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Hematopoietic stem cell transplantation for multiple sclerosis: finding equipoise

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Summary:

Hematopoietic stem cell transplantation of multiple sclerosis is rapidly expanding. Success for this approach requires an understanding of the pathophysiology of multiple sclerosis and design of trials that select patients with active inflammatory disease, low disability scores, and avoidance of conditioning agents that may damage neural stem cell compartments or further compromise already injured axons.
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An evolving literature on Multiple Sclerosis (MS) supports the concept that MS is both an inflammatory demyelinating and axonal degenerative disease.[1–5] Hematopoietic stem cell transplantation (HSCT) was suggested as a treatment in 1995 in alignment with the hypothesis that pathologic events in MS result from an immune-mediated inflammatory attack on myelin.[5,6] In America, the first HSCT for MS was performed in 1996.[7] The toxicity from phase I trials will be reviewed as well as data on efficacy from the Northwestern/Milwaukee and Prague trial. Results from these studies indicate that: (1) HSCT protocols can be carried out with sufficient safety to warrant use in MS, which carries little risk of mortality but significant risk of disability provided that protocol design emphasizes safety, and (2) patients likely to respond to HSCT are likely to be found in earlier phases of disease (ie relapsing remitting MS) where active inflammatory events are more frequent and less irreversible neurological impairment has already occurred.

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Types of multiple sclerosis

Clinically definite (MS) requires at least two demyelinating neurologic events separated both anatomically in the central nervous system (CNS) and temporally in time. Relapsing–remitting MS is defined as relapsing disease without progression between relapses with or without residual neurologic deficits from each relapse. Secondary progressive MS demonstrates gradual neurologic deterioration with or without superimposed relapses after an initial relapsing–remitting course. Primary progressive MS shows gradual, progressive deterioration from onset. MS is a common disease with a North American prevalence of one in a 1000 people. At onset, 85% of cases are relapsing–remitting and 15% are primary progressive. Within 10 years, approximately 50% of relapsing–remitting disease becomes secondary progressive.[8–11]

Hypothesis for design of phase I HSCT trials

The rationale for immune modulation is based on the theory that MS is an autoimmune or at least immune-mediated disease. Support for an autoimmune etiology comes from pathological, animal, and experimental observations. Histologically, the lesions are inflammatory 'plaques' that contain T and B lymphocytes, macrophages, and plasma cells.[12] The MHC locus that is associated with numerous autoimmune diseases has MS-associated MHC haplotypes.[13] An animal autoimmune demyelinating disease, experimental autoimmune encephalomyelitis, resembles MS both clinically and histologically.[14] Traditional treatment for MS employs immune suppressive or immune modulating medications including interferon, copaxone, oral or intravenous (IV) pulse corticosteroids, oral or IV pulse cyclophosphamide, azathioprine, and mitoxantrone. FDA-approved therapies for relapsing-remitting MS interferon beta (Avenox®, Betaseron®) or Copaxone® (copolymer 1 or glatiramar acetate), known as ABC therapy.[15–23] Avenox® and Betaseron® are different formulations of interferon beta. Copaxone® is a mixture of four amino acids in a defined molar residue ratio containing L-glutamate, L-lysine, L-alanine, and L-tyrosine.

Development of new therapies for MS is important since no current treatment is curative and the disease is

associated with extensive morbidity, as demonstrated by natural history studies, and also by the high suicide rate among severely affected MS patients in some studies. For these reasons, phase I toxicity studies of intense immune suppression requiring HSC support were initiated in America beginning in 1996.[7]

Conditioning regimens and toxicity

There are a number of considerations when developing or adopting pre-existing transplant regimens for novel indications such as MS. When considering autologous transplantation regimens for the treatment of MS, the risk of the transplant strategy and conditioning regimen must be in keeping with the degree of risk (morbidity and mortality) of the disease being treated. A number of different regimens have been used in the initial hematopoietic stem cell transplantation (HSCT) trials in MS patients.[24–32] As phase I studies, it is important to evaluate causes of deaths for each trial.

The City of Hope (Duarte, CA, USA) used an intensive conditioning regimen of busulfan (16 mg/kg), cyclophosphamide (120 mg/kg), and antithymocyte globulin (30 mg/kg) along with $CD34^+$ selection to deplete lymphocytes from the graft.[26] Two out of five treated patients died from infections. One patient died 22 days after transplant from influenza and the second died 19 months after HSCT from *Streptococcus pneumonia* sepsis. The Fred Hutchinson Cancer Center Consortium treated 26 patients with progressive MS.[27,28] The conditioning regimen was total body irradiation (TBI) (800 cGy given 200 cGy b.i.d. with lung shields to 650 cGy), cyclophosphamide (120 mg/kg divided 60 mg/kg/day), and ATG (either 90 mg/kg equine or 15 mg/kg rabbit) given for 6 days (days -5, -3, -1, $+1$, $+3$, and $+5$). G-CSF mobilized peripheral blood stem cells (PBSC) were lymphocyte depleted by $CD34^+$ positive selection. The only patient in whom rabbit ATG had been given instead of equine ATG died from Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD).[28] The patient who developed PTLD had received 6 days of rabbit ATG at 2.5 mg/kg/day (total dose of 15 mg/kg). PTLD is a complication of prolonged and aggressive immune suppression occurring in both solid organ transplants and HSC transplantation for malignancies when a lymphocyte or purged graft is infused. Therefore, PTLD is a complication not unique to rabbit ATG but rather secondary to the extent of immune suppression. It is probable that higher doses of equine ATG may also cause PTLD. While not known at the time, the rabbit ATG dose (15 mg/kg) is roughly equivalent to an equine ATG dose of 150–225 mg/kg. The Thessaloniki group in Greece has reported that BEAM (carmustine, etoposide, cytarabine, melphalan) when combined with ATG and lymphocyte depletion of the graft was complicated by mortality from an opportunistic infection (aspergillosis).[29]

In summary, deaths have occurred in the phase I MS trials, which were related to infections. As phase I studies, the results suggest caution in combining lymphocyte-depleted grafts with aggressive immune suppressive conditioning regimens. This may be accomplished by decreasing the dose intensity of conditioning agents, eliminating one of the conditioning agents, or infusing an unmanipulated graft not depleted of lymphocytes. For a disease such as MS, which has significant morbidity but virtually no 5- or 10-year mortality, HSCT must be designed to minimize infectious deaths. These phase I trials suggest that future transplant conditioning regimens should be less intense, especially if combined with $CD34^+$ selection.

Efficacy of HSCT

A recent study by Mancardi *et al*[30] reported 10 subjects undergoing HSCT followed with a frequent MRI protocol, who demonstrated lack of either enhancing lesions or accumulation of T2 burden of disease over an observation period of 4–30 months.[30] While HSCT has a remarkable, sustained, impact on MRI evidence of inflammation, currently there is little evidence for clinical benefit in terms of disability.

The Prague (Czeck Republic) trial treated 15 patients with BEAM (BCNU 300 mg/m^2 i.v., etoposide 800 mg/m^2 i.v., cytosine–arabinoside 800 mg/m^2 i.v., melphalan 140 mg/m^2 i.v.).[32] A serious respiratory infection event, which required temporary intubation and supportive ventilation, occurred in one patient during the early post-transplantation period. Median scores of the Expanded Disability Status Scale (EDSS) were 6.5 (6.0–7.5) pretransplant. One patient improved by 1.0 or more EDSS points (by 1.5 point). One patient worsened by 1.5 point down to 9.0 points on EDSS and died 31 months after the transplantation from disease progression (EDSS 10.0). No other patient changed by more than 0.5 EDSS steps. The Northwestern/Milwaukee study has a median follow-up post-transplant of 36 months (range 6 months to 6 years). Of 18 patients whose pretransplant Kurtzke EDSS was >6.0, 9 have progressed by 1.0 or more EDSS points. Of 10 patients whose EDSS was <6.0, no patient has progressed by 1.0 or more EDSS steps. The only patient with relapsing remitting MS is also the only patient whose EDSS improved by 1.0 or more EDSS points going from 3.5 to 1.0 (manuscript in preparation).

HSCT, like other immune-based therapeutic approaches to the treatment of MS, is likely to offer the most benefit to individuals in earlier more active inflammatory stages of the disease. HSCT does not appear to prevent further progression in patients with progressive disease and high disability scores (EDSS >6.0). Future HSCT studies should focus on patients earlier in disease course with active inflammatory disease (active relapses).

Hypothesis for design of future HSCT trials

Results from neuropathological, MRI natural history studies, and immune suppressive trials, including intense immune suppressive HSCT studies, suggest another hypothesis for MS. It is both an immune-mediated demyelinating disease and an axonal degenerative process. There are several possible explanations for neuronal injury resulting from immune-mediated destruction of myelin. 'Death by

injury' may result from inflammatory cytokines released during demyelination. Even in the absence of inflammation, myelin may insulate axons from blood or local metabolic oxidants. Therefore, demyelinated axons may be subjected to greater oxidant injury. There is likely a trophic interaction between axons and oligodentrocytes, which may function as supporting or 'nurse' cells to nourish the axon. Some axons extend from the brain to the distal end of the spinal cord. This is an astronomical distance for a microscopic cell and the nucleus of a neuron may be unable to provide long-term support to a distant axon without other cells within the CNS having a nurturing role. Indeed, primary cultures of mouse neurons survive significantly longer *ex vivo* if layered over a mixture of non-neuronal CNS cells including oligodendrocytes and astrocytes. Long-standing demyelination may, therefore, lead to 'Death by neglect'.

Candidates for HSCT

Patients for new HSCT trials should be selected earlier in disease with less disability and active inflammation despite primary therapy. The protocol should be aimed at suppressing relapses in patients at risk for progressive disability. Unfortunately, there are no good clinical or MRI markers predictive for worsening disability in patients with relapsing–remitting disease. Weinshanker has reported that the number of relapses within the first 2 years correlates with late disability.[9–11] Confavreux *et al*[33] reported that for patients with an EDSS of 4.0 or greater (attained after a longer disease duration), the number of relapses does not correlate with progression of irreversible disability. An EDSS of 4.0 or more may already be too late for therapy aimed at inflammatory demyelinating events to prevent progression of subsequent disability. However, some level of sustained disability would be required to justify the procedure until more evidence on safety and efficacy have accumulated. Efficacy of earlier intervention in MS is supported by the CHAMPS study, in which over a 3 year interval, treatment with interferon following the first clinical event significantly lowered the probability of developing clinically definite MS.[34] If prevention of early demyelinating events is important in preventing late disability, a safe but intense immune suppressive regimen might be more effective than interferon beta or glatiramer acetate, and thus indicated in patients with definite MS and continuing relapses. Possible criteria for a future HSCT study could be:

(1) An EDSS of 3.0–5.5.
(2) Inflammatory disease despite primary disease modifying therapy with at least 6 months of interferon.

Failure is defined as two or more clinical relapses with documented neurologic changes within the year prior to the study. Relapses must have required treatment with corticosteroids. Sensory only relapses should probably be excluded. Failure may also be defined by evidence on MRI of active inflammation (ie gadolinium enhancement).

Conditioning regimen for future MS HSCT trials

Since patients in these new studies would be earlier in the disease course, a safer conditioning regimen that does not include TBI should be considered. Cyclophosphamide at 200 mg/kg with or without ATG has been used safely in a variety of autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, and aplastic anemia.

As mentioned, MS is probably both an axonal degenerative and demyelinating disease. While no data exist that TBI is associated with axonal injury at the doses used in phase I regimens, radiation's effect is via generation of intracellular-free radicals causing damage to DNA and protein. Theoretically, axonal degeneration may be accelerated by TBI conditioning regimen-related neuronal injury. This is particularly important since permanent disability in MS correlates with axonal degeneration not demyelination. The conditioning regimen should also be designed to minimize damage to CNS repair pathways such as neural stem cells and oligodendrocyte progenitor remyelination. Sensitivity of neural stem cells to either radiation or cyclophosphamide is unknown but hematopoietic stem cells are resistant to cyclophosphamide despite lethal sensitivity to even low doses (200 cGy) of TBI.

TBI has been associated with late myelodysplasia and leukemia (5–8% incidence), solid tumors, hypothyroidism, and cataracts that would be unlikely from cyclophosphamide $\pm$ATG. Patients with relapsing–remitting MS will have a zero 10 year disease-related mortality making the incidence of TBI-related leukemia a serious adverse effect. TBI will cause infertility. In contrast, ATG does not cause infertility and cyclophosphamide-induced infertility is age related. Females under age 26 regain normal ovarian function, while one-third of females over age 26 regain ovarian function. Phase I HSCT studies using triple immune suppressive regimens and $CD34^+$ selection have been associated with lethal opportunistic infections. No lethal infections have been reported for cyclophosphamide $\pm$ATG with or without $CD34^+$ selection in any of 73 RA or 34 SLE patients undergoing HSCT. Unlike TBI containing regimens, a regimen of cyclophosphamide$\pm$ATG is not myeloablative and even if autologous stem cells did not engraft, hematopoiesis would recover spontaneously. By dose reduction in regimen intensity from some of the original phase I protocols and proper selection of patients with relatively early inflammatory disease, HSCT may offer new hope to patients with MS.

References

1 Trapp BD, Peterson J, Ransohoff RM *et al*. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; **338**: 278–285.
2 De Stefano N, Matthews PM, Fu L *et al*. Axonal damage correlates with disability in patients with relapsing–remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain* 1998; **121** (Part 8): 1469–1477.
3 Fu L, Matthews PM, De Stefano N *et al*. Imaging axonal damage of normal-appearing white matter in multiple sclerosis. *Brain* 1998; **121** (Part 1): 103–113.

4 Ferguson B, Matyszak MK, Esiri MM *et al*. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; **120** (Part 3): 393–399.
5 Oksenberg JR, Hauser SL. Emerging concepts of pathogenesis: relationship to multiple sclerosis therapies. In: Rudick RA, Goodkin DE (eds). *Multiple Sclerosis Therapeutics*. Martin Dunitz: London, 1999, pp 215–242.
6 Burt RK, Burns W, Hess A. Bone marrow transplantation for multiple sclerosis. *Bone Marrow Transplantation* 1995; **16**: 1–6.
7 Burt RK, Traynor AE, Cohen B *et al*. T cell-depleted autologous hematopoietic stem cell transplantation for multiple sclerosis: report on the first three patients. *Bone Marrow Transplantation* 1998; **21**: 537–541.
8 Noseworthy JH, Lucchinetti C, Rodriguez M *et al*. Multiple sclerosis. *N Engl J Med* 2000; **343**: 938–952.
9 Weinshenker BG. The natural history of multiple sclerosis: update 1998. *Semin Neurol* 1998; **18**: 301–307.
10 Weinshenker BG. The natural history of multiple sclerosis. *Neurol Clin* 1995; **13**: 119–146.
11 Weinshenker BG. Natural history of multiple sclerosis. *Ann Neurol* 1994; **36** (Suppl): S6–S11.
12 Lassmann H, Raine CS, Antel J *et al*. Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. *J Neuroimmunol* 1998; **86**: 213–217.
13 Jersild C, Fog T, Hansen GS *et al*. Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. *Lancet* 1973; **2**: 1221–1225.
14 Glabinski AR, Tani M, Tuohy VK *et al*. Murine experimental autoimmune encephalomyelitis: a model of immune-mediated inflammation and multiple sclerosis. *Methods Enzymol* 1997; **288**: 182–190.
15 Paty DW, Li DK, UBC MS/MRI Study Group, IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing–remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993; **43**: 662–667.
16 The IFNB Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. Interferon (beta)-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology* 1995; **45**: 1277–1285.
17 PRISMS (Prevention of Relapses and Disability by Interferon (beta)-1a Subcutaneously in Multiple Sclerosis) Study Group. Randomised double-blind placebo-controlled study of interferon (beta)-1a in relapsing/remitting multiple sclerosis. *Lancet* 1998; **352**: 1498–1504. (Erratum, *Lancet* 1999; **353**: 678.)
18 European Study Group on Interferon (beta)-1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon (beta)-1b in treatment of secondary progressive multiple sclerosis. *Lancet* 1998; **352**: 1491–1497.
19 The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing–remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* 1993; **43**: 655–661.
20 Johnson KP, Brooks BR, Cohen JA *et al*. Copolymer 1 reduces relapse rate and improves disability in relapsing–remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. *Neurology* 1995; **45**: 1268–1276.
21 Johnson KP, Brooks BR, Cohen JA *et al*. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology* 1998; **50**: 701–708.
22 Millefiorini E, Gasperini C, Pozzilli C *et al*. Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome. *J Neurol* 1997; **244**: 153–159.
23 Edan G, Miller D, Clanet M *et al*. Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: a randomised multicentre study of active disease using MRI and clinical criteria. *J Neurol Neurosurg Psychiatry* 1997; **62**: 112–118.
24 Burt RK, Traynor AE, Pope R *et al*. Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood* 1998; **92**: 3505–3514.
25 Openshaw H, Stuve O, Antel JP *et al*. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology* 2000; **54**: 2147–2150.
26 Openshaw H, Lund BT, Kashyap A *et al*. Peripheral blood stem cell transplantation in multiple sclerosis with busulfan and cyclophosphamide conditioning: report of toxicity and immunological monitoring. *Biol Blood Marrow Transplantation* 2000; **6**: 563–575.
27 Nash RA, Kraft GH, Bowen JD *et al*. Treatment of severe multiple sclerosis (MS) with high dose immunosuppressive therapy (HDIT) and autologous stem cell transplantation (SCT) (abst 3640). *Blood* 2000; **96** (Suppl A): 842a.
28 Nash RA, Dansey R, Storek J *et al*. Epstein Barr virus (BBV)-associated post-transplant lymphoproliferative disorder (PTLD) after high dose immunosuppressive therapy (HDIT) and autologous CD34-selected stem cell transplantation (SCT) for severe autoimmune diseases (abst 1747). *Blood* 2000; **96** (suppl A): 406a.
29 Fassas A, Anagnostopoulos A, Kazis A *et al*. Autologous stem cell transplantation in progressive multiple sclerosis – an interim analysis of efficacy. *J Clin Immunol* 2000; **20**: 24–30.
30 Mandalfino P, Rice G, Smith A *et al*. Bone marrow transplantation in multiple sclerosis. *J Neurol* 2000; **247**: 691–695.
31 Carreras E, Saiz A, Graus F *et al*. Autologous CD34+ selected hematopoietic stem cell transplantation (CD34+/HSCT) for multiple sclerosis: update of a single center experience in 10 patients. *Biol Blood Marrow Transplantation* (abstr) 2001; **7**: 69.
32 Kozak T, Havrdova E, Pit'ha J *et al*. High-dose immunosuppressive therapy with PBPC support in the treatment of poor risk multiple sclerosis. *Bone Marrow Transplantation* 2000; **25**: 525–531.
33 Confavreux C, Vukusic S, Moreau T *et al*. Relapses and progression of disability in multiple sclerosis. *N Eng J Med* 2000; **343**: 1430–1438.
34 Jacobs LD, Beck RW, Simon JH *et al*. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. *N Engl J Med* 2000; **343**: 898–904.