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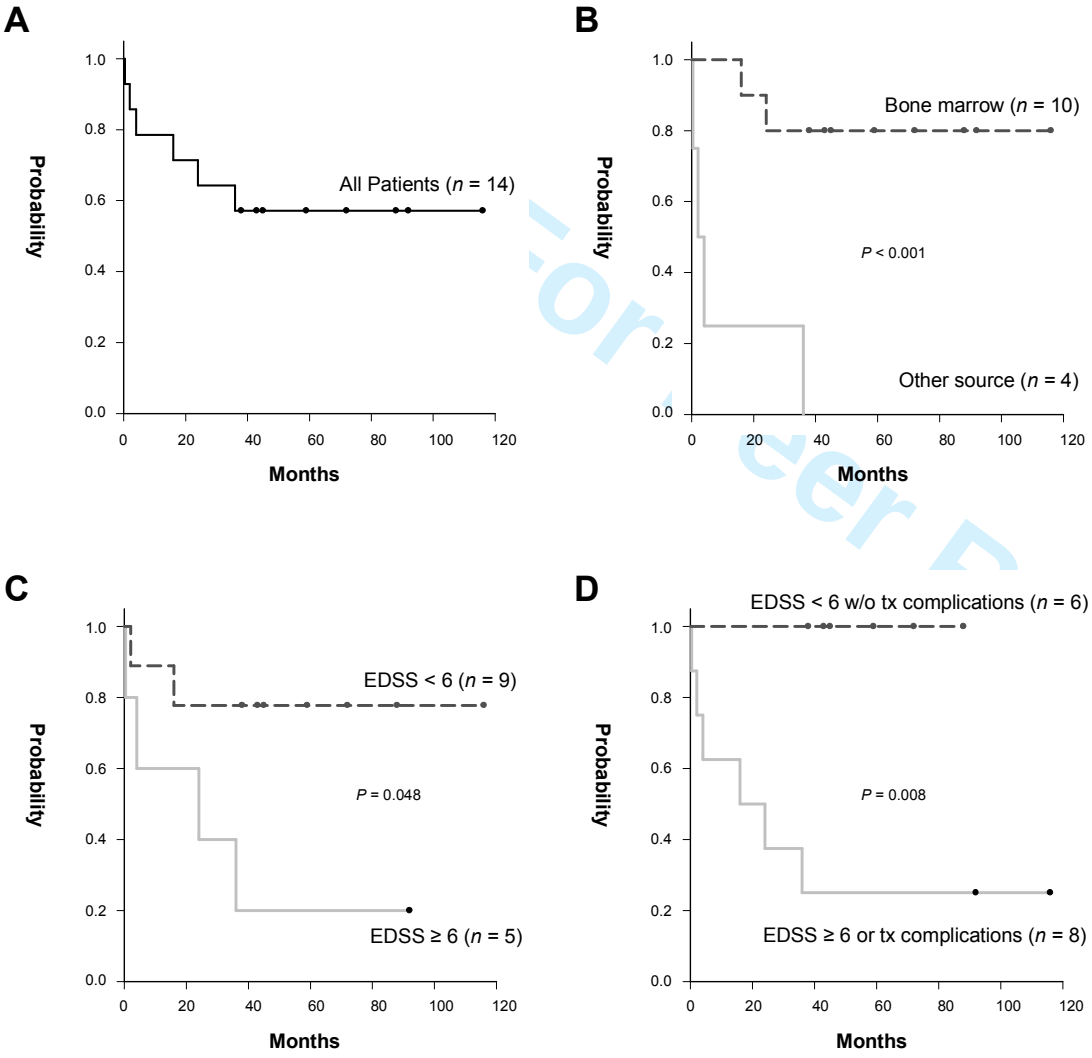

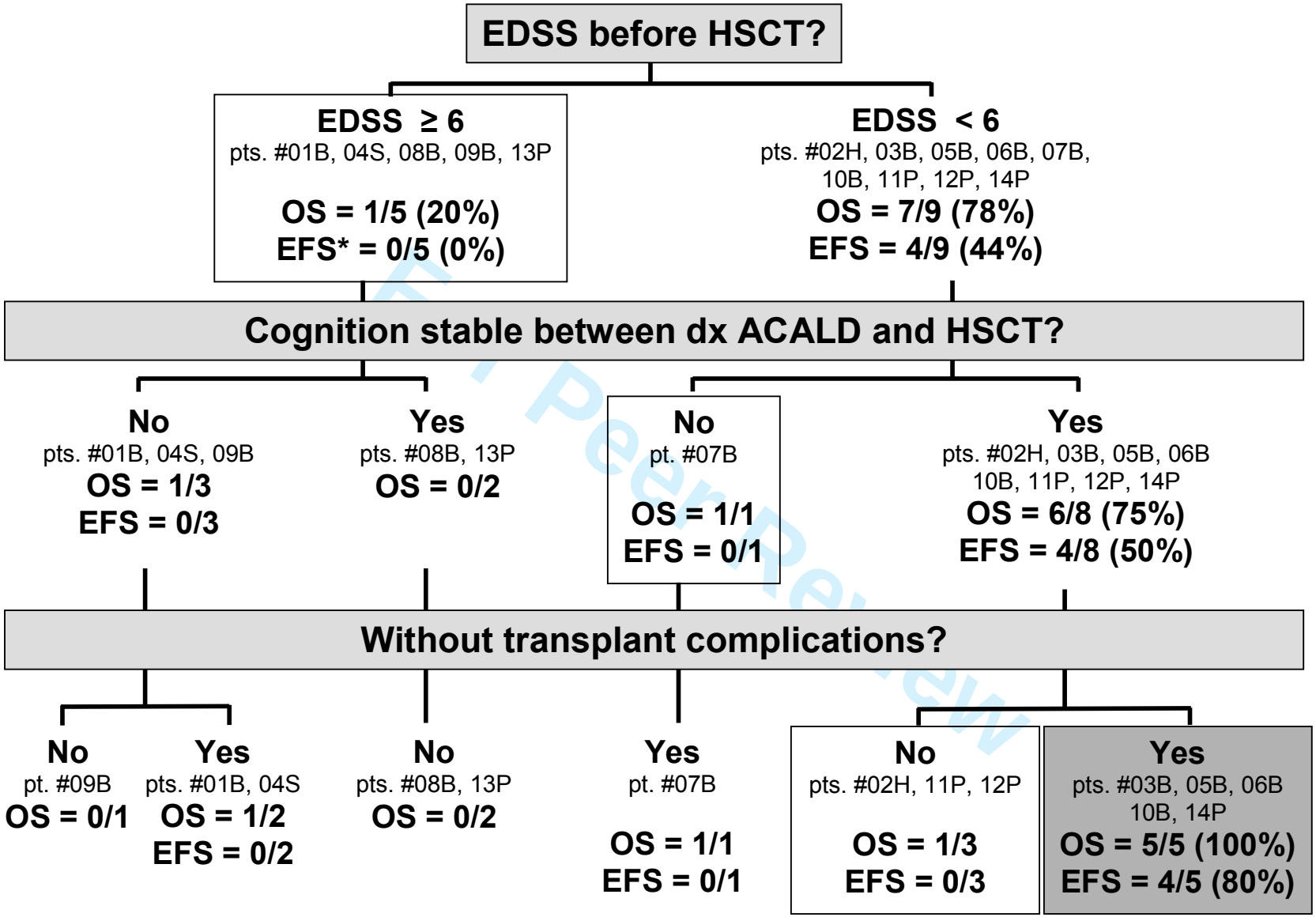

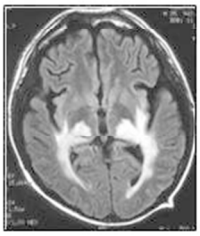

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Long-term outcomes of allogeneic hematopoietic stem cell transplantation for adult cerebral X-linked adrenoleukodystrophy

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*** These authors contributed equally to this work.**

P.A. and W.K. initiated, screened and evaluated the French and German patients, respectively, while F.S. and R.A. were responsible for transplanting the French and German patients, respectively. J.S.K. organized the German series, collected, ~~and~~ analyzed data and wrote the manuscript with P.A and W.K.

Abstract

The adult cerebral inflammatory form of X-linked adrenoleukodystrophy is a rapidly progressive neurodegenerative disease, as devastating as childhood cerebral adrenoleukodystrophy. ~~While~~ Allogeneic hematopoietic stem cell transplantation has been demonstrated to provide long-term neurological benefits for boys with the childhood cerebral form, but results in adults are sparse and inconclusive.

We ~~collected and retrospectively~~ analyzed data from 14 adult males with adult cerebral adrenoleukodystrophy ~~who had been~~ treated with allogeneic hematopoietic stem cell transplantation on a compassionate basis in four European centres. All presented with ~~progression of~~ cerebral demyelinating lesions and gadolinium enhancement. Median age at diagnosis of adult cerebral adrenoleukodystrophy was 33 years (range 21 - 48 years)**.** In addition to cerebral inflammation five patients ~~presented with~~ had established severe motor disability from adrenomyeloneuropathy affecting only the spinal cord and peripheral nerves (expanded disability status scale score ≥ 6).

Eight patients survived (estimated survival 57 ± 13 %) with a median follow-up of 65 months (minimum 38 months). Death was directly transplant-/infection- related (n = 3), due to primary disease progression in advanced adult cerebral adrenoleukodystrophy (n = 1), or secondary disease progression (n = 2) after transient multi-organ failure or non-engraftment ~~respectively~~. Specific complications during stem cell transplantation included deterioration of motor and bladder functions (n = 12) as well as behavioural changes (n = 8). ~~Whilst~~ Arrest of progressive cerebral demyelination and prevention of severe loss of neurocognition was achieved in all eight survivors, but deterioration of motor function occurred in the majority (n = 5). Limited motor dysfunction (expanded disability status scale score < 6) prior to ~~stem cell~~ transplantation was associated with significantly improved survival (78 ± 14 % [n = 9] versus 20 ± 18 % [n = 5]; P < 0.05) and maintenance of ambulation (expanded disability status scale score < 7) post-transplant (78 % versus 0 %; P = 0.021). In contrast, bilateral involvement of

the internal capsule on brain MRI was associated with poorer survival (20 ± 18 % [n = 5] versus 78 ± 14 % [n = 9]; $P < 0.05$).

This study is the first to support the feasibility, complications and potential long-term neurological benefit of allogeneic hematopoietic stem cell transplantation in adult cerebral adrenoleukodystrophy. Further studies are warranted to attempt to improve outcomes through patient selection and optimisation of transplantation protocols. ~~Given the rarity of this disease, it is essential to have clear referral pathways to specialist centres able to assess and treat patients without delay.~~

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Running title: Allogeneic HSCT in adult X-ALD

Key words: X-linked adrenoleukodystrophy; adult cerebral form; hematopoietic stem cell transplantation; brain MRI; long-term outcome

Abbreviations: AACS = adult X-ALD clinical symptom score; ACALD = adult cerebral inflammatory form of X-linked adrenoleukodystrophy; AMN = adrenomyeloneuropathy; CCALD = childhood cerebral inflammatory form of X-linked adrenoleukodystrophy; EDSS = Kurtzke expanded disability status scale; GVHD = graft-versus-host disease; HSCT = hematopoietic stem cell transplantation; IQ = intelligence quotient; X-ALD = X-linked adrenoleukodystrophy

Introduction

X-linked adrenoleukodystrophy (X-ALD) is an inherited peroxisomal disorder caused by a defective *ABCD1* gene leading to a characteristic accumulation of saturated very long chain fatty acids in blood and tissues. The white matter in the brain, spinal cord, adrenal cortex, Leydig cells and hair follicles is typically affected ~~tissues~~ (Moser, 1997; Kemp *et al.*, 2012; Wiesinger *et al.*, 2015). With an estimated combined incidence of 1:17,000 in men and women X-ALD is one of the commonest ~~most prevalent inborn~~ peroxisomal diseases.

Although ~~there is a~~ pronounced phenotypic variation may occur within kindreds, two major forms of X-ALD can be differentiated (Moser, 1997; Kemp *et al.*, 2012; Wiesinger *et al.*, 2015). Firstly, a chronic neuronopathic form, called adrenomyeloneuropathy (AMN), involving spinal cord and to a lesser extent peripheral nerves in adult males as well as heterozygous females, and characterized by a slowly progressive spastic paraplegia with sensory ataxia. Secondly, a cerebral demyelinating variant with neuro-inflammation that causes ~~rapid~~ white matter destruction. Cerebral demyelination results in rapid loss of neurocognitive, motor and sensory functions with vegetative state and death ~~following~~ within ~~months to~~ a few years of onset. To date, no triggers or other factors have been identified to predict the onset of this acute inflammatory cerebral form of X-ALD, which affects about a third of all males in childhood (childhood onset cerebral ALD, CCALD). ~~Although a~~ Primary isolated cerebral disease in adolescence or adulthood is ~~relatively~~ uncommon (5 - 10 %), although up to 63 % of men ~~suffering from~~ with AMN develop secondary cerebral ~~inflammatory~~ demyelination within 10 - 15 years (de Beer *et al.*, 2014). For the purposes of this study, primary and secondary cerebral demyelination with gadolinium enhancement of lesions in adulthood ~~will be~~ is termed adult cerebral ALD (ACALD). ACALD is as devastating as CCALD: ~~with~~ patients rapidly lose cognitive and motor function leading to death.

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established long-term treatment ~~method~~ in boys with CCALD (Aubourg *et al.*, 1990; Shapiro *et al.*, 2000; Baumann *et al.*, 2003; Peters *et al.*, 2004; Beam *et al.*, 2007; Miller *et al.*, 2011), although the ~~exact~~ mechanism of action is not fully understood (Moser and Mahmood, 2007; Schonberger *et al.*, 2007; Cartier *et al.*, 2014). Survival and neurological outcome of CCALD patients after HSCT is clearly superior compared to untreated patients (Mahmood *et al.*, 2007; Miller *et al.*, 2011). Despite the significant risks of HSCT, ~~there is~~ no other effective therapeutic option exists for CCALD. ~~In ACALD, there are~~ Only a few anecdotal reports of allogeneic HSCT in ACALD are available ~~with inconclusive results~~ (Hitomi *et al.*, 2005; Fitzpatrick *et al.*, 2008). In contrast to boys, the cerebral form in men is usually associated with motor and sensory deficits in the lower limbs as well as bladder dysfunction ~~resulting from~~ as a consequence of the AMN. The pattern of CNS demyelination in adults may also be different (Loes *et al.*, 2003; Eichler *et al.*, 2007). Moreover, bone marrow-derived cells infiltrating the brain may react differently in older patients (Barrett *et al.*, 2015). ~~Nevertheless,~~ The extremely poor prognosis with a median overall survival of 2 years once ACALD enters the phase of active neuroinflammation (van Geel *et al.*, 2001; de Beer *et al.*, 2014), combined with lack of any other effective treatment, has justified the extension of allogeneic HSCT into the older age group. ~~with the aim of reducing the rate of disease progression and death~~.

We report ~~here~~ a retrospective analysis of the feasibility, toxicity and long-term neurological outcomes of 14 adult males treated with allogeneic HSCT for ACALD ~~on an individually selected compassionate basis~~ in four European centres. Based on our combined experience, we propose some preliminary guidelines for patient selection and treatment protocols for allogeneic HSCT in this setting. ~~and identify future areas for research and development~~.

PATIENTS, MATERIALS, AND METHODS

Patients

Fourteen adult males underwent HSCT for ACALD in four hematopoietic stem cell transplant centres in Germany, France and UK. Diagnosis of X-ALD was based on elevated concentrations of fasting plasma very long chain fatty acids, and additionally on mutations of the *ABCD1* gene (HGNC: 61) in nine patients. Diagnosis of ACALD required the detection of gadolinium enhancement in cerebral demyelinating lesions by brain MRI.

Patient characteristics are summarized in table 1. Patients had detailed neurological, neurophysiological, and neuropsychological evaluation as well as MRI exams before and sequentially after HSCT. All patients were offered HSCT on an individually selected compassionate basis in accordance with the practice guidelines of the Working Party on Inborn Errors of the European Group for Blood and Marrow Transplantation (Peters and Steward, 2003) ~~and after providing~~ with written informed consent for HSCT. ~~In addition~~ The use of stem cell collections from unrelated donors and cord blood, where appropriate, were approved by the medical advisory boards of the respective national donor search programs for this indication.

Allograft selection, preparative regimen, and graft-versus-host disease prophylaxis

Twelve out of 14 patients received allogeneic HSCT from a matched donor after myeloablative conditioning. A matched donor was either a genotypical HLA-identical sibling (n = 3) or a ≥ 9/10 HLA-matched unrelated donor (n = 9) confirmed by high-resolution DNA typing of HLA class I (HLA A, B, Cw) and class II (HLA DRB1, DQB1) alleles. Ten of these 12 patients were transplanted with bone marrow, and two patients received granulocyte-colony stimulating factor (G-CSF) mobilised peripheral blood stem cells based on donor

preference. Myeloablative conditioning consisted of busulfan and cyclophosphamide. In two patients ~~there was an inability~~ it was not possible to identify a suitably HLA-matched donor, and they underwent unrelated cord blood transplantation after reduced-intensity conditioning. All but two patients received additional serotherapy for graft-versus-host disease (GVHD) prophylaxis: polyclonal antithymocyte globulin from Genzyme® (Saint Germain en Laye, France; *n* = 1), Fresenius® (Fresenius Biotech, Gräfelfing, Germany; *n* = 10), or the monoclonal antibody alemtuzumab (~~from~~ Genzyme®; *n* = 1). Basic transplant characteristics of all patients are summarized in table 2. GVHD prophylaxis and supportive care measures were delivered according to standard of care protocols at the individual centres.

Assessment of engraftment, GVHD, and toxicity

Engraftment with full donor chimerism was defined as > 90% donor cells in total nucleated cells at day +100 post-HSCT as detected by DNA-based techniques (i.e. short tandem repeats analysis). Diagnosis of acute GVHD was primarily based on clinical criteria; overall staging of acute and chronic GVHD was done according to published criteria. Transplant toxicity was described according to the National Cancer Institute common terminology criteria for adverse events version 3.0 (CTCAE v.3.0) with severe toxicity recorded for adverse events grade ≥ 3.

Data acquisition and assessment of neurological outcome

Patient-related clinical information was obtained from retrospective review of medical records in each transplant centre ~~as well as~~ and from their neurologists.

~~To analyse changes in disease status, the following~~ Assessment tools were used to analyse changes in disease status: the Adult X-ALD Clinical Symptom score (AACS) (Köhler and Sokolowski, 1999) to quantify the overall X-ALD neurological impairment, the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) to assess motor dysfunction, the modified Rankin scale (Rankin, 1957) to describe disability status in daily activities, and the

Loes MRI severity score (Loes *et al.*, 1994) to measure the extent of cerebral demyelination. ~~Detailed~~ Neurological examination covered the following areas: motor function, sensation, bladder function, as well as overall CNS function including vision, hearing, and speech. ~~Based on these evaluations and from transplant records scores for~~ AACS, EDSS, and modified Rankin scale were ~~assigned~~ determined for the time points pre-HSCT, worst status during first 6 months post-HSCT, and status approximately 24 months post-HSCT (minimum ≥ 12 months) for evaluable patients. In four patients (#01B, #03B, #04S, and #07B), no MRI scans were available before the onset of ACALD, while in three patients (#12P, #08B, and #09B), post-HSCT MRI could not be obtained due to poor clinical status and early death. In addition to Loes score, MRI scans were reviewed for gadolinium enhancement and patterns of demyelination (parieto-occipital ± long tract pyramidal involvement versus all other patterns). ~~Furthermore,~~ Lesion progression was calculated from sequential MRI scans. Neuropsychometric assessment was performed pre-HSCT and, whenever possible, at various time points post-HSCT. Normalized measures of intelligence quotient (IQ) were generated by appropriate tools according to the centres' preference.

Patients unable to walk without aid or rest for about 100 meters (EDSS ≥ 6), were classified as advanced AMN. Stable motor function post-HSCT was defined as increment in EDSS < 1 point with preserved/maintained ambulation (EDSS < 7). Severe deterioration in motor function was classified as increment in EDSS ≥ 2 points or to EDSS ≥ 7. Stable neurocognition post-HSCT was defined as deterioration in IQ < 15 (< 1 standard deviation) or no ~~relevant~~ cognitive deterioration as detected by care-givers; severe deterioration in intellectual function was classified as obvious cognitive decline or inability to test for IQ anymore. Moderate deterioration was defined as any deterioration less than severe. For the purpose of this study, events were defined in relation to ACALD progression, i.e. event-free survival was used in this study for surviving patients who retained a stable cognitive function after HSCT.

Statistical analysis

Survival was compared by Kaplan-Meier estimates, and comparisons done by the log-rank method. Categorical variables were compared using the z-test or Fisher's exact test. Comparison of continuous variables was performed by non-parametric tests (Mann-Whitney signed rank test, the Kruskal-Wallis ANOVA with following pair-wise comparisons according to Dunn's method). Before/after analyses were performed for single time points with the Wilcoxon signed rank test or for multiple time points with the Friedman repeated measures ANOVA followed by multiple pair-wise comparisons according to the Tukey's test. Calculations were done using Sigmaplot 11.0 (Systat Inc., San José, CA, U.S.A.).

Results

Vital status, engraftment, disease progression-related mortality, and transplant-related mortality

Fourteen ACALD patients transplanted between 2003 and 2012 at a median age of 34 years (range 21 – 48 years) were included in this study. Characteristics of HSCT are summarized in table 2.

Eight of the 14 patients were alive with a median follow-up of 65 months (range 38 – 116 months). The estimated overall survival probability was 57.1 ± 13.2 % (mean ± standard deviation) (Fig. 1A). There were no survivors after cord blood transplantation with reduced intensity conditioning (n = 2) nor after myeloablative conditioning and peripheral blood stem cell transplantation (n = 2), while eight out of 10 men after myeloablative conditioning and bone marrow transplantation survived (estimated survival probability 80.0 ± 12.6 % (log-rank test 11.48, P < 0.001) (Fig. 1B). The small numbers did not permit detection of relevant transplant differences between patients with matched donors.

Full donor chimerism (> 90% donor cells) was detected in 10 out of 11 evaluable patients receiving myeloablative conditioning; one patient showed 80 % donor cells (table 2). ~~One patient with early fatal complications was not tested.~~ Two men who received cord blood after reduced intensity conditioning did not permanently engraft. ~~Among~~ In the 12 patients receiving myeloablative conditioning, neutrophil recovery (peripheral neutrophil count ≥ 500/µl for 3 consecutive days) occurred at a median of 19.5 days (range: 10 – 34 days). ~~(data not shown).~~ The incidence of severe GVHD was low: only one patient (8 % of all men engrafting with donor cells) developed acute GVHD grade ≥ II (grade 4 of the skin), while chronic GVHD was observed in limited form only in four patients.

There was one death from primary disease progression without relevant HSCT complications (patient #4S). Two further patients died from secondary disease progression after life-

threatening infections with transient multi-organ failure (patient #2H) or graft-rejection (patient #13P). Three early deaths (21 %) were directly transplant- or infection- related, either associated with non-engraftment (patient #12P) or immobility due to advanced AMN (patients #8B and #9B).

Survival was related to baseline motor dysfunction prior to HSCT: limited AMN (EDSS < 6) was significantly associated with superior survival compared with advanced AMN (estimated survival probability 77.8 ± 13.9 % (n = 9) versus 20.0 ± 17.9 % (n = 5); log-rank test 3.91, P = 0.048; Fig. 1C). After excluding from the analysis the two patients who failed to engraft, the EDSS gained further prognostic significance with the estimated survival probability increasing to 87.5 ± 11.7 % (n = 8) for EDSS < 6 versus 25.0 ± 21.7 % (n = 4) for EDSS ≥ 6 (log-rank test 5.16, P = 0.003). ~~(data not shown).~~

Neurological status at diagnosis of ACALD and before stem cell transplantation

~~Basic~~ Disease characteristics of the 14 ACALD men before HSCT are summarized in table 1. Cerebral disease was first detected at a median age of 33 years (range: 21 – 48 years). Ten X-ALD men had been ~~followed~~ monitored in the years before the onset of ACALD. Four patients were first diagnosed with Addison's disease during adolescence (median age at diagnosis 16.5 years (range: 10 – 18 years)) in the absence of AMN at that time. Two patients had been diagnosed with AMN at the age of 24 and 31 years because of spastic paraparesis. ~~and~~ Three patients were identified by family screening with either no symptoms (#10B; age 15 years), increasing learning difficulties (#14P; age 18 years), or gait disturbances in combination with undiagnosed Addison's disease (#8B; age 23 years). At diagnosis of X-ALD ~~trait~~, patient #14P presented with extensive parieto-occipital leukodystrophy and marked ventricular dilatation but without gadolinium enhancement as indication for arrested CCALD at that time. The median period between diagnosis of X-ALD and onset of ~~CNS inflammation~~ ACALD was 132 months (range: 19 – 266 months) among these 10 patients. In contrast, four

patients (#1B, #3B, #4S, and #7B) already had evidence of ACALD at their first MRI examination. Although patient #4S had been originally diagnosed at the age of 24 years by family screening, he was not seen again until 8 years later at which stage he had developed visual and auditory deficits and ataxia. Patient #7B presented with seizures as first symptom. The history of AMN symptoms among these four tended to be shorter than in the other 10 patients (median duration 15.5 months versus 37 months; $P = 0.088$).

~~At detection of cerebral disease,~~ All but one patient (#14P) presented with AMN symptoms, which varied considerably in duration (median 24 months, range 0 - 266 months) and severity: while three patients ~~were already severely impaired by~~ had severe motor dysfunction (EDSS ≥ 6), two patients displayed no motor dysfunction, ~~at all~~ and two others showed only a mild limitation in running. There was no correlation between EDSS and age, but some linear correlation between duration of AMN symptoms and EDSS was observed (r = 0.735; $P = 0.003$). Bladder dysfunction was ~~also~~ common and present in 12 patients, with marked impairment in two of them. Sensory dysfunction was overall mild in 13 patients and in patient #6B ~~mild par-/hypoesthesia~~ was the only AMN symptom.

The median interval from detection of ACALD to HSCT was 9 months (range 3 -15 months). During this period there was a ~~relevant~~ clinical disease progression in six patients: AMN symptoms - one, cognition - two, both motor and cognitive functions - three patients. Patient #6B developed a visual field defect, while IQ remained stable. Patient #14P still had a strictly normal neurological exam even before HSCT, while five other patients presented with severe motor disability (EDSS ≥ 6). With regard to occupational status, five men had discontinued their established work/profession at onset of ACALD, four of them because of progressive motor dysfunction. In the period prior to HSCT, four more patients were unable to continue working mainly due to neurocognitive decline. ~~In addition, three men had developed severe weight loss before HSCT, in patient #5B at least in part due to a low-fat diet in combination with "Lorenzo's oil".~~

The median IQ before HSCT was 99 (range 72 – 139). Cognitive deterioration was suspected or reported by relatives in all four patients who showed ACALD in their first brain MRI. Neuropsychological evaluation before HSCT in these patients also suggested deterioration by revealing better verbal IQs than performance IQs (data not shown). However, seven out of the 10 patients who were followed up long-term displayed no relevant IQ changes until admission for HSCT ($P = 0.018$).

Neurological outcome early after HSCT

Significant neurological and/or behavioural changes were observed during the transplant procedure and early follow-up in all but one ACALD patients (table 3).

Exacerbation of motor disability during the first 6 months post-HSCT was a characteristic feature observed in 12 out of 14 patients: The median increment in EDSS was 2.8 points with considerably heterogeneity (range: 0 - 7 points). Only two patients who did not demonstrate obvious gait disturbances before remained unaffected in their motor function (patients #6B and #14P). All other 12 patients deteriorated further; 10 (71%) of them were at least temporarily unable to walk more than few meters even with aid (EDSS ≥ 7). With the exception of patient #14P, bladder (and sphincter) dysfunction also became apparent or aggravated during the transplant period, especially with fever caused by serotherapy or infection. ~~(similar to the Uhthoff phenomenon observed in multiple sclerosis).~~ Two patients developed seizures during the transplant procedure. Deterioration of neurocognitive function, significant hearing or vision impairment as well as severe disorientation and somnolence occurred in five patients (36 %) during early post-transplant phase, four of whom subsequently died. ~~In addition~~ Moderate to severe behavioural changes were observed in 11 men (79 %), which improved markedly or disappeared in all surviving patients after 6 - 12 months.

Patients with limited AMN at admission for transplantation (EDSS < 6) developed

significantly fewer neurological symptoms (other than progressive motor disability) compared to the ones with advanced AMN (1/9 = 11 % versus 4/5 = 80 %; z = 2.001, *P* = 0.045).

Long-term neurological outcome after HSCT

The detailed long-term follow-up for each patient is listed in table 4, excluding the three patients who died within the first 6 months post-transplant. ~~While~~ Three patients progressively deteriorated ~~in all functions~~ during the first year post-HSCT and died from ACALD progression 16 to 36 months after transplant.

Eight patients stabilized beyond 6 months post-HSCT and became long-term survivors. ~~Among these eight survivors~~ Two of these did not deteriorate during the transplant procedure and showed a stable motor function thereafter. Exacerbation of motor disability during the first 6 months post-transplant partly reversed in the other six patients. ~~But~~ However, only three men (21 % of all transplanted patients) could walk without assistance (EDSS < 6) 24 months after HSCT. ~~while~~ Patient #1B was still unable to walk more than a few meters even with aid (EDSS = 7). The median gain in EDSS in these men was 1.5 points (range 0.5 - 5 points) compared to baseline status. After 24 months post-HSCT, motor function further improved in four patients, while two deteriorated and two remained stable. ~~Although~~ Bladder dysfunction ~~also~~ improved partly over time, but five of the eight patients showed deterioration in comparison to the pre-HSCT status.

In contrast to motor and bladder function, basic cognitive functions were preserved in all surviving patients. Pure intellectual function remained stable in five patients (36 % of all transplanted patients): IQ testing was unchanged in three patients (#3B, #5B, and #6B). Patient #11P had unaltered cognitive function, but was classified as “moderate deterioration” due to significant visual loss. Three other surviving patients (#1B, #7B and #10B) showed a moderate cognitive decline. The deterioration in information processing and learning aptitude in patient #10B may also been influenced by prior excessive drug abuse. Among the five

patients, who had maintained their vocational status prior to HSCT, two (patients #6B and #14P) continued as students, one (patient #11P) was unable to resume work due to severe motor dysfunction and visual loss and two died following HSCT (patients #2H and #9B).

IQ before HSCT was not predictive for outcome: the five patients with proven or suspected cognitive impairment before transplantation (patients #1B, #3B, #4S, #7B, and #14P) had a variable transplant course and outcome after HSCT.

MRI results

The Loes MRI severity score varied considerably both at onset of ACALD and before HSCT. When ACALD was detected, median Loes score was 4.5 points (range 2 – 12 points) of maximum 34 points. The four patients with ACALD at first MRI examination (#1B, #3B, #4S, and #7B) tended to have a somewhat higher overall Loes score (median 7.8 points, range 4 – 9 points). Loes score increased during the time period between first detection of ACALD and HSCT to a median 6.5 points (range 2 – 14 points; $P = 0.004$). Five patients remained stable, while in six patients (#1B, #4S, #5B, #7B, #8B, and #12P) ~~a~~ progression of cerebral demyelination resulting in deterioration of at least 2 points was observed. There was no correlation with progression in Loes score and that of EDSS, AACS, or in Rankin score before HSCT. For the 10 patients with MRI scans performed 6 – 36 months before the onset of ACALD, the median Loes score progression rate was 1.2 points per year (range 0 – 5.3 points). For ~~the~~ 12 evaluable patients, the median Loes score progression rate between onset of ACALD and HSCT was 2 points per year (0 – 6 points).

Due to early death or poor condition, three patients did not have MRI follow-up (~~cp.~~ table 3). Only patient #1B showed minimal contrast enhancement 6 months post-HSCT. None of the 11 patients examined beyond 6 months after transplant showed further gadolinium enhancement of cerebral demyelinating lesions. There was no significant increase in Loes

score beyond 12 months post-HSCT in comparison to Loes score before HSCT among the eight survivors (median 8 points (range 2.5 – 12 points) before HSCT; median 10 points (range 5 – 12 points) > 12 months post-HSCT).

The three patients with late death after HSCT tended to show a greater increase in Loes score (median gain 4 points; range 1.5 – 18 points) compared with the four experiencing moderate deterioration in cognitive function (median gain 1.5 points; range 0 - 9.5 points) and the four with stable cognitive function (median gain 0.8 points; range 0.5 – 2 points). ~~Similarly,~~ New demyelinating lesions after HSCT were detected in only one of the four cognitively stable patients, whereas lesion progression and new lesions were seen in all three patients experiencing late mortality. However, none of these differences were ~~not~~ significant.

Based on MRI ~~appearances~~ analysis, 10 patients (71 %) showed involvement of pyramidal long tract fibers, reflecting AMN (~~cp.~~ table 1). With regard to ACALD, only seven out of 14 patients (50 %) were characterized by the typical parieto-occipital pattern without additional involvement of other structures than pyramidal long tract fibers. Among the other seven patients, three patients (21 %) demonstrated predominant involvement of the frontal lobe white matter, while four patients (29 %) showed ~~relevant~~ demyelinating lesions in ~~even~~ other structures (basal ganglia or cerebellum). Although two of the latter (patients #4S and #5B) displayed additional parieto-occipital involvement, they were classified as an atypical pattern. In contrast to the Loes score itself, the MRI demyelination pattern seemed to have an impact on HSCT outcome: Among the 12 men engrafting (cord blood transplantations excluded) the estimated survival of ACALD patients displaying the typical parieto-occipital pattern (with or without projection fiber involvement; $n = 6$) was 100 % versus 33.3 ± 19.2 % ($n = 6$; log-rank test 5.57, $P = 0.018$) for all other patterns.

MRI appearances were further analysed according to the study of Eichler et al. (Eichler et al., 2007): Patient #9B had corticospinal tract involvement without corpus callosum involvement, eight patients (#1B, #3B, #4S, #7B, #8B, #10B, #13P, and #14P) had corticospinal tract

involvement with additional involvement of the splenium or genu, and five patients (patients #2H, #5B, #6B, #11P, and #12P) had no corticospinal tract involvement but other white matter lesions within the brain. Aside from an increased EDSS in the nine patients with corticospinal tract involvement (median EDSS 5.0 versus 2.9), there were no significant differences in age, ratio of progression, rate of lesion progression as well as outcome compared with the other patients. However, the five patients (#1B, #4S, #8B, #9B, and #13P) with advanced corticospinal tract axonopathy involving the two internal capsules were older at ACALD onset, had all an EDSS ≥ 6 before HSCT, and had a significantly poorer post-transplant survival compared with the unaffected patients with a trend for a faster rate of Loes score progression before HSCT among these patients (details summarized in table 5). In this cohort, ~~the factor~~ bilateral involvement of internal capsule identified the same subgroup of patients as the criterion EDSS ≥ 6 just before HSCT (~~ep.~~ Fig. 1C).

Analysis according to baseline status and transplant complications

To ~~further~~ analyze neurological status between patients with excellent versus poor survival further ~~propability~~, patients were divided into two groups depending on motor disability before HSCT and early transplant complications, i.e. life-threatening to fatal infections during the first 6 months post-transplant and/or graft rejection:

Group I (n = 6) included patients #3B, #5B, #6B, #7B, #10B, and #14P with limited AMN symptoms pre-HSCT (EDSS < 6) and absence of severe early transplant complications.

Group II (n = 8) included five patients (#1B, #4S, #8B, #9B, and #13P) with advanced AMN before HSCT (EDSS ≥ 6) and three patients (#2H, #11P, and #12P) with severe transplant complications.

There was a significant survival advantage for group I patients with 100 % in comparison to group II patients with 25.0 ± 15.3 % (log-rank test 7.06; P = 0.008; Fig. 1D). Neurological and disability status of both patient groups was quantitatively assessed before as well as early

(< 6 months) and late (approximately 24 months) after HSCT using EDSS, AACS, modified Rankin, and Loes score (table 6). ~~Because~~ The extent of motor dysfunction was a major criterion to define both groups, consequently the median EDSS at baseline tended to be different between both groups (though not significantly). Patients in both groups deteriorated during the early post-HSCT period in both motor and bladder function as well as cognitive function, reflected by an increase in EDSS (not significant for group I), AACS, and modified Rankin score. ~~Relevant~~ Differences between both groups became apparent during the long-term follow-up: all six patients in group I survived and partly recovered, i.e. these patients had a 67 % and 50 % chance fully to preserve cognitive and motor function, respectively (table 4), and did not demonstrate significant differences in EDSS, AACS, and modified Rankin score late post-transplant in comparison to baseline status. In contrast, the majority of group II patients steadily deteriorated and died while the two long-term survivors remained moderately to severely impaired both in their motor and cognitive function (patient #11P had only visual impairment with preserved cognitive functions). Accordingly, late post-HSCT status was significantly different between group I and II patients for EDSS and AACS score. There was no significant difference in Loes score, but data were either missing (n = 3) or only available from 8 months post-HSCT (n = 1) for half of the group II patients.

Of the five patients with an EDSS ≥ 6 before HSCT, only patient #1B survived experiencing further cognitive decline (Fig. 2). A baseline EDSS < 6 identified a group of nine patients with overall good survival. However, cognitive decline between diagnosis of ACALD and transplantation was associated with further cognitive deterioration post-transplant (patient # 7B). For the remaining eight patients, transplant complications had a major impact on outcome. In the absence of transplant complications, all five patients survived with stable cognitive function, whereas of the three ~~patients~~ who experienced life-threatening infections and/or graft rejection, only patient #11P survived with loss of vision.

Discussion

This retrospective study of 14 ACALD men provides proof-of-principle that allogeneic HSCT can arrest inflammatory cerebral demyelination allowing survival with preserved neurocognitive function, at least in a sub-group of patients. The estimated mean survival time of the engrafted 12 patients was 81 months, in contrast to 37 - 41 months reported for untreated patients with cerebral demyelination ~~before~~ (van Geel *et al.*, 2001; de Beer *et al.*, 2014). Moreover, four patients in our series (29%) maintained ~~a~~ completely stable intellectual function, and another four developed only moderately impaired neurocognition over a median follow-up period of more than 5 years. Patients who did not progress ~~during~~ 6 to 12 months post-transplant became long-term survivors with preserved cognitive function ~~but in most cases severe~~ despite ongoing AMN symptoms. The results of HSCT therefore appear ~~with regard to survival and cognition may~~ to be similar to that observed in CCALD patients (Mahmood *et al.*, 2007), ~~provided the procedure~~ if transplant is performed in patients with stable cognitive function as well as limited AMN symptoms. ~~before transplant.~~ The relatively long ~~median time lag~~ interval between first detection of ~~CNS inflammation~~ ACALD and HSCT of 9 months in comparison to 5.1 months in boys with CCALD (Miller *et al.*, 2011) ~~along with refined transplantation protocols~~ may offer future improvement.

~~Transplant outcome for adults with ACALD is presumably dependent on both neurological baseline status before HSCT as well as the transplant procedure itself.~~

~~With regard to factors that may help to optimise the HSCT procedure~~ The small number of patients allows for only cautious comments regarding factors that may improve outcomes. Similar to established paediatric protocols, most of our adult cohort were treated with a myeloablative busulfan- and cyclophosphamide- based regimen ~~which confirmed the feasibility of engraftment~~. Under these conditions, using bone marrow as stem cell source appeared to be the best single predictor for survival in this series. However, this conclusion

may be biased because of confounding factors related to patients treated with peripheral and cord blood stem cells.

Graft failure occurs more frequently with HLA-mismatched grafts compared to matched donors ~~(relative risk 7.6)~~, in non-malignant versus malignant diseases ~~(relative risk 3.3)~~, and after reduced intensity conditioning compared to myeloablation ~~(relative risk 2.6)~~ (Olsson *et al.*, 2013). According to the same study, graft failure in non-malignant disorders had no effect on survival, whereas graft rejection in our series was associated with death. ~~Moreover,~~ In CCALD, immunoablation alone without donor engraftment does not prevent progression of disease ~~in CCALD~~ (Nowaczyk *et al.*, 1997; Miller *et al.*, 2011). Unrelated cord blood transplantation after myeloablation may be effective in boys with CCALD lacking a matched donor (Beam *et al.*, 2007; Miller *et al.*, 2011). In these studies, however, the overall survival of patients receiving cord blood cells was inferior to other stem cell sources. Therefore, there should be caution regarding unrelated cord blood for ACALD transplant candidates lacking a matched donor, at least with a reduced-intensity conditioning regimen. A promising future alternative option for patients without a ~~closely~~ matched donor may be gene-modified autologous hematopoietic stem cell transplantation (Cartier *et al.*, 2009).

In our series, the two patients who had peripheral blood stem cell transplantation did not survive. Although the reason for failure was most likely due to AMN related immobility with subsequent life-threatening infections, peripheral blood stem cells may also have a negative impact. Transplantation with peripheral blood stem cell in comparison to bone marrow usually results in faster engraftment, more GVHD (Holtick *et al.*, 2015) and higher mortality in non-malignant diseases (Bacigalupo *et al.*, 2015). In ACALD patients, prevention of GVHD may be of particular importance. Rapid clinical deterioration after the onset of severe acute GVHD has been observed in CCALD patients (Peters *et al.*, 2004; Miller *et al.*, 2011) and in a patient with ACALD (Fitzpatrick *et al.*, 2008). In addition, chronic GVHD was associated with temporary deterioration in motor function of an adult patient (Hitomi *et al.*,

2005). Although overall GVHD incidence in this series was low compared to other transplant patients (Socie *et al.*, 2011), the only case of significant acute GVHD was observed after peripheral blood stem cell transplantation.

In summary, we therefore recommend the use of a myeloablative, busulfan-based conditioning regimen in combination with bone marrow for transplanting ACALD patients (see box). However, we recognise that future research could focus on potential improvements in the conditioning regimen using other agents to reduce the toxicity of the procedure.

In addition to transplant-related factors, the neurological baseline status of ACALD patients also determines the outcome after allogeneic HSCT. In boys with CCALD undergoing HSCT, more deaths resulted from disease progression in advanced patients than from transplant related problems (Peters *et al.*, 2004). Although transplant-related mortality in adult patients caused by infection or GVHD may be as high as 30 % (La Nasa *et al.*, 2005; Gooley *et al.*, 2010; Peffault de Latour *et al.*, 2012; Bacigalupo *et al.*, 2015), a survival of less than 60 % in transplanted ACALD patients suggests additional ALD-specific problems. However, an analysis of the impact of neurological status in adults is complicated by the fact that the clinical phenotype of ALD men is usually a combination of ACALD itself plus accompanying AMN symptoms.

~~With regard to~~ In ACALD, preserved cognitive function before HSCT was considered to be important for stable long-term outcome. The four patients with progressive cognitive decline between ACALD diagnosis and HSCT, who already displayed cerebral symptoms such as impaired vision and hearing, attention deficits, or seizures before transplant, suggest a disease stage too advanced for satisfactory outcomes from HSCT. ~~this is the case history of an~~ This is consistent with a previously-reported Irish adult patient with advanced ~~disease~~ ACALD (Fitzpatrick *et al.*, 2008) and boys with symptomatic CCALD. ~~Such patients probably best compare to symptomatic CCALD boys~~ The latter display an inferior survival and a greater

risk for neurological decline after HSCT than ~~nearly asymptomatic or~~ pre-symptomatic CCALD patients (Peters *et al.*, 2004; Miller *et al.*, 2011). ~~Moreover, patients who deteriorate in their cognition during the few months prior to HSCT may simply reflect a highly aggressive cerebral disease.~~ In contrast, the two patients (#3B and #14P) with suspected deterioration in intellectual function before the diagnosis of ACALD but subsequent stabilisation prior to HSCT displayed a stable cognitive function post-HSCT. These courses may indicate cerebral disease with less aggressive dynamics than expected. Uncertainty about the IQ before the onset of ACALD in four patients did not allow definition of a baseline IQ-value as predictor of outcome in this small cohort.

While the Loes score seems to be a valuable predictor of survival and outcome of HSCT in boys with CCALD (Peters *et al.*, 2004; Miller *et al.*, 2011; McKinney *et al.*, 2013), particularly for the typical parieto-occipital demyelination pattern (McKinney *et al.*, 2013), it does not appear to have the same utility in ACALD. In the adults of our series, "atypical" demyelination patterns were observed in 50 % of patients, far more frequent than in childhood with 20 – 30 % (Loes *et al.*, 2003). Whether uncommon MRI patterns and changes in Loes scores immediately prior to transplant predict a poorer survival for ACALD men after HSCT has to be confirmed. ~~per se inflict a poorer survival for ACALD men after HSCT, as indicated in this series, has to be confirmed by a larger number of patients. In addition a larger study has to reveal whether an increment in~~ ~~In addition a larger study has to reveal whether an increment in Loes score immediately before transplant may be a more useful predictor for poor outcome than a high, but stable Loes score: a rapid increase in Loes score between diagnosis of ACALD and HSCT seem to better predict neurologic deterioration (e.g. patient #4S) than extended demyelinating lesions before diagnosis of inflammatory disease which do not show any further increase before transplant (e.g. patient #14P).~~

Independent from Loes score, lesion progression in adult patients with X-ALD has been highly associated with corticospinal tract involvement with a mean progression rate of 1 point

every 10 months (Eichler *et al.*, 2007). In our cohort, lesion progression before HSCT was observed in an overall higher percentage of patients (71 %) independent of corticospinal tract involvement, most likely because gadolinium enhancement was the prerequisite to select patients for transplantation. Contrast enhancement appears to correlate even better with rapidly progressive MRI lesions; median progression rate of Loes score in all 14 adult patients before HSCT was 2.0 points per year (range 0 – 6 points), as high as in children with cerebral X-ALD (Loes *et al.*, 2003) and at a greater rate than previously reported for adults with corticospinal tract involvement (Eichler *et al.*, 2007). Corticospinal tract involvement alone failed to detect a high-risk group of patients in this series. However, bilateral involvement of the internal capsule~~s~~ identified a subgroup of patients with higher age and EDSS at HSCT as well as a poorer prognosis after transplantation in comparison to all other patients. There was also a trend for more rapid lesion progression ~~brain demyelination and possibly also a longer history of AMN before ACALD~~ in this ~~patient~~ group.

Bilateral involvement of the internal capsule~~s~~ may therefore mark an end-stage of ascending axonopathy in adult males with AMN. Further studies are needed to determine if AMN patients with isolated bilateral involvement of internal capsules develop more severe AMN symptoms and are at high-risk to develop ACALD, and, if so, more rapidly progressive ACALD. Our study suggests, however, that ACALD patients with bilateral involvement of the internal capsule~~s~~ have a significantly poorer prognosis after transplant with little benefit, if any, from HSCT.

Advanced AMN, reflected by ~~bilateral~~ involvement of both internal capsule~~s~~, ~~may contribute~~ appeared to be of at least similar importance for survival than extensive ACALD. ~~in this series~~. Severe motor deficits of the lower limbs prior to transplant were frequently associated with immobility and life-threatening infections during the transplant procedure. ~~and subsequently~~. Specifically, poor baseline motor function (EDSS $\geq$ 6) conferred higher morbidity for survivors, even long after transplantation. Whether some patients became

bedridden rapidly after transplant because of myelopathy aggravation or cognitive or psychiatric problems is almost impossible to determine. On the other hand, life-threatening infections and/or non-engraftment may result in severe secondary cerebral disease progression even in patients with limited AMN symptoms prior to transplantation.

~~It is noteworthy, that~~ Nearly all patients in this cohort developed behavioural changes such as disinhibition or depression at least transiently in the early post-transplant period (Rosebush *et al.*, 1999; Chee *et al.*, 2013), which were associated with an additional burden on the patients, ~~and~~ their families, ~~potentially creating additional logistical pressures~~ and on the ~~adult~~ transplant unit. After transplant, these behavioural changes led to fractured relationships. The short- and long- term psychosocial consequences of delivering HSCT to ACALD patients and their families require future consideration.

~~In conclusion,~~ This study indicates for the first time the feasibility, complications and potential long-term neurological benefit of allogeneic HSCT in ACALD. The poor prognosis of patients with advanced AMN symptoms (EDSS ≥ 6) may relate to 1) immobility during the transplant procedure with increased risk of infections, 2) predisposition to rapidly progressive ACALD due to corticospinal tract involvement within the internal capsule~~s~~, and 3) cognitive decline and/or psychiatric problems during the transplant procedure that complicates the management of patients during the transplant procedure. Based on our limited experience, we propose preliminary and tentative recommendations for HSCT in ACALD patients (see box). Further studies are warranted to improve outcomes through careful patient selection and optimisation of HSCT protocols. Given the rarity of this disease, it is essential to have clear referral pathways to specialist centres able to assess and treat patients without delay.

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Figures

Figure 1: Kaplan-Meier estimates for survival of ACALD patients after HSCT based on various transplant and patient characteristics.

(A) Survival of all 14 patients. (B) Patients stratified by stem cell source, i.e. bone marrow versus peripheral blood stem cells or cord blood. (C) Patients stratified by extent of motor dysfunction (EDSS) at admission for HSCT. (D) Patients stratified for EDSS at admission for HSCT and occurrence of life-threatening or fatal infections during first 6 months post HSCT. Dots on probability lines indicate censored patients.

Figure 2: Outcome listed according to EDSS prior to HSCT, stable cognition before HSCT as well as absence of transplant complications.

OS = overall survival; EFS = event-free survival, i.e. no cognitive decline post HSCT.

Preliminary ~~and tentative~~ recommendations for HSCT in ACALD patients:

1) Men at risk of developing ACALD should be routinely monitored by a multidisciplinary team (neurologists, metabolic physicians and transplant haematologists) in specialized centres.
2) Potential family donors should be identified in advance to save time once inflammatory cerebral demyelinating lesions (i.e. with contrast enhancement) ~~occur at~~ are identified on brain MRI.
3) Stable neurocognitive function prior to transplant and limited AMN symptoms (EDSS < 6) are favourable outcome parameters.
4) Patients with bilateral involvement of the internal capsule on brain MRI do not seem to benefit from HSCT.
5) Stem cell source: bone marrow from a matched related or unrelated donor (≥ 9/10 HLA-match confirmed by high-resolution typing) is preferred over peripheral blood stem cells or cord blood.
6) HSCT procedure: busulfan based 'full intensity' or 'myeloablative' conditioning regimens should be used, combined with serotherapy (e.g. antithymocyte globulin) to promote engraftment and limit GVHD. All efforts should be made to prevent fever due to serotherapy or infections, which may be associated with acute neurologic deterioration.
7) Patients should be treated in experienced transplant centres and registered with the European Group for Blood and Marrow Transplantation (EBMT), the Center for

International Blood and Marrow Transplantation Research (CIBMTR) or equivalent transplant registries.

Table 1: Patients demographics and disease characteristics

#[a]	Age at ACALD [years]	EDSS-Score[b] (max. 10)	Addison's Disease	MRI pattern[c] Occipital/frontal/ pyramidal tracts/others	Loes-Score (max. 34)	Clinical symptoms (@ age in years)
11P	23	1	Yes	+ / - / - / -	2.5	Addison's disease (10), mild spastic paraplegia (24)
01B	45	6.5	No	++ / - / ++ / -	7	Sensory symptoms (44.4), spastic paraplegia (45.5), CNS symptoms: attention deficit, disinhibition (46.1). Gadolinium enhancement on 1st MRI [4]
12P	27	4	Yes	++ / - / - / -	5.5	Addison's disease (16), mild spastic paraplegia (25)
02H	48	2.5	Yes	- / + / - / -	2	Addison's disease (18.6), mild spastic paraplegia (45.6)
03B	35	3.5	No	++ / - / + / -	9.5	Intermittent bladder dysfunction (33.0), spastic paraplegia (35.2). Gadolinium enhancement on 1st MRI
04S	32	7	Yes	++ / + / ++ / + (temporal, thalamus)	14	Family screening (24.8) , lost to follow-up; CNS symptoms: impaired hearing/vision (prosopagnosia, field defect), dysphasia, ataxia (31.9), Addison's disease (33.4). Weight loss

#[a]	Age at ACALD [years]	EDSS-Score[b] (max. 10)	Addison's Disease	MRI pattern[c] Occipital/frontal/ pyramidal tracts/others	Loes-Score (max. 34)	Clinical symptoms (@ age in years)
13P	42	6	No	- / - / ++ / + (centrum semiovale)	5	Family screening (35) , spastic paraplegia (35)
05B	33	4	No	+ / (+) / - / ++ (olivo-ponto-cerebellar atrophy)	9	Ataxia (25.5), spastic paraplegia (30), severe bladder dysfunction. Weight loss
06B	31	3	Yes	(+) / + / - / -	6	Addison's disease (17.0), sensory symptoms (25.6), CNS symptoms: mild attention deficit, mild impaired vision (field defect) (31.8)
14P	21	1	No	++ / - / + / -	12	CNS symptoms: learning difficulties (14.5), family screening (18.8) . Cerebral demyelination without Gadolinum enhancement on 1st MRI
07B	32	4	No	++ / - / + / -	11	Spastic paraplegia (31.3), CNS symptoms: seizure (32.4), attention deficit, dysarthria (32.8), impaired vision. Weight loss.

#[a]	Age at ACALD [years]	EDSS-Score[b] (max. 10)	Addison's Disease	MRI pattern[c] Occipital/frontal/ pyramidal tracts/others	Loes-Score (max. 34)	Clinical symptoms (@ age in years)
						Gadolinum enhancement on 1st MRI
08B	42	6.5	Yes	- / ++ / ++ / -	10.5	Spastic paraplegia (23.8), Addison's disease (25.8), CNS symptoms: disinhibition, attention deficit (42.4). Weight loss
09B	46	6.5	No	- / + / ++ / + (cerebellum)	4.5	Spastic paraplegia (24.0), ataxia (44.5). Weight loss
10B	25	4	No	+ / - / + / -	5	Family screening (14.4) , spastic paraplegia (21.4), drug abuse (23)

To table 1:

[a] #: Patients in order of transplantation date. Capital letters indicate transplant center: P = Paris, B = Berlin, H = Hannover, S = Sheffield.

[b] EDSS: Kurtzke Expanded Disability Symptom Score (EDSS) just prior to HSCT.

[c] MRI pattern: MRI lesions differentiated for involvement of parieto-occipital, frontal, pyramidal long tract fibers in internal capsule or pons, and other white matter as indicated. Degree of involvement: -, no; +, moderate; ++, extensive.

Table 2: Patients hematopoietic stem cell transplantation characteristics

#	Δ ACALD to HSCT [Months]	Conditioning	Donor	Source	Donor chimerism (Day +100: >90% donor)	Acute GVHD	Significant Infection	Other significant non-neurological toxicity	Outcome
11P	6	MAC+S	MSD	BM	Yes	Grade I	Life-threatening	Hemorrhagic cystitis (HC), thrombotic microangiopathy	Alive
01B	8	MAC+S	MSD	BM	Yes	No	-	-	Alive
12P	12	RIC+S	UD	1xCB	Not tested (aplastic)	N/A	Fatal (fungal)	-	Dead (TRM)
02H	3	MAC+S	MUD	BM	Yes	Grade I	Life-threatening	Transient multi-organ failure (MOF)	Dead (Progress)
03B	14	MAC+S	MUD	BM	Yes	No	Severe	-	Alive
04S	15	MAC+S	MSD	BM	Yes	No	-	-	Dead (Progress)

#	Δ ACALD to HSCT [Months]	Conditioning	Donor	Source	Donor chimerism (Day +100: >90% donor)	Acute GVHD	Significant Infection	Other significant non-neurological toxicity	Outcome
13P	9	RIC	UD	2xCB	Yes (Day +60) No (<1% donor later) [a]	No	Life-threatening	Immune nephrotic syndrome, end-stage renal failure [1]	Dead (Progress)
05B	9	MAC+S	MUD	BM	Yes	No	Severe	-	Alive
06B	11	MAC+S	MUD	BM	Yes	Grade I	-	-	Alive
14P	6	MAC	MUD	BM	Yes	No	-	-	Alive
07B	12	MAC+S	MUD	BM	Yes	Grade I	Severe	Pneumonia	Alive
08B	9	MAC+S	MUD	PBSC	Yes	No	Life-threatening	Pneumonia, HC, polyserositis, multi-organ failure	Dead (TRM)
09B	5	MAC+S	MUD	PBSC	Not tested	Grade IV	Fatal	Sepsis/pneumonia, HC, multi-organ failure	Dead (TRM)

#	Δ ACALD to HSCT [Months]	Conditioning	Donor	Source	Donor chimerism (Day +100: >90% donor)	Acute GVHD	Significant Infection	Other significant non-neurological toxicity	Outcome
10B	11	MAC+S	MUD	BM	No (80 % donor)	Grade I	-	-	Alive

To table 2:

Δ ACALD to HSCT: Time interval between first detection of gadolinium enhancement in MRI (onset of ACALD) and HSCT in months.

Conditioning: MAC (+S) = myeloablative conditioning (plus serotherapy), i.e. busulfan 16 mg/kg orally (or busulfan i.v. targeted dose in patient #14P) and cyclophosphamide (120 mg/kg over 2 days or 200 mg/kg over 4 days) (plus rabbit antithymoglobulin Genzyme® or Fresenius®).

RIC (+S) = reduced intensity conditioning (+serotherapy), i.e. clofarabine 200 mg/m², busulfan 4 mg/kg orally, melphalan 140 mg/m², and alemtuzumab in patient #12P; cyclophosphamide 50 mg/kg, fludarabine 200 mg/m², total body irradiation with 2 Gy in patient #13P.

Donor: MSD, matched sibling donor; (M)UD, (matched) unrelated donor.

Source: BM, bone marrow, PBSC, peripheral blood stem cells, CB, cord blood with one (1x) or two (2x) units.

Acute GVHD: Acute graft-versus-host disease with maximum grade.

Significant infection: refers to at least severe toxicity (grade ≥3) according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v.3.0) criteria.

Significant non-neurological toxicity: refers to at least severe toxicity (grade ≥ 3) according to CTCAE v.3.0 criteria. HC, hemorrhagic cystitis. ~~MOF, multi-organ failure.~~

Outcome: TRM = transplant-related mortality, progress = ACALD progression

[a] Patient #13P: initially autologous hematopoietic regeneration, since day +30 detection of mixed donor chimerism (maximum >90 % at day +60, 50 % donor at day +120, thereafter <1 % donor chimerism). Toxicity due to allogeneic CB transplantation/non-engraftment suspected.

Table 3: Disease status within 6 months after allogeneic hematopoietic stem cell transplantation and MRI outcome

#	EDSS before	Early post-HSCT changes @ months post HSCT	EDSS @0-6	MRI outcome @months post HSCT
11P	1	Motor dysfunction ↑↑ immediately after tx, walks only few meters with 2 crutches @6; bladder dysfunction ↑↑ (parallel to BK virus cystitis); behavioral disinhibition until @12; vision↓	7	New lesions and progression, LS 12 @13; thereafter stable
01B	6.5	Conditioning: walks only few steps without help until @3; fever after 1st serotherapy: seizure, bladder/stool incontinence (until @1); severe depression until @6	8	No change, minimal Gd+, LS 7(stable) @6; new lesions (frontal), LS 10 @18
12P	4	Motor/bladder dysfunction stable until shortly before death in aplasia (transplant related (TRM)) @2; severe behavioral changes	7	Not tested
02H	2.5	Sepsis after 1st serotherapy: seizure, disorientation, coma, renal failure, bedridden @1; major improvement until @3; still walks only with 2 crutches; thereafter further deterioration	9.5	Many new lesions (PO,frontal,CC), LS 14 @6; progression & atrophy, LS 20 @12

03B	3.5	Sepsis @0.5: bladder/stool incontinence, agitated; walks only few steps w/o aid @1; thereafter motor/bladder function↑; moderate disinhibiton	6.5	Moderate lesion progression (PO), LS 11 @16; stable, CC atrophy, LS 12 @59
04S	7	Motor dysfunction↑↑, hemiplegic @3; vision↓; complete hearing loss@2; major confusion/psychosis@1; severe depression@6; no further deterioration @3-6	8.5	Lesion progression, +atrophy, LS 15.5 @8
13P	6	Spastic paraparesis ↑; moves from wheel-chair to bed with difficulties @6; bladder dysfunction stable; severe behavioral changes	8.0	New lesions and progression, LS 8 @12; minimal progression, LS 9 @24
05B	4	Conditioning: Gait ataxia↑, needs urine catheter; walks only few steps w/o aid @1, thereafter motor function(↑); moderate depression until @6;	6.5	New lesions (PO, frontal), LS 11.5 @7; less lesions detected (PO, CC, visual), LS 8.5 @34
06B	3	Normal motor function; minimal bladder dysfunction unchanged; moderate disinhibition @1	3	New lesions (PO), LS 8 @6; no change @37
14P	1	Normal neurological function preserved; severe behavioural disinhibition @6	1	No progression, LS 12 (stable) @14
07B	4	Sepsis @0.5: walks only few steps with aid, bladder incontinence, attention	7.5	Lesion progression, LS 12 @9;

		deficit; able to walk 300m/bladder function↑@6; major depression until @6; anticonvulsive prophylaxis		less lesions detected, LS 10 @24
08B	6.5	Conditioning: motor dysfunction ↑, bladder/stool incontinence; sepsis @0.5: bedridden, disoriented, "frontal brain syndrome", cognition↓; continued decline until death@4 (TRM)	9.5	Not tested
09B	6.5	Conditioning: severe dizziness, bladder/stool incontinence, dysarthria; sepsis@day 2: can not stand; bedridden; GVHD@ day 7: severe disorientation, hallucination; death@0.5 (TRM)	9.5	Not tested
10B	4	No ~~significant~~ change @1; ataxia↑@3 (needs bilateral aid); rarely stool incontinence @6	6.5	Internal capsule↑, LS 5 (stable) @38

To table 3:

EDSS@0-6 = maximum EDSS score during first 6 months post HSCT.

MRI: LS = Loes score, PO = parito-occipital, CC = corpus callosum

Table 4: Long-term disease status after allogeneic hematopoietic stem cell transplantation

#	EDSS before	Long-term outcome @ months post HSCT	EDSS @ 24 months	Motor function >36 months	Follow-up (months)
11P	1	Disinhibition resolved @12; cognition stable (PIQ 89), but visual loss (1/10, 2/10 with major cuts in visual fields)@18; motor function slowly improved after @24 (walks 500m with 1 crutch @108); severe bladder dysfunction unchanged; good QoL despite motor and visual handicap	6	↑	116
01B	6.5	Depression improved @12; motor dysfunction (↑) @12: transfers alone to wheelchair; moderate bladder dysfunction unchanged; cognition↓ (IQ 75) @20; teaches at handicapped institution; no changes after @24	7	=	92
02H	2.5	Deterioration in all functions since @3; unable to walk unassisted @6; diabetes mellitus/chronic inflammation @12; rapid further deterioration; death from secondary ACALD progression at @16	10	N/A	(16)

03B	3.5	Behavioural changes resolved @12; Cognition stable (IQ 99) @16; walks without aid >500 m @53; limited bladder dysfunction unaltered; completely stable @88; good QoL; retired due to spasticity.	4	↑	88
04S	7	Depression improved @12; further deterioration in motor/bladder/cognitive function after @6; death from ACALD progression @24	10	N/A	(24)
13P	6	Repeated septicemias under dialysis after @6; paraplegic, unable to transfer from bed to wheelchair alone, can speak and understands, but severe cognitive decline @24; very poor QoL, aware of his status; death from secondary ACALD progression at @36 (also related to non-engraftment)	9	N/A	(36)
05B	4	Best clinical status @12: able to walk 200m unassisted; requires crutch for walking @41; severe bladder dysfunction unaltered; cognitive function stable (PIQ 108) @12; depression↓↓, but moody; still unemployed	6	↓	72
06B	3	Stable neurocognition: IQ 111 @13; no relevant change until @31; motor function ↑: walks >5 km @59; no bladder dysfunction @59; bachelor completed; information processing moderately↓; excellent QoL	3	↑	59

14P	1	Severe behavioural disinhibition resolved @12; normal neurological status @14; cognitive function stable (IQ 72 before!); back to university (with great difficulties); good QoL	1	=	43
07B	4	Best clinical status @12: able to walk 100m unassisted; hemiparesis; requires crutch for walking @18; bladder incontinence @18; cognitive function↓ (concentration↓); depression improved@12; still low QoL	6	↓	45
10B	4	Moderate improvement of ataxia after @22 (walks >1 km with bilateral aid); intermittent stool incontinence unchanged; cognition: memory↓, attention↓, information processing↓ @13; formal thinking↑ @38 (moderate decline in neurocognition also due to drug abuse?); retired; good QoL	6	↑	38

To table 4:

Motor function >36 months (at last follow-up): ↑ improved, = stable, ↓ deteriorated. QoL = quality of life.

Table 5: Characteristics of patients with or without bilateral involvement of internal capsules on brain MRI

	Group A [*n* = 5]: With bilateral involvement of internal capsule	**Group B** [*n* = 9]: Without bilateral involvement of internal capsule	*P* =
Progressive disease detected	5 out of 5	6 out of 9	ns
Rate of demyelinating lesion progression [Loes score points per year]	**2.9** (1 – 6)	**0.6** (0 – 6)	0.078
Age at ACALD [years]	41.9 ± 5.6	30.7 ± 8.1	<0.05
Time period AMN to ACALD [months]	90 (15 - 235) *	27 (24 - 49) *	ns
Loes score before HSCT	**6.0** (4.4 - 9.9)	**7.0** (4.9 - 11.4)	ns
EDSS before HSCT	**6.5** (6.4 - 6.6)	**3.5** (2.1 - 4.0)	<0.01
Probability of survival [%]	20 ± 18 *	78 ± 14	<0.015

To table 5:

All numbers in bold represent median values (with 25th and 75th percentile). Significance calculated by rank sum test. *Age and probability of survival: Mean ± standard deviation. Significance calculated by student-t-test (age) or log-rank test (survival). ns = not significant.

Table 6: Changes in neurological performance scales and MRI severity score before and after HSCT according to baseline status and transplant complications

	Group I [*n* = 6]:: **Baseline EDSS < 6 w/o transplant complications**			**Group II** [*n* = 8]:: **Baseline EDSS ≥ 6 or early transplant complications***		
	Before HSCT	**Early post HSCT** [≤ 6 months]	**Late post HSCT** [approx. 24 months]	**Before HSCT**	**Early post HSCT** [≤ 6 months]	**Late post HSCT** [approx. 24 months]
EDSS [max. 10 points]	**3.8** (3.0 - 4.0)	**6.5** (3.0 - 6.5)	**5.0** (3.0 - 6.0)	**6.3** (3.3 - 6.5)	**8.3** (7.5 - 9.5) ++ #	**9.0** (6.8 -1 0.0) ++ # [*n* = 5]
AACS [max. 24 points]	**7.5** (7.0 - 10)	**13.0** (10.0 - 15.0) ++	**9.0** (7.0 - 12.0)	**11.5** (5.5 - 12.5)	**18.0** (16.5 - 22.5) ++ #	**21.0** (15.8 - 24.0) ++ # [*n* = 5]
Modified Rankin Score	**2.0** (1.0 - 3.0)	**4.0** (3.0 - 5.0) ++	**3.5** (2.0 - 4.0)	**3.5** (1.5 - 4.0)	**5.0** (5.0 - 6.0) ++ #	**6.0** (4.0 - 6.0) ++ [[*n* = 5]
Loes Score (MRI) [max. 34 points]	**9.3** (6.0 -11.0)		**9.3** (8.0 - 11.0)	**5.3** (3.5 - 8.8)		**11.0** (9.5 - 16.0) [*n* = 4]

All numbers in bold represent median values (with 25th and 75th percentile). Differences in baseline scores between group I and group II were not significant. * Early transplant complications, i.e. at least life-threatening infections during early transplant phase or graft rejection.

++ Friedman repeated measures ANOVA on ranks with following pair wise multiple comparisons (Tukey test)): $P < 0.05$ in comparison to respective baseline status before HSCT. Missing values of the three early deceased patients for the late time point were supplemented with the respective values from the earlier time point (last value before death) except for Loes score.

Kruskal-Wallis ANOVA on ranks with following multiple comparisons versus control according to Dunn's method: $P < 0.05$ in comparison to late post HSCT status of group I patients.