adult patients the slow disease progression may allow less stringent criteria. Additionally, brain abnormalities (rated with the MLD-Loes score) are predictive for outcome: patients with an MRI score above 15 at diagnosis are likely to have an unsuccessful outcome. Quantitative MRS can further be of aid in determining eligibility for HCT in ambiguous cases: severely reduced concentrations of (NAA) indicate low probability of a successful outcome.

In **chapter 6** we compared brain tissue of transplanted and non-transplanted patients to compare the inflammatory response and oligodendrocyte numbers between these two groups to gain further insight into the exact mechanism by which HCT halts further demyelination or even improves myelination. We found that in transplanted patients, there is presence of metabolically competent macrophages that are able to digest sulfatides, with a polarization of these macrophages towards an M2-like phenotype. There was a higher number of oligodendrocyte precursors and mature myelin forming oligodendrocytes in transplanted than untreated patients. These data suggest additional beneficial effects of HCT beyond cross-correction of enzyme deficiency that could be further exploited in order to improve outcome. That these changes could be demonstrated in spite of the fact that transplantation was not successful, underlines the robustness of these findings.

**Quantitative MRI techniques**

Quantitative MRI techniques such as proton MRS and DTI broaden our knowledge about the pathomechanisms involved in the disease. In **chapter 7** we described that MRS at diagnosis is predictive for clinical outcome.\(^7\) Patients with abnormal concentrations (severely reduced NAA, Glu
and Glx and increased Lac and Ins) had poor outcome, whilst patients with concentrations closer to normal had moderate outcome. NAA was the main explanatory variable. Notably, in some patients in whom we observed a normalization in the ratio of Cho/NAA, this improvement did not coincide with an improvement in MRI score or reduction in lesion volume. The MRI score also includes other WM regions and cerebral and cerebellar atrophy, which forms a partial explanation. MRS concentrations at diagnosis are of aid in deciding whether HCT will be beneficial, especially for patients with a borderline neurological and cognitive examination. If baseline metabolite concentrations are severely abnormal, there is low probability of a good outcome. In **chapter 8** we studied DTI parameters at diagnosis and follow-up in MLD patients and found decreased FA and increased MD and RD in NAWM, corpus callosum, and pyramidal tracts in patients compared to controls. In the thalamus no differences in FA were observed, but all diffusivities were increased in both patient groups. We found increased AD in the thalamus but decreased AD in the corpus callosum and NAWM of patients. These changes are most likely reflective of different pathological processes occurring in MLD, reflecting a balance between neuro-axonal loss and intracellular storage accumulation, depending on region and disease stage.

**Extra-neurological involvement**

Despite the fact that ASA activity has been shown to return to normal reference values after HCT, both sulfatide excretion in urine (own unpublished findings) as sulfatide accumulation in visceral organs and in the peripheral nervous system are not affected by HCT. In our transplanted patients, we saw remarkably large intrasubject fluctuations of sulfatide excretion in urine after HCT (unpublished data). All values remained above normal references values. We do not understand the reason for this large intrasubject fluctuations. Measurements were corrected for dilution of urine, and a catabolic versus anabolic state would not be expected to be of influence. For the increased sulfatide excretion itself after HCT we hypothesize that damage to kidney tissue, not repaired by the transplantation, or clearance of sulfatides in a tissue dependent pace (possibly slower in the kidney than in the central nervous system), results in this increased excretion. An alternative hypothesis is that the degradation of stored sulfatides requires higher amounts of ASA enzyme than the prevention of storage, implying that HCT does prevent further deterioration but does not restore ASA activity enough to ameliorate stored sulfatides. Another explanation might be that the donor macrophages do not reach the visceral organs (including the gallbladder and kidney) and the peripheral nervous system. The peripheral neuropathy can severely hamper motor function, especially in patients with an earlier onset, after transplantation.

All in all, the precise mechanism for the ongoing accumulation after HCT is not yet understood but definitely requires further attention since it will help us to optimize treatment options for MLD. It is evident that with the limitations of HCT, other therapy strategies are needed, perhaps even in combination with HCT, certainly for patients with early onset and for those in more advanced stages of the disease. This will be discussed under future perspectives.

In **chapter 9** we reported a high incidence of gallbladder abnormalities found in our MLD patients, suggestive of a causal relationship between MLD and the development of gallbladder polyps and
eventual carcinoma. The difficulty with gallbladder carcinoma is the extremely fast evolution, implying that usually, once it is symptomatic, curative treatment is no longer possible. Due to the various pathological abnormalities we found in our patients, including hyperplastic polyps, a known precancerous condition, we believe screening of the gallbladder by abdominal ultrasound should be added to the standard clinical care of MLD patients. If the ultrasound shows no abnormalities we recommend a follow-up ultrasound every 2 years. A cholecystectomy is advised for polyps exceeding 5 mm in HCT treated patients and untreated patients in good clinical condition in order to prevent untimely death from a preventable cause and improve quality of life. It is important to bear in mind that, due to the thickness of the gallbladder wall and a small collapsed gallbladder often found in MLD patients, there is a substantial risk of missing a polyp on ultrasound, with an increased risk of evolving into carcinoma. To minimalize this risk, we therefore advice considering cholecystectomy when polyps cannot be ruled out on the ultrasound. Kim et al also report a large MLD patient cohort with a high incidence of gallbladder abnormalities. Remarkably, they do not recommend to add an ultrasound to the standard clinical care of patients, but only for patients who present with abdominal pain. Additionally, they only advise cholecystectomy for patients with gallbladder abnormalities in the setting of relevant clinical symptoms, and not a prophylactic cholecystectomy in the case of asymptomatic polyps found on ultrasound.

**Follow-up of transplanted patients**

A standard, uniform treatment and follow-up protocol would be beneficial for patient care. Our current protocol contains a first assessment for transplanted patients 6 months after HCT, including neurological examination with scoring of gross motor function (GMFC-MLD), brain MRI (rated by the MLD-Loes score) including MRS, measurement of ASA activity, sulfatide excretion in urine and assessment of nerve conduction velocity. These assessments are repeated a year after HCT and consequently each year until 5 years after HCT. Cognitive function is evaluated one year after HCT, depending on age of the patient through the Bayley Scales of Infant Development-II, the Wechsler Intelligence Scale for Children-III or the Wechsler Adult Intelligence Scale-III. Chimerism analysis is usually performed at day 60 after HCT and subsequently every year after HCT, at least during the first 5 years. After that time, follow-up is adapted per patient and clinical status.

As previously stated, screening of the gallbladder by abdominal ultrasound should be added to the standard clinical care of MLD patients and included in the general evaluation prior to HCT; follow-up depends on the findings, but even in patients with normal ultrasound it should be repeated every 2 or 3 years.

We see ovarian dysfunction after chemotherapy in a substantial number of our female HCT-treated patients. Hormonal substitution is advised to prevent or treat symptoms related to estrogen deficiency such as osteoporosis and climacteric symptoms. Bone density should be followed as well.

**Palliative care**

Unfortunately, many patients are diagnosed when the disease has already progressed to a point where HCT or gene therapy would no longer be beneficial. For these patients, it is important that
they receive the best possible care for their inevitably progressing symptoms, to maintain good quality of life.

ITB as potent treatment of spasticity has been described above. Another treatment option for spasticity aside from ITB is a selective dorsal rhizotomy (SDR), in which the posterior lumbosacral rootlets from the spinal cord are partially transected in order to reduce the excitatory sensory input. The advantage of SDR is that it requires only one surgical intervention, whereas ITB requires multiple hospital visits for adjustment and refill of the pump. We have little experience with SDR for our MLD patients, but it has been shown that it can have a positive effect on comfort in non-walking children with spasticity, but pain is not always completely alleviated and daily care problems often persist. Additionally, patients are at increased risk for developing dystonia, which should closely be evaluated when considering SDR.

Epilepsy is a frequent symptom, especially in more advanced patients. Frequently occurring seizures should be treated in order to prevent possible co-morbidities as trauma, encephalopathy, aspiration and hospitalization. Seizures are usually well under control with medication.

With disease progression, drooling and dysphagia usually occur, which ultimately makes feeding via gastrostomy necessary. Timely placement of a gastrostomy, when first signs of swallowing dysfunction develop, is important to reduce the risk of aspirations and malnutrition. Another frequent problem is sialorrhoea, which is often treated with anticholinergics, of which glycopyrronium is the first choice. In advanced cases glycopyrronium may no longer be sufficient. Botulinum toxin injections in salivary glands can also be used to suppress salivation. It should be used with caution since a possible side effect is deterioration of the dysphagia and thickening of secretions.

Chronic pain and irritability are unfortunately not uncommon, especially in later stages of the disease. One should be aware of possible underlying causes such as neuropathic pain, spasticity, joint dislocation, bone fractures, constipation, bowel obstruction, appendicitis, gastroesophageal reflux and dental injury. Gallbladder colics and urinary retention, the latter due to neuropathy, are other possible causes of pain in MLD patients. It is also important to distinguish pain from discomfort as a response to strong stimuli or overstimulation. The response to analgesics or sedation can help discriminating between these causes. Sleep can be affected by the irritability, pain and discomfort. Melatonin is recommend as first step, other options are alimemazine and gabapentine. Gabapentine is a calcium channel modulator and is typically used to treat chronic pain or epilepsy. Apart from its regular indication, we used gabapentine in three patients suffering from irritability accompanied by increased muscle tone not sufficiently treated by ITB, with positive effect.

**Future perspectives**

**Novel treatments**

It is likely that in the future, therapy for MLD will be multimodal, including HCT-GT and enzyme replacement therapy. We learned from patients in whom HCT was successful that there are still obstacles to overcome such as the previously mentioned cognitive decline and the continuing peripheral neuropathy.
**Hematopoietic Stem Cell- Gene Therapy (HSC-GT)**

In HSC-GT, HSCs culture and manipulation are essential steps to achieve gene transfer. Vectors integrate into the host genome, thereby expressing the corrective gene in their progeny. Preliminary results of a lentiviral mediated HSC-GT clinical trial of 9 patients with presymptomatic late-infantile and early symptomatic early juvenile patients report safety of the procedure. At a median follow-up of 3 years after treatment, all patients were alive with halted disease progression or prevention of disease onset. One patient, who did have disease progression between enrollment and treatment initiation, did not benefit from the treatment. Remarkably, peripheral neuropathy (already present at diagnosis) improved in one third of patients 2 years after HSC-GT. This suggests that the above normal enzyme expression reached by HSC-GT has an advantage in correcting MLD. Remyelination, by local Schwann cell precursors, is thought to take place after removal of sulfatides from nerve tissue.

Intrathecal GT with viral vectors encoding ARSA has the advantage of more rapid and significant expression of ARSA in the brain over lentiviral mediated HSC-GT. Disadvantages are the invasiveness of the procedure and the risk of an immune reaction against the transgene. Intrathecal GT is thought to target mostly neurons, but the lysosomal enzyme could be secreted by transduced neurons and recaptured by other cells and thereby also correct the enzyme deficiency in oligodendrocytes. A phase 1/2 clinical trial to assess the safety of and efficacy of intrathecal GT with AAVrh.10hARSA into the white matter of both hemispheres (NCT01801709; clinicaltrials.gov) was stopped because of lack of efficacy (P. Aubourg, personal communication).

Intravenous ERT has not been efficient in controlling CNS disease manifestations, and therefore intrathecal ERT agent delivery and trials are ongoing to prove its efficacy. One such trial, using a biological recombinant of human ASA, has now completed, but results are pending (clinical trials.gov: NCT01510028).

**Newborn screening**

Criteria for inclusion of diseases in screening programs are broadly based on frequency, severity of the disease in the untreated population, availability of reliable testing methodology, effective treatment options and cost effectiveness. Other metabolic diseases such as X-linked adrenoleukodystrophy (X-ALD) and mucopolysaccharidosis type I (MPS-I) have recently been recommended for newborn screening (NBS) in the Netherlands. Krabbe disease, another lysosomal storage disorder affecting the CNS and PNS, has a disease course comparable to MLD. NBS for Krabbe disease has been implemented in the US (in the state of New York) since 2006. Early diagnosed infants can be treated with HCT. The inclusion of MLD in the NBS program is complicated by the fact that HCT is not an effective therapy for all subtypes. HSC-GT is now emerging and results are promising and suggestive of a safe and effective therapy also for the late-infantile subtype. This would make MLD a disease with (possible) effective treatment options for all subtypes, warranting therefore implementation within NBS. Still, it is essential to know what subtype a patient would develop; most of all to determine the best moment for treatment. Subtype determination would require mutation screening after ASA activity has been found low, but is complicated by the heterogeneity of disease causing mutations. However, it is known that if a patient is homozygous for mutations predicted to lead to complete loss of ASA activity, they will develop the late-infantile form of the disease. In general, the more effective ASA is produced, the later the onset of the disease.
Regarding the best moment for treatment, the question arises whether the earlier is per definition the better. For late-infantile patients this seems to be the case, but for adult patients one could question whether the benefit of treatment earlier than needed at a young age outweighs the possible risk of treatment related mortality and morbidity, risking otherwise healthy years. The best moment for treatment will therefore always remain a decision of both doctor and patient, different for each individual. More experience with the above-mentioned therapies and combinations of these will broaden our knowledge and will allow physicians to provide the best possible counseling.

Better understanding of the disease and its pathomechanisms will in the future optimize treatment options and will hopefully eventually make this devastating disease a treatable disorder, for all patients.
References


