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Cellular Transplant Therapies for Globoid Cell Leukodystrophy: Preclinical and Clinical Observations

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Abstract

Globoid cell leukodystrophy (GLD) is a progressive neurodegenerative disorder caused by the deficiency of galactocerebrosidase (GALC), resulting in accumulation of toxic metabolites in neural tissues. Clinically variable based on age of onset, infantile GLD is generally a rapidly fatal syndrome of progressive neurologic and cognitive decline, whereas later-onset GLD has a more indolent, protracted clinical course. Animal models, particularly the twitcher mouse, have allowed investigation of both the pathophysiology of and the potential treatment modalities for GLD. Cellular therapy for GLD, notably hematopoietic cell transplantation (HCT; transplantation of bone marrow, peripheral blood stem cells, or umbilical cord blood cells) from a normal related or unrelated allogeneic donor provides a self-renewing source of GALC in donor-derived cells. The only currently available treatment option in human GLD, allogeneic HCT, can slow the progression of the disease and improve survival, especially when performed in presymptomatic infants. Because persistent neurologic dysfunction still occurs after HCT in GLD, preclinical studies are evaluating combinations of HCT with other treatment modalities.

Keywords

globoid cell leukodystrophy; Krabbe's disease; galactocerebrosidase; GALC; hematopoietic cell transplantation

Globoid cell leukodystrophy (GLD), an autosomal recessive condition also known as *Krabbe's disease* in its infantile form, results from a deficiency of galactocerebrosidase (GALC). This enzyme, which is essential for the normal myelination process, catalyzes the hydrolysis of galactosylceramide and other sphingolipids such as psychosine (Wenger et al., 2000). In the absence of GALC, central and peripheral demyelination occurs because of the accumulation of psychosine and other toxic metabolites, leading to the death of

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CONFLICT OF INTEREST STATEMENT

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ROLE OF AUTHORS

KRM and AMY designed and wrote this Review.

oligodendrocytes and Schwann cells required for maintenance of myelin sheaths. The globoid cells for which the disease is named are reactive macrophages with periodic acid-Schiff (PAS)-positive inclusions that are composed of degraded myelin products. The biochemical diagnosis of GLD is made by demonstrating deficient GALC activity (typically 0–5% of normal). Asymptomatic individuals are sometimes diagnosed prenatally via chorionic villus sampling (Wenger et al., 2000) or shortly after birth when testing is prompted because of family history.

Clinically, GLD has variable presentations and can be broadly divided into early- and late-onset types. The early-onset type (Krabbe's disease) typically presents in early infancy and is characterized by rapid neurologic deterioration, including seizures, spasticity, cognitive decline, and death. The late-onset type has a more insidious onset, can manifest from age 6 months to adulthood, and may or may not affect cognition. With the exception of the G270D mutation, which is associated with a more protracted clinical course and juvenile or adult onset, neither specific changes in the *GALC* gene nor residual GALC levels are correlated with disease severity or prognosis (Wenger et al., 2000). Because the late-onset type tends to have a much more variable clinical course, lack of prognostic markers in this group can further complicate treatment decisions.

The treatment of human GLD has been largely limited to supportive care and symptom management. Allogeneic hematopoietic cell transplantation (HCT; transplantation of bone marrow, peripheral blood stem cells, or placental/umbilical cord blood cells) from healthy donors has been used since the late 1960s to treat patients with leukemia, blood disorders, and cellular immunodeficiencies. By providing a self-renewing cellular source of the deficient enzyme, HCT is a potential therapy for GLD. This Review addresses preclinical and clinical results of HCT for GLD and considers combined-modality treatment strategies to augment the beneficial effects of HCT.

RATIONALE FOR HCT

The concept of enzymatic replacement by HCT as a potentially curative approach to various leukodystrophies, including GLD, has been in development since the early 1980s, initially in studies of bone marrow transplantation (BMT) from healthy related or unrelated allogeneic human leukocyte antigen (HLA)-compatible donors. After HCT, donor-derived cells replace not only hematopoietic and immune cells such as erythrocytes, granulocytes, platelets, and lymphocytes but also the entire reticuloendothelial system, including pulmonary alveolar macrophages, osteoclasts, Kupffer and Ito cells in the liver, cutaneous Langerhans cells, and mononuclear cells (microglia) in the CNS (Yeager, 1988). The spleen is the first organ outside of the bone marrow to demonstrate these donor-derived cells, followed by other sites such as lung, kidney, liver, and CNS (Wu et al., 2000). Repopulation of mononuclear cells in the CNS with enzymatically normal donor-derived cells is crucial to the treatment of Krabbe's disease by HCT (Yeager, 1990; Yeager et al., 1991). It has been conclusively shown in murine HCT models that donor-derived enzyme and/or hematopoietic cells can be found in the CNS (Hoogerbrugge et al., 1988; Yeager et al., 1991; Wu et al., 2000; Galbiati et al., 2009), and this finding has been confirmed in postmortem studies of human recipients of gender-mismatched allogeneic HCT (Unger et al., 1993). However, repopulation of CNS

mononuclear cells is a gradual process that occurs over several months after HCT. The progressive demyelination in the CNS that occurs before complete posttransplant replacement by enzymatically normal donor-derived cells may be one of the most substantial challenges for improving clinical outcomes of HCT for human GLD.

ANIMAL MODELS OF GLD

Animal models for the study of human GLD have existed since the 1960s and include canine, primate, and murine models. In 1963, the Cairn terrier was the first animal model of GLD to be described; in 1970, it was confirmed that affected canines had deficiency of GALC, thus confirming this model as a biochemical as well as a neuropathological correlate for human GLD (Suzuki et al., 1970). Other canine breeds with GALC deficiency have since been identified (Wenger, 2000). As reviewed by Wenger (2000), GLD was identified in rhesus monkeys in 1989; just as with humans, affected monkeys had the characteristic globoid PAS-positive macrophages in the CNS. Use of primate and canine models to evaluate treatments for GLD has been limited by the relatively small numbers of animals available for study, bioethical considerations, and challenges and costs of maintaining breeding colonies (Duffner et al., 2009). The twitcher mouse, first discovered at The Jackson Laboratory in 1976 and described in 1980 (Duchen et al., 1980; Kobayashi et al., 1980; Suzuki and Suzuki, 1983), is perhaps the best animal model of human GLD to date. Homozygous twitcher mice (*twi/twi*) are indistinguishable from heterozygous carriers (*+/-twi*) or homozygous wild-type (WT; *+/+*) littermates at birth and for approximately the first 3 weeks of life. The first neurologic signs begin about postnatal day 20 and include tremor (hence the term *twitcher*, especially involving head and trunk), limb incoordination, and decreased activity; diminished weight gain compared with normal mice can be seen a few days before neurologic symptoms (Duchen et al., 1980; Suzuki and Suzuki, 1983). Feeding difficulty is noted early in the course of disease because of cranial nerve involvement (Suzuki and Suzuki, 1983). These progressive deficits lead to paralysis and wasting, and the average life span of a twitcher mouse is approximately 45 days; in contrast, the median life span of a normal mouse is 600–700 days. Autopsy studies reveal white matter changes, with demyelination in brainstem, spinal cord, and peripheral nerves and with appearance of abnormal multinucleated PAS-positive globoid cells, which are macrophages that contain products of myelin degradation (Duchen et al., 1980). Kobayashi et al. (1980) confirmed that the manifestations noted in twitcher mice, as in human GLD, were due to deficiency of GALC. One important difference in the clinical manifestations experienced by twitcher mice and humans is that the peripheral nervous system is much more involved in the former (Duchen et al., 1980; Kobayashi et al., 1980).

CELLULAR TRANSPLANTATION IN ANIMAL MODELS OF GLD

The availability of a robust murine model of GLD has made comprehensive preclinical studies of HCT possible. Yeager et al. (1984) first reported the beneficial effects of HCT in the twitcher mouse. Twitcher mice (in which GALC deficiency was confirmed by assays of tail-tip tissue in presymptomatic mice) underwent congenic HCT by intraperitoneal injection of bone marrow and spleen cells from healthy mice with normal GALC activity after a preparative regimen of 9 Gy (900 rad) total body irradiation (TBI). Complete donor

hematopoiesis occurred approximately 6–7 weeks after transplantation. The mean life span was 80 days (range 56–108 days) in twitcher mice that underwent HCT compared with 40 days (range 28–47 days) in untreated twitcher mice ($P < 0.001$). Treated twitcher mice in this study demonstrated patchy areas of remyelination 30–50 days after transplantation and extensive remyelination by day 80. Behaviors such as gait and foraging were qualitatively improved in transplanted twitcher mice, whereas truncal tremor (a manifestation of CNS involvement) was unchanged (Yeager et al., 1984). Likewise, there were no improvements in other CNS signs and symptoms, and globoid cells continued to increase in number after transplantation. Transplanted twitcher mice nevertheless died with progressive CNS neurodegeneration (Yeager et al., 1984).

Since that time, these findings have been corroborated by other laboratories, although occasionally with differences in certain measures of disease that might reflect differences in BMT conditioning regimens (discussed in further detail below) or study design.

Hoogerbrugge et al. (1988) also performed myeloablative BMT with 9-Gy TBI as a conditioning regimen and demonstrated a rise in GALC activity in the CNS of 15% of that observed in normal controls. These investigators also showed a median survival of almost 100 days in transplanted twitcher mice when animals that had died early from post-BMT complications were excluded from analysis. Toyoshima et al. (1986) studied nerve conduction in transplanted twitcher mice and found that, in addition to the pathologic improvement in myelination as seen by Yeager et al. (1984), there was also a clinical improvement in motor nerve conduction velocity (MCV). Tibial-nerve MCV was measured throughout the life span of transplanted and control twitcher mice as well as control healthy mice. A decrease in MCV was noted as early as day 15, before overt motor symptoms, in twitcher mice and progressed throughout the life span of affected mice. After HCT, MCV in twitcher mice was slower than in normal controls but, in contrast to nontransplanted twitcher mice, did not worsen over time. Although transplanted twitcher mice had truncal tremor, they did not develop hindlimb paralysis and remained active. Slowed weight gain, which has been observed by other researchers, was noted in this study as well; life span was not reported (Toyoshima et al., 1986).

Relative lack of improvement in the CNS despite (albeit incomplete) improvement in pathologic and clinical markers of disease in the peripheral nervous system in twitcher mice treated with BMT was initially attributed largely to the impermeability of the blood–brain barrier to donor-derived GALC after HCT. This was examined further in several investigations (Ichioka et al., 1987; Hoogerbrugge et al., 1988; Yeager et al., 1988). GALC activity and psychosine levels were measured in the brains and other tissues of both transplanted and untransplanted twitcher mice. Psychosine levels in the brains and sciatic nerves of untreated twitcher mice were up to 70- and 40-fold higher, respectively, than psychosine levels of control mice. GALC activity in the brains of transplanted twitcher mice reached normal levels by 90 days post-HCT (Ichioka et al., 1987). Similarly, levels of GALC in hematopoietic sites (bone marrow and spleen) were similar to those of controls, although levels were lower in other organs, including brain, liver, heart, lung, and kidney (Hoogerbrugge et al., 1988). Importantly, a definite rise in GALC levels in the CNS continued posttransplant, even in the absence of treatments to disrupt or modify the blood–brain barrier. Brain psychosine levels remained significantly lower in transplanted twitcher

mice compared with control twitcher mice, and these levels were relatively stable until day 90 (at which point a slight increase was noted). However, psychosine levels in the brains of twitcher mice remained higher than psychosine levels of normal mice throughout the posttransplant period. The observation of significant GALC activity and decreased psychosine levels in the CNS of transplanted twitcher mice provided evidence of enzyme replacement by donor-derived cells. These studies also indicated that simple impermeability of the blood–brain barrier to either donor-derived cells or peripherally produced enzyme is not the primary factor limiting the use of HCT for treatment of murine GLD because GALC levels increased and levels of toxic metabolites levels decreased posttransplant. In contrast, the relatively prolonged period without normal levels of GALC during the prenatal, neonatal, transplant, and engraftment processes along with other as yet unidentified factors may allow for irreversible and progressive neurologic damage. Hoogerbrugge et al. (1988) demonstrated that, over time after transplant, the number of pathognomonic globoid cells decreased and the number of foamy macrophages increased in the CNS, most notably in areas most affected by the disease (including cerebellum and spinal cord). The foamy macrophages did not have galactosylceramide inclusions, indicating the presence of GALC and metabolism of this substrate. Moreover, immunohistochemical studies demonstrated that those macrophages were of donor origin. Alterations in levels of globoid cells and donor-derived macrophages in the CNS were not appreciated until at least 100 days after transplant (Hoogerbrugge et al., 1988), and, as noted earlier, this delay in GALC replacement is highly relevant to the clinical application of HCT for GLD, especially the early-onset infantile form.

Deficiency of sphingolipid activator protein-A (SAP-A) in mice is a model of late-onset GLD, with affected animals exhibiting a clinical course similar to that of the human disorder (Yagi et al., 2005). Although SAP-A is essential for proper GALC activity, some residual enzymatic activity is still present in SAP-A-deficient mice, which may account for the protracted clinical course compared with the fulminant neurodegeneration in twitcher mice, in which levels of SAP-A are normal (Shigematsu et al., 1990). After performing HCT, Yagi et al. (2005) were able to demonstrate donor-derived cells in the peripheral and central nervous systems as early as postnatal day 90 (approximately 82–83 days posttransplant). Compared with untransplanted affected mice, transplanted SAP-A-deficient mice had decreased psychosine levels, increased life span, and improvement in CNS pathology. The different posttransplant clinical courses in these two instructive murine models have clinical implications for cellular therapies of human infantile-onset vs. late-onset GLD.

CELLULAR TRANSPLANTATION IN THE TWITCHER MOUSE: EFFECTS OF CONDITIONING REGIMEN

Although there is guarded enthusiasm for the use of HCT as a potential treatment option for GLD, there have been longstanding concerns about potential toxic effects of the pretransplant conditioning regimens, especially in young infants. An appropriate conditioning regimen is crucial to a successful transplant because it allows for ablation of the host bone marrow and, thus, subsequent engraftment of donor-derived cells. An inadequate preparative regimen may lead to rejection or incomplete engraftment of

allogeneic donor hematopoietic cells. Once again, the twitcher preclinical model allows evaluation of various effects of conditioning regimens. The earliest studies of HCT for murine GLD used TBI, which is both myeloablative and immunosuppressive, as the conditioning regimen. Since then, chemotherapy with busulfan (BU), an alkylating agent that is myeloablative but not immunosuppressive, has also been proved effective. In a proof-of-concept experiment, Yeager et al. (1991) used high-dose BU (100 mg/kg) as a conditioning regimen and showed that treated twitcher mice lived longer, had improved gait, and did not develop hindlimb paralysis. At 100 days post-HCT, sciatic nerves had decreased edema with only rare globoid cells, and levels of GALC were increased in the liver, spleen, and central and peripheral nervous systems. The effects of pretransplant conditioning with graded doses of BU on survival, clinical manifestations, and enzymatic replacement in neural and other tissues were compared with outcomes of HCT after TBI (9 Gy; Yeager et al., 1993). Survival in twitcher mice that had undergone HCT after a low dose of BU (10 mg/kg) was similar to that of untreated twitcher mice because that preparative regimen was inadequate for engraftment of donor cells. Survival was prolonged, and gait, foraging, and grooming behaviors were all improved in twitcher mice that received HCT after higher doses of BU (35–50 mg/kg). Donor chimerism (the percentage of lymphohematopoietic cells derived from donor stem cells) was negligible in mice treated with 10 mg/kg BU but improved to approximately 88% by 90 days post-HCT in mice that received 35–50 mg/kg BU. In those treated with TBI, donor-derived cells were initially higher (days 30–60) in the peripheral blood and spleen and lower in the bone marrow compared with mice conditioned with optimal doses of BU, but these differences were no longer noted at day 90. Increased GALC activity was observed after HCT with both BU and TBI conditioning regimens, even with relatively modest doses of BU. These findings showed that pretransplant conditioning with BU led to sustained donor engraftment, biochemical improvement, and prolonged survival in murine GLD. Importantly, donor-derived GALC was detected in the CNS after either BU or TBI. A caveat of these studies is that the HCT was from congenic (same strain, same H-2 type, and same minor histocompatibility antigens) donors, a transplant system that requires myeloablation but does not require immunosuppression. In clinical transplantation, in which the donors are allogeneic (HLA-compatible, but likely mismatched at minor histocompatibility antigens), immunosuppression is an essential component of the preparative regimen to prevent graft rejection (host-vs.-graft reaction) and facilitate engraftment.

Although successful conditioning regimens have been described, concern remains that these regimens themselves may be neurotoxic and thus responsible for some of the neurologic changes noted in transplanted mice. Galbiati et al. (2007) examined the proliferation, migration, and differentiation of neural cells in twitcher mice that had undergone BMT after TBI and found that the irradiated mice had smaller brains, increased markers of neuroinflammation, and decreased neural precursor cells. Reddy et al. (2012) studied pretransplant conditioning with TBI in 3- or 4-day-old twitcher and WT mice at day 3 or 4 and quantitatively evaluated tremor post-HCT. Although transplanted twitcher mice had more tremor (measured by peak power and frequency of peak power) and decreased movements than WT HCT recipients, WT mice that had undergone HCT after TBI conditioning also were noted to have decreased movement posttransplant compared with

untransplanted age-matched WT mice. The possible detrimental effect of TBI even in normal healthy mice undergoing HCT highlights concern for neurotoxicity of conditioning regimens that may have an impact in clinical HCT as well.

CELLULAR TRANSPLANTATION IN HUMAN GLD

To date, over 500 patients have received HCTs for lysosomal and peroxisomal metabolic disorders; among these are a number with GLD, including both late- and early-onset forms (Krivit, 2004). Currently, only allogeneic HCTs have been explored, with both bone marrow and umbilical cord blood (UCB) as the source of donor hematopoietic cells. The use of UCB may be particularly attractive for HCT in human GLD; the extensive, readily available source of transplantable cells in cord-blood banks worldwide potentially decreases the time required to identify a suitable allogeneic donor and to proceed promptly with HCT (Kurtzberg, 2009). It is important to consider that, without respect to the source of donor cells, allogeneic HCT carries with it substantial risks of morbidity and mortality (Wingard et al., 2011). Pretransplant conditioning regimens can have both immediate and long-term sequelae, including risks of growth retardation and secondary malignancies as well as possible neurotoxicity (as indicated in the preclinical studies described above; Duffner et al., 2009; Majhail et al., 2011). Graft-vs.-host disease (GVHD), which can be acute or chronic, occurs when donor-derived immune cells attack healthy recipient cells, tissues, and organs. Even with HLA-matched related or unrelated HCT, GVHD can occur and can range in severity from relatively self-limited to fatal (Pidala, 2011). Infectious complications are common both during the neutropenia in the immediate posttransplant course and in the weeks to months after as a consequence of the immunosuppressive effects of treatments for acute or chronic GVHD. Although TBI has been used extensively in murine models of HCT, as described earlier, this modality has many early and late associated complications; thus, preparative regimens that use moderate- to high-dose chemotherapy are often preferred in clinical HCT, especially for young patients (Gyurkocza and Sandmaier, 2014).

The first human allogeneic BMTs for GLD were reported by Krivit et al. (1998). In this series of five patients, four had late-onset GLD; from disease biology, these individuals would be expected to have a milder phenotype and a more protracted course compared with the infantile-onset form of the disease. The age at HCT ranged from 2 months to 11 years. Four patients received BMTs from HLA-matched siblings, and one received an unrelated HLA-antigen D-related mismatched UCB transplant. All received a myeloablative conditioning regimen of BU and cyclophosphamide, with addition of TBI for the UCB transplant. Cyclosporine was used posttransplant for prevention of acute GVHD. Technical aspects of the transplant were well tolerated; neutrophil engraftment was prompt, and, when observed, acute GVHD was stage II (moderate) or less. All patients had improvement in or stabilization of neurophysiologic and/or neurocognitive parameters, albeit not all to the same clinical extent. This study demonstrated both the feasibility of HCT as a treatment for human GLD and substantial clinical improvement post-HCT, especially in those with late-onset GLD who were either asymptomatic or only mildly symptomatic at the time of HCT. Kurtzberg et al. (2002) reported similar findings for a group of six infants transplanted in the neonatal period with UCB (five patients) or bone marrow (one patient). All had been diagnosed with GLD early on the basis of having a sibling who had the disease. All

recipients had successful engraftment and complete donor chimerism; at a median followup of 2.5 years posttransplant, all patients had longer survival compared with that of their previously affected siblings. Improvement in clinical parameters such as neurocognitive functioning was noted as well as improvement in myelination and nerve conduction velocities (Kurtzberg et al., 2002). Posttransplant nerve conduction studies to assess improvement in the functional status of the peripheral nervous system have also shown largely positive findings, with the caveat that, as previously observed in murine GLD transplant models, those who received HCTs earlier postdiagnosis had better outcomes than those transplanted later (Siddiqi et al., 2006). Furthermore, although early clinical improvement may occur, it is not always sustained. A long-term review of 16 asymptomatic infants with GLD who underwent UCB transplants at Duke University Medical Centers described improvements in neurologic and cognitive functions but persistent and significant motor dysfunctions in the 14 evaluable patients (two patients died in the immediate posttransplant period; Escolar et al., 2005). Another multicenter report of HCT in six asymptomatic children with GLD indicated neurodevelopmental delay in the five evaluable patients (one patient died in the immediate posttransplant period; Duffner et al., 2009). In both groups, patients had very low heights and weights for age; it is not known whether this growth retardation is related to the underlying GLD, to the pretransplant conditioning regimen, or to other factors (Escolar et al., 2005; Duffner et al., 2009).

Outcomes of HCT in symptomatic patients with infantile GLD are not as encouraging as are those in asymptomatic patients with the infantile form or in patients with late-onset GLD. Escolar et al. (2005) performed unrelated UCB transplants in 11 asymptomatic newborns and 14 symptomatic infants with GLD. The asymptomatic individuals were diagnosed prenatally or at birth on the basis of an affected sibling; the symptomatic individuals ranged in age from 4 to 9 months. The conditioning regimen included BU and cyclophosphamide as well as antithymocyte globulin to reduce the risks of GVHD, and cyclosporine was used for GVHD prophylaxis. Sustained and complete donor chimerism was observed in 16 of the 17 surviving patients. Two of the seventeen patients developed severe GVHD, which was fatal in one of those patients. This study underscored the findings that asymptomatic newborns with GLD but not symptomatic infants have a significant survival advantage when treated with HCT. In addition to survival advantage, the asymptomatic cohort did not develop seizures; improvement in gross motor function was observed in many of these patients, although nerve conduction velocities improved in a minority. In contrast, neurologic outcomes for the symptomatic group were poor, with evidence of disease progression by MRI evaluation in most of these patients from 3 months to 5 years after HCT, development or worsening of seizure activity, and lack of improvement in neurodevelopmental status. A report of related HLA-matched allogeneic HCT in a 4-month-old infant with symptomatic GLD showed similarly poor results; despite adequate engraftment and improvement in GALC levels, the patient died of progressive neurodegeneration 180 days after transplant (Caniglia et al., 2002). Altogether, this clinical experience supports the use of related or unrelated HCT in asymptomatic patients with GLD because both life span and quality of life are improved with treatment. However, these benefits are much less likely after HCT in patients already showing neurological manifestations of infantile GLD.

In utero cellular transplantation of fetal liver cells and hematopoietic cells has been evaluated in a number of hematologic, immunologic, and metabolic disorders for which antenatal diagnosis is feasible (Touraine et al., 1992; Flake and Zanjani, 1997). Prenatal cellular therapy is especially appealing in GLD because this method would, in theory, decrease the time for which neurologic tissue is subject to toxic metabolites and, because the fetus is in an immune-tolerant state, would eliminate the requirement for a potentially toxic conditioning regimen. The earliest report of in utero cellular transplantation for GLD demonstrated successful engraftment of haploidentical paternal bone marrow (Bambach et al., 1997). The fetus was diagnosed with GLD at 10 weeks of gestation and received an intraperitoneal injection of 5×10^9 CD34⁺ cells/kg at approximately 13 weeks of gestation. Although normal fetal heart tones and growth patterns were documented through 18 weeks of gestation, fetal death occurred at 20 weeks of gestation. Postmortem evaluation showed that the liver (the primary hematopoietic organ at this gestational age) was largely of donor origin, confirming donor cell engraftment. In this case, fetal death might have resulted from leukostasis and dysregulated hematopoiesis following the exceptionally large dose of donor hematopoietic cells (about 3 log greater than that given in conventional allogeneic HCT; Bambach et al., 1997). This group performed a second in utero HCT at approximately 13 weeks of gestation using haploidentical paternal bone marrow and an intraperitoneal cell dose of 4.2×10^8 CD34⁺ cells/kg. This infant was born at 37 weeks and appeared normal; however, blood samples showed only 2% donor cells and undetectable GALC activity, indicating failure of engraftment. At age 6 months, this infant underwent successful unrelated allogeneic HCT, which led to sustained donor cell engraftment (Blakemore et al., 1999). To the best of our knowledge, in utero transplantation has not yet been successful in human GLD.

Decreasing transplant-related morbidity and mortality is of great concern if HCT is to be a viable treatment option for GLD. In a retrospective review of HCT for lysosomal storage diseases, transplant-related mortality was about 10% in recipients of HLA-matched grafts from siblings and was 20–25% in recipients of HLA-mismatched grafts; approximately 24% of patients had acute GVHD (Hoogerbrugge et al., 1995). Thus, improvements in both conditioning regimen-related toxicities and the prevention and treatment of GVHD are crucial. Reduced-intensity preparative regimens have recently gained favor for a variety of HCT indications and have been used successfully in late-onset GLD (Lim et al., 2008). Depletion of donor T cells to prevent GVHD has been explored in a variety of metabolic conditions. In nine children who underwent HCT with T-cell-depleted grafts for metabolic disorders (including three with GLD), none developed GVHD, and all had successful engraftment (Corti et al., 2005). All three patients with GLD had complete donor chimerism at day 100 posttransplant and were alive at day 1,022 to day 1,659 posttransplant (Corti et al., 2005). However, lymphocyte depletion of allogeneic HCTs delays posttransplant immune recovery and increases the risks of infectious complications.

PRECLINICAL EXPLORATION OF FUTURE TREATMENTS: CELLULAR TRANSPLANT COMBINED WITH OTHER TREATMENT MODALITIES

Despite the improvements observed in preclinical models and clinical settings, HCT is clearly far from curative in GLD. One major obstacle (discussed above) is the amount of time required for donor-derived cells to repopulate the mononuclear cells in the CNS and provide a source of GALC, given that GLD is quite rapidly progressive. Previous studies of both gene therapy and enzyme replacement therapies in murine models have demonstrated benefit (Weinberg, 2005; Neri et al., 2011; Lattanzi et al., 2014). Investigations that combine these techniques with HCT are ongoing (Weinberg, 2005; Reddy et al., 2011; Ricca et al., 2015), and several have been evaluated in the preclinical setting. In theory, direct enzyme replacement therapy (ERT) into the CNS would rapidly increase GALC activity while patients await migration of donor-derived cells and repopulation of CNS mononuclear cells to provide a self-renewing source of GALC. Qin et al. (2012) treated twitcher mice with both intrathecal and bilateral intracerebroventricular ERT injections on days 2 and 3 of life, followed by TBI and BMT 1 day later. As expected, ERT dramatically increased GALC activity to supraphysiologic levels in treated mice, but the effect was transient; several weeks later, GALC levels were similar in treated and untreated twitcher groups. Psychosine levels, however, remained lower in treated mice and were lower in the group that received both ERT and BMT than in the ERT-alone group. Moreover, the recipients of both ERT and BMT had decreased markers of neuroinflammation, increased life span, and improvement in neurobehavioral function. Mice that underwent both ERT and BMT had decreases in some neurophysiologic and neurobehavioral measures of peripheral nervous system and cerebellar function (e.g., the wire hang test), possibly as a consequence of neurotoxicity of the TBI conditioning regimen (Qin et al., 2012).

Several preclinical studies have evaluated the combination of HCT and GALC gene transfer (Lin et al., 2007; Galbiati et al., 2009; Reddy et al. 2011; Rafi et al., 2015). Using a nonmyeloablative preparative regimen (4-Gy TBI), BMT, and six intracerebral injections of the GALC-expressing AAV2/5 vector, Lin et al. (2007) showed significantly increased life spans of treated twitcher mice (average 104 ± 7 days, range 38–151 days). Mice that received both BMT and AAV2/5-GALC treatment had greater body weight at 35 days of age and improved behavioral measures at 40 days of age and beyond compared with untreated twitcher mice or twitcher mice treated with either modality alone. Combining infusion of bone marrow cells without a preparative regimen along with an intravenous injection of a GALC-lentiviral vector into neonatal twitcher mice, Galbiati and colleagues (2009) demonstrated that recipients of combination treatment lived longer (maximum 126 days) than those given either treatment modality alone. Although mice that had been given both marrow infusion and GALC-lentiviral vector injection had more myelination and improved neurologic symptoms initially, they too died with progressive neurodegeneration. Similarly, Reddy et al. (2011) reported increased efficacy of BMT in murine GLD when combined with CNS-directed gene therapy with a viral vector (AAV) to the forebrain, cerebellum, and spinal cord. The investigators postulated that the potential synergy of these two treatment modalities might be related to decreased CNS inflammation provided by BMT. Donor-derived mononuclear cells were first noted in the CNS just over 30 days posttransplant,

substantially earlier than observed in other studies with HCT only. Nevertheless, levels of psychosine decreased only in recipients of both AAV and BMT. Ricca et al. (2015) observed improved outcomes with a combination of BMT and either intracerebral gene therapy or transplantation of GALC-overexpressing neural stem cells. In this study, twitcher mice that received combination therapy had significantly prolonged life spans and improved neurologic functions, including less twitching and improved gait.

Recently, Rafi et al. (2015) investigated combined BMT (at postnatal day 9 or 10) with a single intravenous injection of AAVrh-10-GALC vector 1 day later and demonstrated remarkable results. Sixteen animals were included in the combined treatment arm, and 10 were still living at the time of analysis, including one mouse over 340 days old. Importantly, these twitcher mice did not exhibit abnormal behaviors after receiving combined treatment. Two mice that died, one at postnatal day 218 and the other at postnatal day 334, did not demonstrate neurologic deterioration or weight loss prior to death. One mouse that died at 178 days had increasing tremors before death. Moreover, GALC levels in the CNS of combination-treated mice were comparable to those found in WT mice; sciatic nerves had above-normal levels of GALC activity. These researchers postulated that synergy between the two treatment modalities contributed to these impressive results; BMT may have decreased some of the inflammatory mediators that contribute to disease progression, and gene therapy given at day 10 supplied critical enzyme activity. Moreover, AAVrh-10-GALC provided enzymatic activity to the peripheral nervous system as well, an important consideration in clinical therapeutics for human Krabbe's disease. However, although these results are strikingly positive compared with previous work, challenges remain; characteristic PAS-positive macrophages were still seen in some tissues, suggesting that the strategy is not curative.

With the understanding that GLD is a complex disease and that its hallmark neurodegeneration is likely multifactorial and influenced by GALC deficiency, dysregulation of inflammation, and other factors, Hawkins-Salsbury et al. (2015) attempted a "triple therapy" approach in the twitcher mouse model, combining AAV2/5-GALC and BMT (as evaluated by Galbiati et al., 2009) with substrate reduction by thrice-weekly subcutaneous injections of L-cycloserine, which decreases psychosine production. Recipients of this triple treatment had a median life span of 298 days, with the oldest mouse living to 454 days, arguably the longest twitcher mouse life span reported to date. Additionally, GALC activity in the brains of treated mice was greater than that in the brains of WT mice, and psychosine levels in the brains of treated mice were not statistically different from those in normal mouse brains. Weight gain was still less than in WT mice; likewise, performance on certain motor tests (rotarod and inverted wire hang tests), although much improved from that of untreated twitcher mice and even late into the life span of treated twitcher mice, was worse than in WT mice. Moreover, pathologic changes in peripheral nerves were still noted after triple therapy.

Although combined therapies for GLD have not yet been tested in the human setting, these encouraging preclinical investigations suggest both safety and increased efficacy of such approaches and justify additional studies to evaluate clinical utility.

SUMMARY

GLD remains a fatal progressive neurodegenerative disorder without ideal, definitive treatment options. Although HCT is able to ameliorate some of the clinical sequelae, this approach is not curative and is associated with procedure-related morbidity and mortality. However, for select individuals with GLD, especially asymptomatic infants and those with later-onset GLD, HCT is the only currently available approach to ameliorate the clinical manifestations and the course of the disorder. From the observation that the best outcomes of HCT occur in asymptomatic infants, early identification of affected individuals may be considered. Several states have neonatal screening programs for GLD; however, one may debate the utility of screening for GLD and other rare diseases for which current treatment options are limited and not curative (Lantos, 2011). Ongoing preclinical work to examine combining cellular transplant techniques with other therapies may help to improve the current outlook for this otherwise devastating disease.

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REFERENCES

- Bambach BJ, Moser HW, Blakemore K, Corson VL, Griffin CA, Noga SJ, Perlman EJ, Zuckerman R, Wenger DA, Jones RJ. Engraftment following in utero bone marrow transplantation for globoid cell leukodystrophy. *Bone Marrow Transplant.* 1997; 19:399–402. [PubMed: 9051254]
- Blakemore KJ, Moser HW, Corson VL, Griffin CA, Noga SJ, Zuckerman R, Wenger DA, Evans MI, Jones RJ. An update on in utero bone marrow transplantation for Krabbe disease. *J Mol Neurosci.* 1999; 13:238–239.
- Caniglia M, Rana I, Pinto RM, Fariello G, Caruso R, Angioni A, Dionisi Vici C, Sabetta G, De Rossi G. Allogeneic bone marrow transplantation for infantile globoid-cell leukodystrophy (Krabbe's disease). *Pediatr Transplant.* 2002; 6:427–431. [PubMed: 12390432]
- Corti P, Peters C, Balduzzi A, Bertagnolio B, Biondi A, Bugarin C, Dassi M, Furlan F, Gaipa G, Longoni D, Maglia O, Parini R, Perseghin P, Uderzo C, Uziel G, Masera G, Rovelli A. Reconstitution of lymphocyte subpopulations in children with inherited metabolic storage diseases after haematopoietic cell transplantation. *Br J Haematol.* 2005; 130:249–255. [PubMed: 16029453]
- Duchen LW, Eicher EM, Jacobs JM, Scaravilli F, Teixeira F. Hereditary leucodystrophy in the mouse: the new mutant twitcher. *Brain.* 1980; 103:695–710. [PubMed: 7417782]
- Duffner PK, Caviness VS Jr, Erbe RW, Patterson MC, Schultz KR, Wenger DA, Whitley C. The long-term outcomes of presymptomatic infants transplanted for Krabbe disease: report of the workshop held on July 11 and 12, 2008, Holiday Valley, New York. *Genet Med.* 2009; 11:450–454. [PubMed: 19346954]
- Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, Wenger DA, Pietryga D, Wall D, Champagne M, Morse R, Krivit W, Kurtzberg J. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med.* 2005; 352:2069–2081. [PubMed: 15901860]
- Flake AW, Zanjani ED. In utero hematopoietic stem cell transplantation. A status report. *JAMA.* 1997; 278:932–937. [PubMed: 9302247]
- Galbiati F, Clementi G, Superchi D, Givogri MI, Bongarzone ER. Effects of irradiation on the postnatal development of the brain in a genetic mouse model of globoid cell leukodystrophy. *Neurochem Res.* 2007; 32:377–388. [PubMed: 17203404]
- Galbiati F, Givogri MI, Cantui L, Lopez Rosas A, Cao H, van Breemen R, Bongarzone ER. Combined hematopoietic and lentiviral gene-transfer therapies in newborn twitcher mice reveal contemporaneous neurodegeneration and demyelination in Krabbe disease. *J Neurosci Res.* 2009; 87:1748–1759. [PubMed: 19185028]

- Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. *Blood*. 2014; 124:344–353. [PubMed: 24914142]
- Hawkins-Salsbury JA, Shea L, Jiang X, Hunter DA, Guzman AM, Reddy AS, Qin EY, Li Y, Gray SJ, Sands MS. Mechanism-based combination treatment dramatically increases therapeutic efficacy in murine globoid cell leukodystrophy. *J Neurosci*. 2015; 35:6495–6505. [PubMed: 25904800]
- Hoogerbrugge PM, Poorthuis BJ, Romme AE, van de Kamp JJ, Wagemaker G, van Bekkum DW. Effect of bone marrow transplantation on enzyme levels and clinical course in the neurologically affected twitcher mouse. *J Clin Invest*. 1988a; 81:1790–1794. [PubMed: 3290253]
- Hoogerbrugge PM, Suzuki K, Suzuki K, Poorthuis BJ, Kobayashi T, Wagemaker G, van Bekkum DW. Donor-derived cells in the central nervous system of twitcher mice after bone marrow transplantation. *Science*. 1988b; 239:1035–1038. [PubMed: 3278379]
- Hoogerbrugge PM, Brouwer OF, Bordigoni P, Cornu G, Kapaun P, Ortega JJ, O'Meara A, Souillet G, Frappaz D, Blanche S, Fischer A, Ringden O. Allogeneic bone marrow transplantation for lysosomal storage diseases. *Lancet*. 1995; 345:1398–1402. [PubMed: 7760610]
- Ichioka T, Kishimoto Y, Brennan S, Santos GW, Yeager AM. Hematopoietic cell transplantation in murine globoid cell leukodystrophy (the twitcher mouse): effects on levels of galactosylceramidase, psychosine, and galactocerebrosides. *Proc Natl Acad Sci U S A*. 1987; 84:4259–4263. [PubMed: 2884662]
- Kobayashi T, Yamanaka T, Jacobs JM, Teixeira F, Suzuki K. The twitcher mouse: an enzymatically authentic model of human globoid cell leukodystrophy (Krabbe disease). *Brain Res*. 1980; 202:479–483. [PubMed: 7437911]
- Krivit W. Allogeneic stem cell transplantation for the treatment of lysosomal and peroxisomal metabolic diseases. *Springer Semin Immunopathol*. 2004; 26:119–132. [PubMed: 15452666]
- Krivit W, Shapiro EG, Peters C, Wagner JE, Cornu G, Kurtzberg J, Wenger DA, Kolodny EH, Vanier MT, Loes DJ, Dusenbery K, Lockman LA. Hematopoietic stem-cell transplantation in globoid-cell leukodystrophy. *N Engl J Med*. 1998; 338:1119–1126. [PubMed: 9545360]
- Kurtzberg J. Update on umbilical cord blood transplantation. *Curr Opin Pediatr*. 2009; 21:22–29. [PubMed: 19253461]
- Kurtzberg J, Richards K, Wenger D, Provenzale J, Escolar M, Wall D, Champagne M, Krivit W. Correction of Krabbe disease with neonatal hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2002; 8:97.
- Lantos JD. Dangerous and expensive screening and treatment for rare childhood diseases: the case of Krabbe disease. *Dev Disabil Res Rev*. 2011; 17:15–18. [PubMed: 22447750]
- Lattanzi A, Salvagno C, Maderna C, Benedicenti F, Morena F, Kulik W, Naldini L, Montini E, Martino S, Gritti A. Therapeutic benefit of lentiviral-mediated neonatal intracerebral gene therapy in a mouse model of globoid cell leukodystrophy. *Hum Mol Genet*. 2014; 23:3250–3268. [PubMed: 24463623]
- Lim ZY, Ho AYL, Abrahams S, Fensom A, Aldouri M, Pagliuca A, Shaw C, Mufti GJ. Sustained neurological improvement following reduced-intensity conditioning allogeneic haematopoietic stem cell transplantation for late-onset Krabbe disease. *Bone Marrow Transplant*. 2008; 41:831–832. [PubMed: 18246117]
- Lin D, Donsante A, Macauley S, Levy B, Vogler C, Sands MS. Central nervous system-directed AAV2/5-mediated gene therapy synergizes with bone marrow transplantation in the murine model of globoid-cell leukodystrophy. *Mol Ther*. 2007; 15:44–52. [PubMed: 17164774]
- Majhail NS, Brazauskas R, Rizzo JD, Sobeks RM, Wang Z, Horowitz MM, Bolwell B, Wingard JR, Socie G. Secondary solid cancers after allogeneic hematopoietic cell transplantation using busulfan-cyclophosphamide conditioning. *Blood*. 2011; 117:316–322. [PubMed: 20926773]
- Neri M, Ricca A, di Girolamo I, Alcalá-Franco B, Cavazzin C, Orlacchio A, Martino S, Naldini L, Gritti A. Neural stem cell gene therapy ameliorates pathology and function in a mouse model of globoid cell leukodystrophy. *Stem Cells*. 2011; 29:1559–1571. [PubMed: 21809420]
- Pidala J. Graft-vs.-host disease following allogeneic hematopoietic cell transplantation. *Cancer Control*. 2011; 18:268–276. [PubMed: 21976245]
- Qin EY, Hawkins-Salsbury JA, Jiang X, Reddy AS, Farber NB, Ory DS, Sands MS. Bone marrow transplantation increases efficacy of central nervous system-directed enzyme replacement therapy

- in the murine model of globoid cell leukodystrophy. *Mol Genet Metab.* 2012; 107:186–196. [PubMed: 22704480]
- Rafi MA, Rao HZ, Luzi P, Wenger DA. Long-term improvements in life span and pathology in CNS and PNS after BMT plus one intravenous injection of AAVrh10-GALC in twitcher mice. *Mol Ther.* 2015; 23:1681–1690. [PubMed: 26329589]
- Reddy AS, Kim JH, Hawkins-Salsbury JA, Macauley SL, Tracy ET, Vogler CA, Han X, Song S-K, Wozniak DF, Fowler SC, Klein RS, Sands MS. Bone marrow transplantation augments the effect of brain- and spinal cord-directed adeno-associated virus 2/5 gene therapy by altering inflammation in the murine model of globoid-cell leukodystrophy. *J Neurosci.* 2011; 31:9945–9957. [PubMed: 21734286]
- Reddy AS, Wozniak DF, Farber NB, Dearborn JT, Fowler SC, Sands MS. Bone marrow transplantation alters the tremor phenotype in the murine model of globoid-cell leukodystrophy. *J Clin Med.* 2012; 1:1–14. [PubMed: 24013457]
- Rica A, Rufo N, Ungari S, Morena F, Martino S, Kulik W, Alberizzi V, Bolino A, Bianchi F, del Carro U, Biffi A, Gritti A. Combined gene/cell therapies provide long term and pervasive rescue of multiple pathological symptoms in a murine model of globoid cell leukodystrophy. *Hum Mol Genet.* 2015; 24:3372–3389. [PubMed: 25749991]
- Shigematsu H, Morimoto S, Kishimoto Y, Weiler S, Tomich J, Barranger J, Shinohara M, Yeager AM, O'Brien JS. Saposins (sphingolipid activator proteins) in the twitcher mutant mouse. *J Neurochem.* 1990; 55:1659–1662. [PubMed: 2120388]
- Siddiqi ZA, Sanders DB, Massey JM. Peripheral neuropathy in Krabbe disease: effect of hematopoietic stem cell transplantation. *Neurology.* 2006; 67:268–272. [PubMed: 16864820]
- Suzuki K, Suzuki K. The twitcher mouse. A model of human globoid cell leukodystrophy (Krabbe's disease). *Am J Pathol.* 1983; 111:394–397. [PubMed: 6859223]
- Suzuki Y, Austin J, Armstrong D, Suzuki K, Schlenker J, Fletcher T. Studies in globoid leukodystrophy: enzymatic and lipid findings in the canine form. *Exp Neurol.* 1970; 29:65–75. [PubMed: 5478365]
- Touraine JL, Raudrant D, Rebaud A, Roncarolo MG, Laplace S, Gebuhrer L, Betuel H, Frappaz D, Freycon F, Zabot MT, Dubernard J. In utero transplantation of stem cells in humans: immunological aspects and clinical followup of patients. *Bone Marrow Transplant.* 1992; 9(Suppl 1):121–126. [PubMed: 1354520]
- Toyoshima E, Yeager AM, Brennan S, Santos GW, Moser HW, Mayer RF. Nerve conduction studies in the twitcher mouse (murine globoid cell leukodystrophy). *J Neurol Sci.* 1986; 74:307–318. [PubMed: 3525759]
- Unger ER, Sung JH, Manivel JC, Chenggis ML, Blazar BR, Krivit W. Male donor-derived cells in the brains of female sex-mismatched bone marrow transplant recipients: a Y-chromosome-specific in situ hybridization study. *J Neuropathol Exp Neurol.* 1993; 52:460–470. [PubMed: 8103085]
- Weinberg KI. Early use of drastic therapy. *N Engl J Med.* 2005; 352:2124–2126. [PubMed: 15901868]
- Wenger DA. Murine, canine, and nonhuman primate models of Krabbe disease. *Mol Med Today.* 2000; 6:449–451. [PubMed: 11074371]
- Wenger DA, Rafi MA, Luzi P, Datto J, Constantino-Ceccarini E. Krabbe disease: genetic aspects and progress toward therapy. *Mol Genet Metab.* 2000; 70:1–9. [PubMed: 10833326]
- Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, Sorror ML, Horowitz MM, Bolwell B, Rizzo JD, Socie G. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. *J Clin Oncol.* 2011; 29:2230–2239. [PubMed: 21464398]
- Wu YP, McMahon E, Kraine MR, Tisch R, Meyers A, Frelinger J, Matsushima GK, Suzuki K. Distribution and characterization of GFP⁺ donor hematogenous cells in twitcher mice after bone marrow transplantation. *Am J Pathol.* 2000; 156:1849–1854. [PubMed: 10854208]
- Yagi T, Matsuda J, Tominaga K, Suzuki K, Suzuki K. Hematopoietic cell transplantation ameliorates clinical phenotype and progression of the CNS pathology in the mouse model of late onset Krabbe disease. *J Neuropathol Exp Neurol.* 2005; 64:565–575. [PubMed: 16042308]
- Yeager AM. Bone marrow transplantation in children. *Pediatr Ann.* 1988; 17:694–714. [PubMed: 3070470]

- Yeager AM. Bone marrow transplant in lysosomal storage diseases: a prelude to gene-insertion therapy. *Md Med J.* 1990; 34:337–341. [PubMed: 2110281]
- Yeager AM, Brennan S, Tiffany C, Moser HW, Santos GW. Prolonged survival and remyelination after hematopoietic cell transplantation in the twitcher mouse. *Science.* 1984; 225:1052–1054. [PubMed: 6382609]
- Yeager AM, Ichioka T, Kishimoto Y. Hematopoietic cell transplantation in murine globoid cell leukodystrophy (the twitcher mouse). *Ann N Y Acad Sci.* 1988; 529:242–245.
- Yeager AM, Shinohara M, Shinn C. Hematopoietic cell transplantation after administration of high-dose busulfan in murine globoid cell leukodystrophy (the twitcher mouse). *Pediatr Res.* 1991; 29:302–305. [PubMed: 2034480]
- Yeager AM, Shinn C, Shinohara M, Pardoll D. Hematopoietic cell transplantation in the twitcher mouse: effects of pretransplant conditioning with graded doses of busulfan. *Transplantation.* 1993; 56:185–190. [PubMed: 8101401]

SIGNIFICANCE

Globoid cell leukodystrophy (GLD), or Krabbe's disease, is caused by a deficiency of galactocerebrosidase (GALC), which is essential for normal myelination. The prognosis of GLD is poor, with progressive neurologic deterioration and early death, especially in infantile forms of the disorder. Studies of hematopoietic cell transplantation (HCT) in a murine model of GLD and in infants and children with GLD have provided insights into the effects of cellular transplantation on the clinical, biochemical, and neuropathological manifestations of this demyelinating leukodystrophy. The combination of HCT with other modalities, including infusions of GALC and viral-GALC vectors, is under investigation to improve treatment outcomes in GLD.