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# Challenges Enrolling Patients with Acute Ischemic Stroke into Cell Therapy Trials

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Infusion of autologous bone marrow-derived mononuclear cells (MNCs) is a promising investigational therapeutic approach for patients with acute ischemic stroke. Preclinical models indicate that MNCs can reduce neurological deficits and enhance recovery. We recently concluded a phase I clinical trial to determine the safety and feasibility of these cells in patients with acute ischemic stroke. In this article, we discuss practical barriers and challenges encountered during the trial and provide lessons learned for the design and planning of future clinical trials testing novel cell therapies for acute ischemic stroke.

## Overview of Stem Cell Therapy for Stroke

Over the past decade, significant advances have been made in exploring the potential of cellular therapies to enhance recovery after stroke. There is a substantial body of evidence proving safety and efficacy of various types of cell therapies in animal stroke models [1]. Presently, a number of phase I and phase II clinical trials are underway to study cell therapies for a variety of neurological disorders, including stroke. We have recently completed a phase I clinical trial testing the intravenous administration of autologous bone marrow-derived mononuclear cells (MNCs) in patients with acute ischemic stroke. The cells were harvested from the patients' bone marrow within 24–72 h after symptom onset. We began with a 10-patient pilot study, and then expanded to enroll a total of 25 patients. The detailed methods and safety analysis of the first 10 patients is published elsewhere [2].

## Choice of Cell Type

Among the various cell therapies under investigation, bone marrow-derived MNCs have several advantages. They permit autologous applications, thus negating concerns for immune rejection. They can be separated from marrow within hours and do not require cell culture, which allows for cell administration in the acute setting of stroke. Several independent laboratories have shown that these cells enhance recovery in rodent models of ischemic stroke [3–6].

## Choice of Time Window

Increasing evidence from animal models suggests that the acute post stroke inflammatory period may be an important window to gain optimal effects from adult tissue-derived cell therapies. The literature suggests the first few days after the onset of stroke may be the ideal window for treatment with MNCs [5,6].

## Clinical Trial

We conducted a study to assess the feasibility of a bone marrow harvest followed by intravenous administration of autologous bone marrow-derived MNCs within 24–72 h after ischemic stroke. Other centers have tested MNCs in later time windows beyond the acute setting [7,8]. The phase I trial was registered with the US Food and Drug Administration, and was approved by the Institutional Review Board of the University of Texas, and the ethics committee at Memorial Hermann Hospital at Texas Medical Center. Bone marrow was successfully harvested from the patients and MNCs were infused within the subsequent 6 h. In the course of the study, we encountered a number of barriers that posed challenges to clinical trial enrollment using an autologous cell therapy approach. With the recent growth and interest in conducting clinical trials of cellular therapies for acute stroke, understanding what are the common issues that impact feasibility of trial enrollment is critically important. We therefore discuss practical experiences that may be useful to colleagues seeking to plan and implement similar clinical trials in acute stroke and other acute medical disorders.

## Practical Barriers to Enrollment

Using illustrative cases, we provide a descriptive account of the challenges faced in enrolling patients into a clinical trial testing autologous cells in acute stroke. We highlight unexpected clinical issues that prevented or hampered enrollment. We classify these issues into 5 broad categories and will present illustrative cases exhibiting practical scenarios that we encountered.

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### *Naturally expected variation in clinical course*

Patients after the onset of ischemic stroke often improve or deteriorate in the subsequent days during hospitalization. Factors that predict in-hospital neurological worsening are well described [9–12]. In particular, large hemisphere infarctions are prone to expansion, cerebral edema, midline shift, and sometimes herniation necessitating life-saving hemicraniectomy and surgical decompression [so-called malignant middle cerebral artery (MCA) infarction]. Hemicraniectomy has been proven to improve mortality and morbidity in patients with large infarctions and is performed electively before the patients show brainstem herniation [13]. In our study, it was not always predictable which patients would require hemicraniectomy by the time window for enrollment. Some patients with large infarcts fell out of the study after consent was obtained due to neurological deterioration and the need for surgical decompression. As the study progressed, we modified our inclusion criteria to include upper lesion size cutoffs that are associated with the development of malignant MCA syndromes. We also excluded patients already showing radiographic evidence of significant swelling or patients who were lethargic, indicative of raised intracranial pressure.

### *Tissue plasminogen activator*

As per American Heart Association (AHA) guidelines, patients with acute ischemic stroke are administered intravenous tissue plasminogen activator (IV t-PA/t-PA) as standard of care if they present to our hospital within 4.5 h of symptom onset [14]. IV t-PA leads to thrombolysis and reperfusion, and adds to the variation in clinical course of ischemic stroke patients in the acute setting [15]. A 55-year-old woman was consented for the trial after receiving IV t-PA, but she improved to such an extent that she fell below the range of clinical deficits to remain in the study and was deemed to be a screen failure. Conversely, thrombolysis can cause hemorrhage within the infarct and neurological deterioration. There is a 2%–8% risk for symptomatic brain hemorrhage associated with use of t-PA in stroke patients as reported in prospective clinical trials [16–18]. A 64-year-old woman initially treated with t-PA, consented for the clinical trial at 24 h after stroke. She subsequently developed intracerebral hemorrhage and deterioration to such an extent that she exceeded the study's clinical severity criteria and was then deemed a screen failure.

### *Therapeutic requirement for anticoagulation*

Anticoagulation with intravenous heparin and warfarin is given to some patients with acute ischemic stroke for secondary prevention [19]. The most common indications for anticoagulation are atrial fibrillation, cardiac thrombus, and extra cranial arterial dissection. Physicians differ in their preferences with regard to the time to start anticoagulation in the acute setting of ischemic stroke. These decisions are made based on the degree of embolic risk from the source, clinical severity, infarct size, and presence of hemorrhage within the infarct. However, patients on therapeutic doses of heparin or warfarin cannot undergo invasive procedures, such as a bone marrow harvest. Consequently, patients were excluded within the 24–72 h window if placed on anticoagulation during this time frame. For example, an 81-year-old woman met criteria for the clinical trial and consented to participate, but a transthoracic echocardiogram, done routinely to identify possible etiologies of stroke, showed a possible cardiac mass. A transesophageal echocardiogram did not confirm a mass, but did find a small thrombus in the left atrial appendage for which she had to be anticoagulated, and therefore was not deemed suitable for the bone marrow harvest procedure. However, there is no class I evidence from randomized trials that intravenous heparin is effective for prevention of stroke or reduces stroke progression. The choice to use heparin in the acute setting of stroke is therefore clinician-dependent, but the AHA recommends delaying heparin and Coumadin at least a few days in patients with moderate to large-sized strokes given the concern that anticoagulation could increase the risk for hemorrhagic transformation [20–22]. Therefore, for patients with larger strokes in whom anticoagulation was indicated and delayed several days while in the hospital, we were able to enroll eligible patients without disrupting clinical care. Antiplatelets are typically given to stroke patients in the acute setting, but they did not pose any barriers to enrollment, as the bone marrow can be safely harvested while on aspirin or clopidogrel or both. Similarly, the routine use of subcutaneous heparin or low-molecular weight heparin for prophylaxis against developing deep venous thrombosis was also not an exclusion to participate in the trial.

### *Tolerating conscious sedation*

During the acute setting of ischemic stroke, the brain has lost cerebral auto-regulation [23]; consequently, cerebral perfusion pressure is directly dependent on mean arterial pressure. Titration of blood pressure within narrow limits therefore is an important component of stroke management in the acute and subacute poststroke setting [24]. The bone marrow harvest in this study requires conscious sedation, which can lower blood pressure [25]. For each enrollment, we had a neurointensivist at the bedside during the harvest to directly manage blood pressure reductions. There were no definite severe adverse events related to the harvest. If consented patients initially had a diagnostic procedure in their stroke work-up, before the bone marrow harvest, such as a transesophageal echocardiogram requiring conscious sedation that caused hypotension (below 90 mmHg systolic), we elected to then exclude them from the study.

### *Patients undergo carotid revascularization during hospitalization*

Carotid stenosis is a common cause for ischemic stroke. When diagnostic work-ups reveal carotid stenosis as the cause of the patients' stroke, AHA guidelines recommend carotid revascularization by endarterectomy or angioplasty/stenting [20–22]. These procedures carry peri-procedural risks, including further embolic strokes, which confound safety analyses [26–29]. Patients were deemed screen failures if the clinical team intended to pursue elective carotid revascularization procedures.

## Lessons Learned

In completing a trial testing an autologous cell therapy from the bone marrow, we confronted a number of issues

that are relevant to the design and planning of subsequent cell therapy studies. There are predictable and unpredictable factors in the natural course of acute stroke that pose challenges to enrollment. Feasibility of enrollment and subsequent time projections for completion of clinical trials should be based on the following points and practical expectations.

(1) Some patients fluctuate in the acute setting of ischemic stroke and it is important to consider waiting on enrollment until the patients are neurologically stable. Larger infarcts progress during the time window of 1–3 days. In subsequent clinical trials testing cell therapies in the acute stroke setting, if there is concern for infarct progression or clinically significant cerebral edema that may lead to neurological worsening and subsequent hemicraniectomy, we favor delaying enrollment, which may, however, potentially lead to exclusion from trial participation given the 1–3-day time window. For an acute patient initially enrolled into a cell therapy trial who then shows deterioration in the next few days and requires hemicraniectomy, it may be difficult to determine if morbidity is solely associated with the surgical procedure or if the bone marrow harvest and/or cell infusion has some contribution. To reduce the chances of enrolling patients who may need elective hemicraniectomy, we believe that selection criteria for a clinical trial should require maximum lesion size cutoffs that predict deterioration. Infarct sizes can be calculated on magnetic resonance imaging, which is routinely obtained at hospitals with stroke centers. In addition, we also recommend excluding patients who show clinical evidence for significant swelling and raised intracranial pressure that also predict the development of malignant infarction. These patients tend to be lethargic and are not easily arousable. Another approach would be to monitor patients for infarct progression with repeated brain imaging before bone marrow harvest and cell infusion. However, the costs of repeated imaging will need to be taken into account as a study-related procedure.

(2) Patients who receive IV t-PA are at risk to develop significant hemorrhagic transformation and should be clinically monitored as well as imaged before enrollment. There are validated clinical prediction scores for t-PA-associated symptomatic intracerebral hemorrhage [30,31].

(3) Patients who require anticoagulation within the 1–3-day study time frame will not be candidates for an autologous bone marrow-derived cell therapy study. These limitations might also apply to other acute medical conditions, such as unstable angina treated with t-PA, anticoagulation, and GPIIb/IIIA inhibitors. However, larger strokes may necessitate delaying anticoagulation during the same time window, which consequently might permit enrollment. If, however, interventional procedures, such as carotid revascularization or stenting of intracranial vessels, which carry embolic risks, are electively planned during the first few weeks after stroke, we recommend caution enrolling such patients into cell therapy studies if there is concern for the cells to cause thrombosis. For example, there is an evolving literature suggesting that some types of mesenchymal stem cell therapies could predispose to thrombosis [32].

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