Cell Therapy in Demyelinating Diseases

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Summary: Multiple sclerosis presents particular and serious problems to those attempting to develop cell-based therapies: the occurrence of innumerable lesions scattered throughout the CNS, axon loss, astrocytosis, and a continuing inflammatory process, to name but a few. Nevertheless, the limited and relatively focused nature of damage to oligodendrocytes and myelin, at least in early disease, the large body of available knowledge concerning the biology of oligodendrocytes, and the success of experimental myelin repair, have allowed cautious optimism that therapies may be possible. Here, we review the clinical and biological problems presented by multiple sclerosis in the context of cell therapies, and the neuroscientific background to the development of strategies for myelin repair. We attempt to highlight those areas where difficulties have yet to be resolved and draw on a variety of more recent experimental findings to speculate on how remyelinating therapies are likely to develop in the foreseeable future. **Key Words:** Multiple sclerosis, remyelination, stem cells, cellular therapy, demyelination.

INTRODUCTION

Within the past year or so (formal publication is awaited), the first patients with multiple sclerosis (MS) have, by way of preliminary clinical experiment, received intracerebral implants of cells, in the hope that these (autologous Schwann) cells would effect myelin repair (http://www.myelin.org/12082003.htm). Early and informal indications suggest that proof of efficacy is lacking, but there is a drama underlying this work: reparative cell therapy in multiple sclerosis has finally begun its journey from laboratory to clinic.

What has been the experimental rationale for this work, and, no less important, what is the future of cell therapy in multiple sclerosis? Does it lie with Schwann cells, or with stem cells, with other glia or none? Here we propose that, while serious and substantial clinical and biological problems remain to be solved, remyelination treatments by cell-based therapy represent an approachable challenge, offering a realistic prospect of successful implementation for the current generation of patients with multiple sclerosis.

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THE CLINICAL SCIENTIFIC BACKGROUND TO CELL THERAPY FOR DEMYELINATION

The complexity of the CNS poses daunting challenges to reparative medicine. Not for nothing did Cajal coin the immortal if lugubrious phrase "everything may die, nothing may be regenerated," and affirm the perception that the brain has traded flexibility in response to damage, and a capacity for functional, regenerative repair, for prodigious sophistication and complexity. In the context of this challenging background, there are, however, three good reasons to believe that demyelinating diseases such as multiple sclerosis might have significant advantages over many other CNS disorders in their inherent eligibility for cell therapies.

The first is that, despite the valuable concentration on axon loss in multiple sclerosis in recent years, it remains primarily a demyelinating disease. Axon loss undoubtedly occurs earlier in the course of MS than previously believed, and is more than likely to represent the principal pathophysiological cause of disability in chronic progressive disease, but none of the recent experimental, imaging, or neuropathological studies have challenged the concept that disease processes in MS are primarily directed against oligodendrocytes and/or myelin, and that axons are relatively spared until late disease.[1,2] The importance of considering the mechanism of axon loss is addressed below, but the key implication of the primary

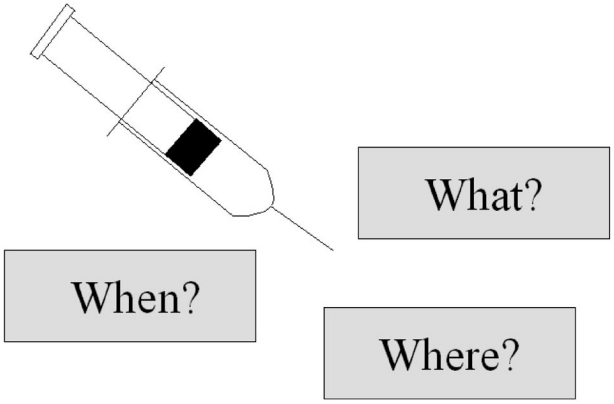

FIG. 1. Questions to be considered in the development of reparative cell therapy.

targeting of myelin in MS is that, in the main, axon pathways remain intact. Cell therapies therefore aim "only" to reinvest axons with myelin, rather than address the almost overwhelming challenge presented by other neurological diseases: that of re-establishing connectivity in highly complex but fragmented axonal circuitry.

The second positive feature of MS, in terms of developing cell therapies, is found in the clear evidence of spontaneous if partial myelin repair in multiple sclerosis.[3–6] The aim of cell therapies thus ceases to be the artificial imposition of a repair phenomenon *de novo*, and becomes one of enhancing or supplementing a spontaneous process. How this might be done must depend on a better understanding of the clinical biology of the disease, including the reasons why endogenous repair is not more successful, and how these limitations can be overcome.

Thirdly, there is now available a very large body of experimental evidence, employing a wide variety of animal models of demyelination and a range of sources of remyelinating cells, collectively providing proof of principle: cell therapy can, without question, achieve successful remyelination. Thus, implanted dissociated rat Schwann cells remyelinate the rodent CNS,[7,8] and can restore normal conduction[9]; transplantation of purified oligodendrocyte lineage cells[8,10–15] or cell lines[12,16,17] likewise achieves myelin repair, accompanied by improved conduction[18] and also demonstrable functional recovery.[19] Olfactory ensheathing cells (OECs), upon transplantation into lesions containing demyelinated axons, also lay down (Schwann cell-like) myelin.[20,21] Rodent embryonic stem cells and neural stem cells expanded from adult rodent brain both possess significant remyelinating potential.[22–25] Adult bone marrow also contains stem cells with neurogenic (and other) potential: these cells too, upon injection, are associated with successful myelin repair.[26,27]

THE REMAINING OBSTACLES TO DEVELOPING EFFECTIVE CELL THERAPIES IN MS

Against this positive experimental and pathological background to therapeutic remyelination in MS, why is it that cell therapies, or at least clinical experimental therapeutic trials, are not already widespread in this disease? What are the significant remaining hurdles, and how serious are they? What still needs to be done to develop at least potentially effective interventions? At least four significant problems readily suggest themselves: each will be briefly considered (FIG. 1).

The timing of implantation: early or late?

The firstprinciple in any new therapeutic endeavor must always remain "first, do no harm." In late MS, when progressive disability is established, and hope of spontaneous recovery extinguished, the possibility of doing damage or at best compromising spontaneous repair is remote. However, compelling arguments must be offered if the early exhibition of any potentially hazardous intervention in MS is to be justified; at this stage, little disability is present and therefore there is much to lose, and the natural history is such that some patients will never develop significant disability. In addition, implanting cells into early lesions exposes remyelinating glia, and their new myelin, to ongoing inflammatory activity. Concurrent use of potent immunosuppressive agents, required in any case with allogenic transplants to prevent graft rejection, might help protect cells, but no current therapies reliably stop myelin destruction.

Despite this, we believe earlier intervention might well have the best prospect of success. Spontaneous remyelination appears to occur maximally in acute inflammatory lesions,[5,28] suggesting an optimally propitious environment. Indeed, some suggest that anti-inflammatory drugs[29] or the suppression of inflammation in general[30] may impair myelin regeneration.

The clinical impact of the accumulating axon loss in secondary progressive disease[2,31] provides a more potent reason for earlier remyelinating intervention. Quite apart from the futility of attempting to remyelinate axons long since departed, recent findings indicate that changes in the cell surface expression of various molecules (e.g., polysialic acid–neural cell adhesion molecule) in chronically demyelinated axons actively inhibit myelination.[32] Also apparent is a profound inhibitory effect on remyelinating glia of chronic astrocytosis,[33] a key feature of the chronically demyelinated lesion. These considerations all seriously mitigate against deferred intervention but, in addition, the underlying cause of axon loss also must be considered.

The course of secondary progression—and by implication, of axon loss—appears to be influenced neither by early inflammatory disease activity[34–36] nor, sadly, by

even the most profound immune suppressant or anti-inflammatory treatments. These and other observations have fueled the hypothesis that progressive axonal damage is (at least in part) a consequence of persistent myelin loss.[2,37,38] Pathological studies have indicated that chronic axon loss does not correlate with inflammatory cell infiltrate, tumor necrosis factor (TNF) expression, nitric oxide expression, or demyelinating activity, but is related to the overall extent of established myelin loss.[34,36] It is seen in lesions which are demyelinated but which exhibit sparse or no inflammation, but is rare in remyelinated lesions.[36] Demyelination-induced axon loss might occur by several possible mechanisms: directly, through the loss of oligodendrocyte-derived trophic support,[37,39,40] or sustained demyelination-induced conduction block and electrical silence,[41] or indirectly through increased vulnerability of the exposed axon to injurious agents.[42] A further important driver for early intervention thus emerges: the restoration of a normal oligodendroglial environment to sustain (previously demyelinated) axons. Therefore, the earlier the intervention, the greater the potential gain.

The site of implantation

Clearly, multiple inoculations of cells into widely distributed lesions in the brain and spinal cord of patients with MS is unrealistic. What should not be overlooked, however, is that many plaques are clinically silent, while a disproportionate degree of disability frequently emanates from a few critical lesions in eloquent areas. Thus, implantation into a very small number of carefully selected lesions, for example, the optic nerves, the spinal cord, or the superior cerebellar peduncle, could yield a useful therapeutic dividend.[43]

A more global myelin repair strategy, applicable not only for multiple sclerosis but also for the significantly rarer group of patients with inherited disorders of myelin metabolism, however, is not impossible to foresee. Transplanted cells would need to be encouraged to migrate widely, as occurs during development. Supplementing cellular transplantation with growth factor infusions,[44] cotransplantation with growth factor-secreting cells,[45] and suppressing molecules that inhibit migration[33,46] have all been tried experimentally, with limited success. An alternative approach would exploit both the circulation of the brain and the blood–brain barrier disruption present at sites of active inflammation to disseminate and deliver cells, relying on the tropism of certain reparative cells for diseased tissue, as discussed below (in relation to bone marrow-derived and other adult stem cells).

Monitoring success or otherwise

At present, the MRI detection of new myelin is not reliably feasible, but new techniques continue to emerge, of which magnetization transfer contrast is the strongest candidate for imaging remyelination.[47] Magnetic resonance spectroscopy measurement of N-acetyl-aspartate levels might offer means of assessing any impact on local neuron/axon survival.[48,49] Using paramagnetic particles to label cells before transplantation, enabling their dispersion to be tracked by MRI[50–52] has promise, although from a safety perspective, even the most trivial manipulation of cells before implantation would be better avoided. Furthermore, graft survival cannot be inferred from migration, since dead cells remain visible,[50] and this method not only fails to show new myelin formation but may also impair the ability of other MR modalities to do so.

Serial neurophysiology may prove valuable, and monitoring conduction times may provide evidence of returning saltatory conduction in the targeted pathway(s). The optic nerve has particular advantages in this respect, but various approaches to more generalized neurophysiological assessment have been described and may prove useful for any intervention aimed at multifocal or more diffuse myelin repair.[53]

Finally, remyelination without clinical improvement would be a hollow victory, so robust and reproducible methods of clinical assessment need to be applied *ab initio*. Specific clinical outcome measures of function, disability, and handicap must be adopted and tailored for each type of intervention. Ultimately, success will need to be measured using properly designed clinical trials, in which clinical outcomes should be paramount. Considerable advances in clinical scale design have improved physical and functional measurement in multiple sclerosis,[54] so that the tools for assessing clinical outcome, on which remyelination therapies must stand or fall, are becoming available.

Choice of reparative cell

Cells of the oligodendrocyte lineage. Oligodendrocytes are the most obvious candidates. These are the cells lost in multiple sclerosis, and it is their normal function to myelinate the CNS. Immature oligodendrocytes and oligodendrocyte precursors are found in fresh lesions[55–60] and are generally considered responsible for the majority of spontaneous remyelination.[61–64] Consideration of the stage within the oligodendrocyte lineage optimal for transplantation is important. Although some studies have suggested that mature differentiated oligodendrocytes are useful myelinating cells,[10] the majority view is that mitotic[65]and migratory[14,66,67] capacities are vital prerequisites for successful remyelination, and that postmitotic oligodendrocytes, lacking these competencies, do not readily recapitulate their development to form myelin sheaths again.[14,68,69]

Despite their motility, oligodendrocyte progenitors show poor survival and migration when implanted into normal white matter, although they are able to populate

and remyelinate when injected into, or very close to, lesioned tissue.[70] By contrast, these cells survive well in x-irradiated tissue, which depletes endogenous progenitors.[71] Part of this increased survival may reflect competition between endogenous and implanted cells for survival factors, because progenitor numbers increase with increased availability of platelet-derived growth factor (PDGF)[72] or glial growth factor 2 (GGF2).[73] The possibility of improving graft survival and proliferation by the use of growth factors has been explored *in vivo* with some success,[45] but this introduces a further complication in developing clinical therapy.

A further difficulty of "using" oligodendrocyte progenitors is that investigations of human CNS glia have consistently demonstrated significant biological differences from rodent cells, so that data concerning rodent oligodendrocyte progenitor cells (OPCs) cannot be directly extrapolated to human glia. Early studies identified glia similar to the rodent OPCs in cultures derived from the fetal human CNS[74]; these cells can synthesize myelin in the dysmyelinated rodent CNS, even after cryopreservation.[75] Initial studies of the more recently identified adult human oligodendrocyte progenitors[76–78] suggested a very limited capacity for remyelination (in the irradiated rodent spinal cord).[79] However, elegant methods for selection of these cells (for experimental purposes) from samples of human white matter have since been perfected[80] and, interestingly, very recent comparative studies suggest that adult human CNS-derived oligodendrocyte progenitors have a significantly greater remyelinating capacity than their fetal counterparts.[81]

Schwann cells. Perhaps surprisingly, Schwann cells make a significant contribution to endogenous myelin repair in multiple sclerosis, particularly in the spinal cord.[6,82,83] Experimental methods have been established for preparing cultures of Schwann cells from adult peripheral nerve biopsies and for purifying and expanding the cells *in vitro* to generate large populations of Schwann cells.[84,85] When so purified, human Schwann cells successfully lay down new myelin in the mouse[86] and the rat spinal cord.[87,88]

Autologous Schwann cell harvesting from peripheral nerve biopsy, expansion *in vitro*, and transplantation into patients with multiple sclerosis offers the considerable attractions of relative ease of availability, and the avoidance of rejection. Furthermore, by contrast with oligodendrocyte-established new myelin, Schwann cells and their myelin sheaths should be resistant to continuing disease-related immunological attack. Firm evidence is required, however, that expanded human Schwann cells do not form tumors *in vivo*, a hazard described when rodent Schwann cells immortalized by growth factor expansion were transplanted[89]; unpurified preparations of human peripheral nerve cells result in substantial fibroblast overgrowth with axon destruction[87]; this obviously

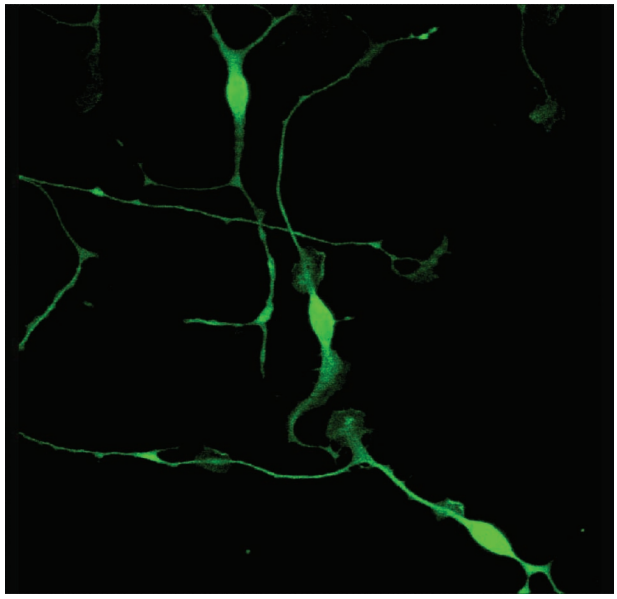

FIG. 2. Cultured Schwann cells, stained with S100.

presents an imposing barrier to the clinical application of Schwann cell transplants. The apparent inhibitory effect of astrocytes on Schwann cell-mediated CNS remyelination[90–92] represents another potential problem for the use of Schwann cells in remyelination therapy (FIG. 2).

Olfactory glia. OECs ensheath the axons emanating from olfactory epithelial neurons that penetrate the olfactory bulb of the CNS (FIG. 3). They are found in the olfactory bulb, nerves, and epithelium. Normally, OECs are nonmyelinating, but rodent OECs assume a myelinating phenotype closely resembling that of Schwann cells when transplanted into lesions containing demyelinated axons.[20,21] The ability of OECs to promote CNS axon regeneration and ensheath and myelinate demyelinated axons has led to considerable interest in olfactory glia in the field of CNS repair.[93]

One of the potential advantages of OECs over Schwann cells relates to their relationship with astro-

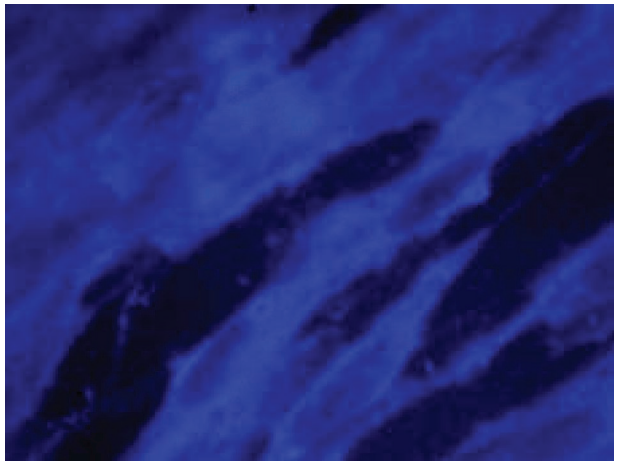

FIG. 3. Adult human olfactory glia *in vitro*.

cytes. In health, OECs coexist alongside astrocytes within the olfactory bulb, whereas in experimentally demyelinated lesions, OECs can (in contrast to Schwann cells) ensheath and myelinate axons unimpeded by the astrocytic environment.[20] *In vitro*, rodent OECs migrate far more successfully over astrocytes than Schwann cells.[94] Human OECs, like rodent OECs, are capable of remyelination following transplantation into the demyelinated rodent spinal cord.[95,96]

Stem cells. There is general agreement that stem cells have enormous therapeutic potential, perhaps particularly for the treatment of neurodegenerative disease.[97–100] To date, most studies have concentrated on using embryonic tissue as a source of stem cells, and certainly rodent embryonic stem cells possess considerable remyelinating potential.[22] However, to develop therapies would obviously require the dissection of human embryos as the stem cell source, and this raises significant practical, immunological, and ethical concerns. One serious risk is that of teratoma formation[101]; removing this capacity from embryonic stem cells with absolute success may pose considerable problems. In addition, the emergence of significant chromosomal abnormalities in cultured human embryonic stem cells raises further concerns about their safe use.[102] The problem of rejection would also have to be circumvented. Although this might be overcome using stem cells from embryos cloned (by cell nuclear transfer) from individual putative recipients (recently legalized uniquely in the United Kingdom), the implication that every patient requiring a transplant would first have to be cloned seems quite unrealistic and would not bypass the major ethical difficulties associated with the use of human embryonic material.

These problems have helped stimulate the largely successful search for alternative sources of stem cells.[103] There is increasing evidence that adult stem cells have a greater capacity to differentiate into a wider range of cell types than previously anticipated, and the use of adult stem cells, particularly autologous cells would avoid many of the difficulties associated with embryonic stem cells.[103–106]

It is now clear that neural stem cells are present in the adult rodent brain[107]; large numbers of oligodendrocyte lineage cells can be generated using neurosphere/oligosphere techniques, which, upon transplantation, successfully remyelinate axons. Neural stem cells are also present in the adult human brain (FIG. 4).[108] Recently, it has been reported that adult CNS-derived rodent stem cells will repair multifocal demyelinating lesions (in experimental allergic encephalomyelitis-affected rodents) even after intravenous delivery.[24]

Bone marrow-derived stem cells (FIG. 5) are also capable of homing to damaged tissue(s) from the circulation,[109,110] and this tropism includes movement toward injured CNS tissue.[111,112] It is now beyond doubt that adult bone marrow harbors a subpopulation of potentially highly proliferative stem cells,[106,112–120] whose differentiation capacity includes glial cells and neurones.[121–124] Transplant studies in rodents have confirmed the ability of bone marrow-derived cells to express neural phenotypes in the CNS of recipients. Directly or peripherally injected bone marrow-derived cells will repair damage, often with demonstrable functional as well as anatomical recovery, in rodent models of traumatic, degenerative, and ischemic CNS damage.[112,125–131] Remyelination is reported not only after

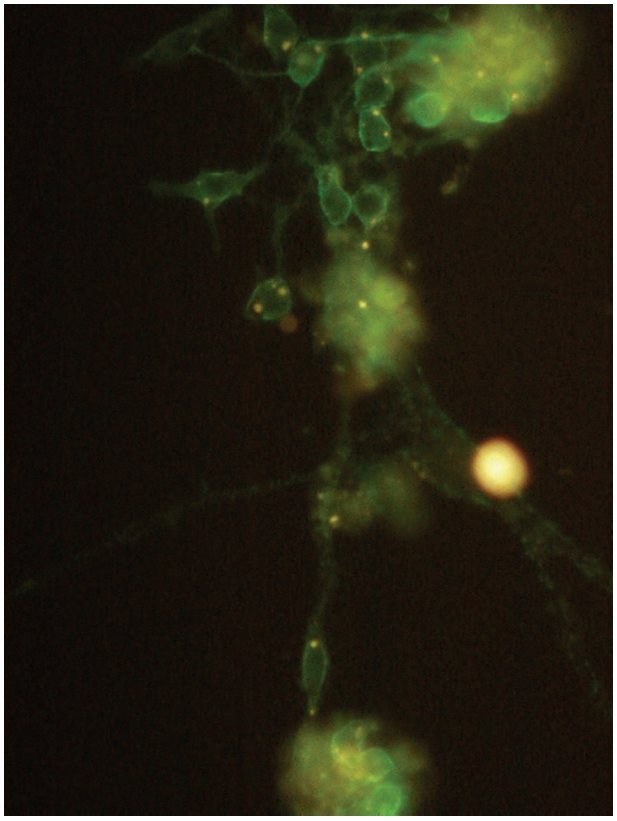

FIG. 4. Adult human brain-derived neural stem cells differentiated into oligodendrocyte progenitors and stained with NG2.

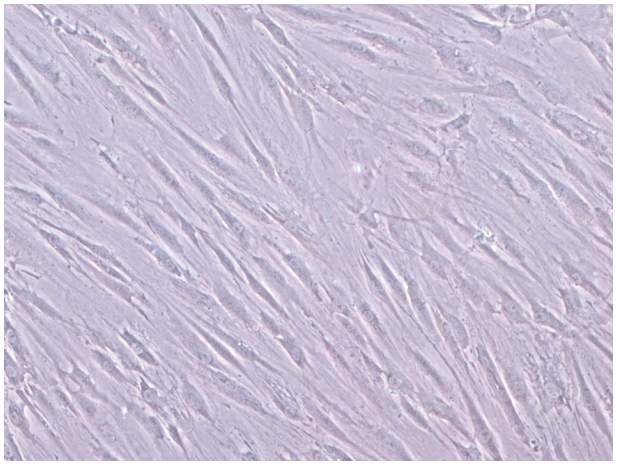

FIG. 5. Adult human mesenchymal stem cells in culture.

direct injection into lesions,[27,132] but also, in more recent studies, after peripheral injection.[26]

These properties, together with their easy accessibility, ethical robustness, and significant track history in the treatment of hematological disease makes bone marrow cells ideal candidates for use in cellular therapies for CNS disease. Further elucidation of the mechanisms involved may allow for mobilization of endogenous cells, perhaps even obviating the need for transplantation.

The possibility has been raised that cell fusion of bone marrow-derived cells with host cells provides an alternative explanation for apparent transdifferentiation.[133,134] However, cell fusion cannot explain the extensive *in vitro* data indicating multipotentiality (see above). *In vivo* studies confirm transdifferentiation without fusion in a variety of tissues.[135–137] Furthermore, from a pragmatic perspective, fusion may simply be part of the means by which bone marrow-derived stem cells stimulate successful regeneration[138]; bone marrow–host cell fusion in a liver disease model[133,134] occurs in the context of metabolic rescue by transplanted cells with functional liver repair and survival of treated animals.[139] Recent studies indicate that polyploidy is in fact a far more common phenomenon that previously realized; the possible occurrence of fusion does not necessarily imply diminished regenerative capacity in a putative reparative cell.[140,141]

CONCLUSION

We believe that cellular therapy holds considerable promise for patients with demyelinating disease, and are optimistic that this promise may begin to be realized within the relatively near future. We would caution against expecting reports of great benefit from trials restricted to recruiting patients with chronic disease who already have established stable (or progressive) disability, in whom one might predict a minimal response. Nevertheless, we look forward to the time when eligibility for cellular therapy trials will be less restrictive and, in the interim, continue to accrue the safety data and basic understanding of the mechanisms involved that will make this a reality.

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