THE LANCET
Neurology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Hess DC, Wechsler LR, Clark WM, et al. Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol* 2017; published online March 17. http://dx.doi.org/10.1016/S1474-4422(17)30046-7.

Appendix

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria

1. Male or female patients between 18 and 83 years of age (*initially 18 and 79*)*, inclusive;
2. Clinical diagnosis of cortical cerebral ischemic stroke;
3. Occurrence of a moderate to moderately severe stroke with clear motor or speech deficit documented by National Institutes of Health Stroke Scale (NIHSS) score of 8 to 20 (inclusive) that did not change by 4 points or more from the screening to the baseline assessment.
4. Onset of stroke must have occurred 24 to 48 hours *(initially 24 to 36 hours)** prior to administering the investigational product. *
5. Confirmation of hemispheric cortical infarct with brain magnetic resonance imaging (MRI) including diffusion-weighted imaging demonstrating an acute lesion measuring >5 mL and <100 mL;
6. A Rankin score of 0 or 1, by either self-report or family report, prior to the onset of symptoms of the current stroke;
7. Patients who received either tissue plasminogen activator (tPA), and/or underwent mechanical reperfusion, according to the approved labels of tPA and the mechanical device, are eligible if they meet all other eligibility criteria; (*initially tPA OR underwent mechanical thrombectomy but not both*) *
8. Female patients who are either:
a. Not pregnant, not breastfeeding, and not planning on becoming pregnant during the study;
b. Not of childbearing potential
c. If of childbearing potential, must agree to use an effective method of avoiding pregnancy to the end of the trial.
9. Male patients with female partners of childbearing potential must agree to use adequate contraceptive methods if engaging in sexual intercourse;
10. Patients or legal representatives must freely sign the informed consent form
11. Willing and able to comply with all aspects of the treatment and testing schedule; and
12. Willing and able to return to the trial site for the post-treatment evaluations.

Exclusion Criteria

1. Presence of a lacunar or a brainstem infarct on MRI as the etiology of current stroke symptoms;
2. Reduced level of consciousness (score of >2 for item 1a of NIHSS);
3. Occurrence of a hemorrhagic transformation of ischemic stroke as evidenced by computerized tomography or brain MRI scan that is clinically significant in the opinion of the investigator;
4. Ipsilateral focal neurological deficits from prior lesions in the brain that would complicate evaluation;
5. History of arrhythmias or QTc prolongation that is clinically significant in the opinion of the investigator;
6. Experienced seizures since the onset of stroke;
7. Experienced a major neurological event such as stroke or clinically significant head trauma within 6 months of screening;
8. Uncontrolled hypertension, defined as persistent systolic blood pressure >220 mmHg or diastolic blood pressure >120120 mmHg, despite antihypertensive therapy;
9. Blood glucose level <50 mg/dL or >350 mg/dL (<2.755 mmol/L or > 19.284 mmol/L) at baseline;
10. Significant abnormal laboratory tests at Screening;
a. >2 × upper limit of normal (ULN) for alanine aminotransferase or aspartate aminotransferase;
b. >1.5 × ULN for total bilirubin;
c. >2 × ULN for serum creatinine; or
d. any other abnormal laboratory results at screening that are considered to be clinically significant in the opinion of the investigator;
11. Patients who have a significant comorbid medical condition(s), including, but not

limited to:
a. severe kidney disease requiring hemodialysis or peritoneal dialysis;
b. advanced liver disease such as hepatitis or liver cirrhosis;
c. severe congestive heart failure or history of ejection fraction <30%;
d. severe lung disease requiring home oxygen; or
e. active unstable angina requiring daily treatment with nitrates or other medications;
12. Known human immunodeficiency virus, ongoing systemic infection, severe local infection or who are immunocompromised;
13. Have Alzheimer's disease or other dementias, Parkinson's disease, or any other neurological disorder that would affect their ability to participate in the trial or confound study assessments;
14. Have a history of malignancy of any type, with the exception of adequately treated basal or squamous cell carcinoma of the skin;
15. Have a contraindication for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, body weight, or claustrophobia;
16. Have thrombocytopenia (platelet count <75,000/mm3) or heparin-induced thrombocytopenia;
17. Have a life expectancy less than 90 days;
18. Have a known allergy or religious objections to human tissue or bovine or porcine products;
19. Prior participation in any other trial involving investigational pharmacological agents or devices within 30 days prior to investigational product infusion or planned participation in investigational rehabilitation stroke recovery program;
20. Other serious medical or psychiatric illness that is not adequately controlled and, in the investigator's opinion, would not permit the patient to be managed according to the protocol;
21. Previous surgical removal of the spleen;
22. Major fluctuation in neurological status since the onset of stroke indicating progression or expansion of stroke, or possible transient ischemic attack
23. Plan to have a neurovascular procedure (eg, carotid endarterectomy, stent placement, etc.) within the first year following stroke.

*These inclusion criteria were modified by amendment during the course of the trial. The original criteria are shown in *italics*

Supplement Table 2

Treatment Effect of MultiStem on Immune Cell Subtypes in the Blood of Stroke Patients

Percentage of CD3+ Cells in the Blood	**ITT**	**Day 0**	**Day 2**	**Δ Change**	
MultiStem Treatment	n=38	18.53%	18.45%	-00.08%	**p = 0.001**
Placebo Treatment	n=35	18.84%	23.33%	+4.49%	
Percentage of FoxP3+ Cells in the Blood	**ITT**	**Day 0**	**Day 2**	**Δ Change**	
MultiStem Treatment	n=38	1.15%	1.19%	+0.04%	**p = 0.010**
Placebo Treatment	n=35	1.20%	1.47%	+0.27%	

Supplement

Table 3

Change in Blood Cytokines from Baseline to Day 7. The values presented are mean log of the ratio between the (time point value/baseline value). Any recorded values of zero are treated as missing.

[A: Please write out all the abbreviations below]	**Placebo (n)**	**MultiStem (n)**	**Δ**	**P-value**
Interleukin 1 beta (IL1beta)	1.04 (42)	0.86 (46)	-0.18	0.04
Tumor Necrosis Factor Alpha (TNF alpha)	1.14 (45)	0.90 (50)	-0.24	0.03
Interleukin 6 (IL6)	2.74 (45)	0.77 (48)	-1.98	0.01
Interleukin 12 (IL12)	0.92 (44)	0.84 (48)	-0.08	0.09
Interferon Gamma (IFNgamma)	2.89 (42)	1.08 (48)	-1.80	0.13
Interleukin 2 (IL2)	1.52 (44)	0.93 (47)	-0.58	0.22
Monocyte Chemoattractant Protein-1 (MCP1)	1.21 (45)	1.38 (49)	0.18	0.18

Supplement Table 4

Efficacy Outcomes for Early-Treatment (<36 hours) MultiStem Patients Compared to All Placebo Patients, Excluding All tPA+ET Patients

Outcome	Day 90			1 Year		
	MultiStem (n=27)	**Control (n=52)**		**MultiStem (n=27)**	**Control (n=52)**	
Primary						
Global Stroke Recovery (GSR)	OR:2.28 95% CI: 0.98 – 5.30		p=0.06	OR:1.84 95% CI: 0.81 – 4.20		p=0.15
Secondary						
mRS≤2 (in GSR), n (%)	13 (48.1)	16 (30.8)	p=0.14	13 (48.1)	20 (38.5)	p=0.45
NIHSSΔ75% (in GSR), n (%)	14 (51.9)	16 (30.8)	p=0.08	15 (55.6)	23 (44.2)	p=0.37
BI≥95 (in GSR, EO), n (%)	15 (55.6)	20 (38.5)	p=0.15	19 (70.4)	22 (42.3)	p=0.0168
mRS≤1 (in EO), n (%)	5 (18.5)	3 (5.8)	p=0.09	9 (33.3)	5 (9.6)	p=0.0102
NIHSS≤1 (in EO), n (%)	9 (33.3)	8 (15.4)	p=0.07	10 (37.0)	8 (15.4)	p=0.0309
Excellent Outcome (EO), n (%)	5 (18.5)	2 (3.8)	p=0.0344	8 (29.6)	3 (5.8)	p=0.0040
Excellent Neurological Outcome (NIHSS≤1 or ≥11 Point Improvement, n (%)	12 (44.4)	15 (28.8)	p=0.17			
mRS Shift			p=0.03			p=0.03
Safety						
LTAE or Death, n (%)	n/a			3 (11.1)	14 (26.9)	p=0.11
Secondary infections, n (%)				4 (14.8)	28 (53.8)	p=0.0010
Initial hospitalization days, mean	6.7 (2.73)	10.1	P=0.0094	n/a		

Note: each endpoint was tested independently; no adjustments made for multiplicity.
LTAE = Life threatening adverse events

Supplement Table 5

Efficacy Outcomes for Early-treatment (<36 hours) MultiStem and Placebo Patients

Outcome	Day 90			1 Year		
	MultiStem (n=31)	**Control (n=19)**		**MultiStem (n=31)**	**Control (n=19)**	
Primary						
Global Stroke Recovery (GRS)	Odds ratio: 2.07 CI: 0.70-6.10		p=0.19	Odds ratio: 1.14 CI: 0.38-3.43		p=0.81
Secondary						
mRS≤2 (in GRS), %	45.2	26.3	p=0.16	48.4	52.6	p>0.99
NIHSSΔ75% (in GRS), %	48.4	31.6	P=0.23	51.6	52.6	p>0.99
BI≥95 (in GRS, EO), %	58.1	42.1	p=0.27	67.7	52.6	p=0.37
mRS≤1 (in EO), %	16.1	5.3	p=0.39	32.3	10.5	p=0.10
NIHSS≤1 (in EO), %	32.3	26.3	p=0.76	35.5	21.1	p=0.35
Excellent Outcome (EO), %	16.1	0.0	p=0.02	29.0	0.0	p=0.01
mRS Shift	p=0.09			p=0.27		
Safety						
LTAE or Death, %	9.7	26.3	p=0.15	n/a		
Secondary infections, %	16.1	31.6	p=0.22			
Initial hospitalization days	6.8	11.4	p=0.63			