STATISTICAL ANALYSIS PLAN

IND 19204

HBTBI01

"A Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences Autologous Mesenchymal Stem Cell Therapy for the Treatment of Traumatic Brain Injury and Hypoxic-Ischemic Encephalopathy"

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"A Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences Autologous Mesenchymal Stem Cell Therapy for the Treatment of Traumatic Brain Injury and Hypoxic-Ischemic Encephalopathy"

8. STATISTICAL ANALYSIS PLAN

8.1 Sample Size and Power

A total of 24 patients will be enrolled in the study. We do not provide a power analysis for any outcomes since no formal hypothesis testing will be conducted. Instead, we will calculate estimates of treatment effect and 95% confidence intervals for all measures. We will also calculate probabilities of treatment benefit using Bayesian analyses and conservative neutral priors. Given that this is a Phase 1/2a study, there are no prior studies from which we could derive informative priors. Therefore our approach will be to use neutral priors meaning that a priori they will be centered at a null treatment effect. However, we will restrict the prior range (ie, 95% prior credible interval) to exclude large treatment effects that are almost never observed in human clinical studies.

8.2 Statistical Analyses

Volumetric, DT-MRI, neuropsychological, and functional outcome measures will be analyzed using general linear mixed models (GLMMs) to estimate differences across time points (pre- and post-treatment). We will examine all variables to determine that they are relatively symmetric and unimodal and examine residuals from each model to ensure reasonable adherence to model assumptions. The models will include time of assessment (pre- or post-treatment), and age (at study entry) as covariates and a random effect for subject to account for within subject correlation. Dependent variables will be the volume of whole brain gray matter, white matter, and CSF. Each dependent variable will be evaluated separately. Estimated differences at 6 months post treatment compared to pre-treatment will be reported along with 95% confidence intervals for all outcomes. We will also calculate probabilities of treatment benefit or clinically important effects.

8.2.1 Volumetric Analysis and Neurocognitive Outcomes

For volumetric analyses and neurocognitive outcomes, we will use the global test procedure recommended by Bagiella et al (Bagiella, 2010). This procedure allows analysis of multiple outcome measures in the assessment of intervention effects while controlling Type I error and maximizing power. To examine brain-behavior relations, we will first examine the distribution of variables to determine whether assumptions are met for Pearson or Spearman correlation approaches. To evaluate strength of brain-behavior relations, outcome scores will be correlated with specific macrostructural and microstructural metrics from the DTI. For global outcome, the GOS-EC score will be correlated with gray and white matter volumes and corpus callosum FA. For neurocognitive outcomes, the accompanying table shows the expected relations between outcomes and gray and white matter microstructural metrics. We expect positive correlation of dependent variables with FA and white and gray matter volume and negative correlation of dependent variables with MD.

Table 4:

Expected Relations of Neurocognitive Outcomes with Gray and White Matter Microstructure from Diffusion Tensor Imaging		
Neuropsychological Outcome	**Microstructure: Gray Matter MD**	**Microstructure: White Matter FA**
Processing Speed WAIS-IV Coding		Corpus callosum
Fine Motor 9-hole Pegboard Dexterity Test		Corticospinal
Verbal D-KEFS Verbal Fluency NIH Picture Vocabulary Test		Arcuate/superior longitudinal fasciculus
Working Memory NIH List Sorting Working Memory Test	Dorsolateral prefrontal cortex	
Declarative Memory Rey Auditory Verbal Learning Test	Lateral temporal cortex, hippocampus	
Attention NIH Flanker Inhibitory Control and Attention Test	Dorsolateral & ventrolateral prefrontal cortex	

8.2.2 Cytokine and Pro/Anti-Inflammatory Marker Analysis

For inflammatory cytokines, time course data will be analyzed using a GLMM to test differences across time between pre- and post-treatment. The dependent variables will be the neuroinflammatory biomarkers with time (to model the trajectory) and GOSE (3-4 or 5-8) as covariates and a random subject effect (to account for within subject correlation). Point estimates of pre- and post-treatment differences will be reported along with 95% confidence interval

8.2.3 PET Imaging Analysis

PET imaging analysis: Following completion of PET scanning, two main types of PET analyses will be completed:

8.2.3.1 Volume of Interest Analysis:

A hypothesis-driven VOI analysis where selected brain regions are examined will be conducted. Advantage of narrow search within regions involved in regulating the pain experience (e.g., anterior cingulate, insular cortex, nucleus accumbens, thalamus, amygdala, periaqueductal gray). Predefined VOIs obtained from data of previous studies are applied to image data and transferred to [^{11}C]ER-176 maps.

8.2.3.2 Statistical Parametric Mapping:

In these types of analyses, statistical parametric maps are obtained using whole brain image subtraction routines. Differences between volunteer groups (and covariances with phenotypic data) will be compared using the general linear model on a voxel by voxel basis within SPM8 and Matlab software with correction for multiple comparisons. Resulting Z-maps of statistical significance are mapped onto stereotactic space using volunteers' anatomically standardized T1-weighted MRIs. Only gray matter pixels and regions with specific binding will be included in the statistical parametric analyses (voxels with BPND values > 0.2). To compensate for small residual anatomic variation between subjects and to improve signal to noise ratio, a 3D Gaussian filter (FWHM 6 mm) will be applied to each scan. For

each subtraction analysis, one- or two-sample t-statistic values will be calculated for each voxel using a pooled smoothed variance across voxels. Areas of significant differences will be detected with a statistical threshold set to control for a Type-I error rate at $p = 0.05$ for multiple comparisons, estimated using the Euler characteristic based on the number of voxels in the grey matter and image smoothness. This threshold typically corresponds to $z=4.3$ to 4.6, lower for large size regions after cluster volume correction is applied. Plans for implementing corrections based on false discovery rate (FDR) are underway. The standard Euler characteristic method controls for Family-wise Type-I error, the chance of any false positives. FDR is the proportion of false positives among suprathreshold voxels. The FDR is a more lenient measure of false positives than Family-wise Error and hence more powerful, while offering a principled way to control false positives. After significant statistical effects are obtained in SPM, regional data is extracted into SPSS, plotted to assess for outliers, and used in additional analyses (i.e. correlations with dependent variables). Data is tested for normality (uniformly encountered) and if not obtained, equivalent non-parametric analyses will be used.