Cell Therapy for Stroke

Lawrence R. Wechsler, M.D.
Henry B. Higman Professor of Neurology
Chairman, Department of Neurology
University of Pittsburgh

STROKE UPDATE 2018
Disclosures

- Steering committee:
  - SanBio trials
  - MASTERS trials
Progress in Treatment of Stroke

Prevention
1980-90s

Acute Therapy
1995-2000s

Recovery
2000-
Cell-based therapies
- Growth factors
- Small molecules
- Physiotherapy
- Robotics
- Electromagnetic stimulation
- Motor imagery, cognitive Rx
- Brain-computer interface
Cell Therapy and Medical Tourism

Tony Dorsett turns to controversial stem cells thanks to old friend

“When I was taking the stem cells, I was able to figure things out a little better and not get as frustrated,” Dorsett told USA - TODAY Sports. Likewise, Sherrill says the treatment dramatically helped his ailing knees and shoulder.

Bart Starr walking again after stem cell treatment

In July, Cherry Starr said her husband could walk and feed himself and that his cognition had improved dramatically.
Cell Types

- Neural stem cells
  - Embryonic, fetal, adult
- Immortalized tumor cells
- Mesenchymal stem cells (MSC)
  - Autologous, allogeneic
- Induced pluripotent stem cells (IPSC)
What we have learned about cell therapy for brain recovery: Preclinical

- Cells migrate to site of injury
- Cells survive at transplant site
- Differentiate at site of injury
- Integrate with local environment
- Improve function in animal models
- Multiple potential cell types effective
- Mechanism, route of delivery, timing vary with cell type
How: Route of Administration

- Intravenous
  - Most convenient and applicable
  - Filtered in the lung
  - Distribution to other organs

- Intra-arterial
  - Bypass lungs
  - Blood brain barrier
  - Small vessel occlusion

- Intrathecal
  - Must cross BBB
  - Limited penetration to cortex

- Intraparenchymal
  - Reliable delivery of cells
  - Stereotactic technique
  - Complications
When: Timing of Brain Repair for Stroke

Hess et al. Cell Proliferation 2010
Where: Stroke Location

- **IC** – Basal ganglia
  - Small volume stroke with substantial motor deficit
  - Ease of stereotactic targeting
  - Minimal volume injected for cell delivery
  - Avoids cerebral cortex and potential complications

- **IA** – MCA
- **IV** – Systemic spleen
<table>
<thead>
<tr>
<th>Study</th>
<th>Pts</th>
<th>Window</th>
<th>Cell Type</th>
<th>Route</th>
<th>Location</th>
<th>Safety</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SanBio</td>
<td>18</td>
<td>6-60 mo</td>
<td>Allogeneic MSC</td>
<td>IC</td>
<td>Subcortical</td>
<td>No cell related</td>
<td>Trend to improvement</td>
</tr>
<tr>
<td>Athersys</td>
<td>126</td>
<td>24-48 hrs</td>
<td>Allogeneic MSC</td>
<td>IV</td>
<td>Cortical</td>
<td>No cell related</td>
<td>Improve v. cntrl &lt; 36 hrs</td>
</tr>
<tr>
<td>PISCES</td>
<td>12</td>
<td>6-60 mo</td>
<td>NSC</td>
<td>IC</td>
<td>Subcortical</td>
<td>No cell related</td>
<td>Improved NIHSS 2 yrs</td>
</tr>
<tr>
<td>PISCES2</td>
<td>41</td>
<td>28-56 d</td>
<td>NSC</td>
<td>IC</td>
<td>Subcortical</td>
<td>No cell related</td>
<td>15/21 clinically relevant improved</td>
</tr>
<tr>
<td>Aldagen</td>
<td>10</td>
<td>13-19 d</td>
<td>Autologous MSC</td>
<td>IA</td>
<td>Cortical</td>
<td>No cell related</td>
<td>NA</td>
</tr>
<tr>
<td>Aldagen – RECOVER</td>
<td>29</td>
<td>13-19 d</td>
<td>Autologous MSC</td>
<td>IA</td>
<td>Cortical</td>
<td>No cell related</td>
<td>No change v. sham</td>
</tr>
<tr>
<td>InveST</td>
<td>120</td>
<td>7-29 d</td>
<td>Autologous MSC</td>
<td>IV</td>
<td>Cortical</td>
<td>No cell related</td>
<td>No diff v. controls</td>
</tr>
<tr>
<td>Honmou</td>
<td>12</td>
<td>24-72 hrs</td>
<td>Autologous MSC</td>
<td>IV</td>
<td>Cortical</td>
<td>No cell related</td>
<td>Increased rate of change NIHSS</td>
</tr>
<tr>
<td>Bhasin</td>
<td>6</td>
<td>3-12 mo</td>
<td>Autologous MSC</td>
<td>IV</td>
<td>Cortical</td>
<td>No cell related</td>
<td>No difference in outcome, fMRI</td>
</tr>
<tr>
<td>Savitz</td>
<td>10</td>
<td>24-72 hrs</td>
<td>Autologous MNC</td>
<td>IV</td>
<td>Cortical</td>
<td>No cell related</td>
<td>No worse than historical controls</td>
</tr>
<tr>
<td>Moniche</td>
<td>10</td>
<td>5-9 days</td>
<td>Autologous MNC</td>
<td>IA</td>
<td>Cortical</td>
<td>No cell related</td>
<td>No neurological diff at 6 mo</td>
</tr>
</tbody>
</table>
Intracerebral Delivery
SanBio SB 623 MSCs

- Cryopreserved SB623 Cells
- Expression Vector
- Bone Marrow Aspirate
- MASC Cells
- Transfection and Selection
- Expansion
- SanBio SB 623 MSCs
- NICD
- 7879 bp
- Plasmid
Trophic Factors in Conditioned Medium

- Custom antibody array (RayBiotech)
- 30 cytokines

BDNF  HGF
BMP-4  IGF-I
BMP-6  IL-1α
BMP-7  IL-6
b-NGF  IL-8
CNTF  LIF
**DKK-1**  MCP-1
**DKK-4**  MMP-1
EGF  NT-3
Erythropoietin R  PDGF-AA
FGF-2  SDF-1
**FGF-7**  TGFα
GCSF  TGFβ
GDNF  TNFα
**HB-EGF**  VEGF

Blue indicates factors from MASC, SB623 cells. Underlined factors preferentially secreted by SB623 cells
SanBio SB623 Safety Trial

- **Phase I/IIA** open label safety trial
- **Number of patients** – 18
- **Sites** - 2 (Stanford/Pittsburgh)
- **Cell type** – modified allogeneic MSC
- **Timing** – chronic stroke 6-60 mo
- **Administration** – intracerebral
- **Dose** – 2.5, 5, 10 million cells
- **Location** – subcortical
- **Followup** – 24 mo.
- **Safety endpoints** – Adverse events
- **Efficacy endpoints** – ESS 6 mo (primary), NIHSS, mRS, F-M
- **Other** – PET, Cognitive tests

Steinberg et al. Stroke 2016
SanBio: Key inclusion/exclusion criteria

- **Inclusion**
  - 18-75 years old (33-75 yo tx)
  - Ischemic stroke in subcortical region of MCA or lenticulostriates with or without cortical involvement
  - 6-60 mos post-stroke (7-36 mos); stable for > 3 weeks prior
  - Modified Rankin Score 3 or 4
  - NIHSS Score >7

- **Exclusion**
  - Cerebral infarct size >100 cm³ (on MRI)
  - Presence of serum antibodies to donor SB623 cells (HLA Class I or II)
## SB623 Safety: Serious Adverse Events

<table>
<thead>
<tr>
<th>Cell Dose/Injection</th>
<th>Serious Adverse Event</th>
<th>Relationship to Treatment</th>
<th>Relationship to Procedure</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5x10^6</td>
<td>Seizure (70 d)</td>
<td>Unrelated</td>
<td>Probably</td>
<td>Recovered/resolved</td>
</tr>
<tr>
<td></td>
<td>Stenting of asymptomatic carotid artery stenosis</td>
<td>Unrelated</td>
<td>Unrelated</td>
<td>Recovered/resolved</td>
</tr>
<tr>
<td>5x10^6</td>
<td>Asymptomatic subdural hematoma/hygroma</td>
<td>Unrelated</td>
<td>Definitely</td>
<td>Recovered/resolved</td>
</tr>
<tr>
<td>5x10^6</td>
<td>Transient ischemic attack (16 mo)</td>
<td>Unrelated</td>
<td>Unrelated</td>
<td>Recovered/resolved</td>
</tr>
<tr>
<td>10x10^6</td>
<td>Urinary tract infection/sepsis</td>
<td>Unrelated</td>
<td>Unrelated</td>
<td>Recovered/resolved</td>
</tr>
<tr>
<td>10x10^6</td>
<td>Pneumonia</td>
<td>Unlikely</td>
<td>Possibly</td>
<td>Recovered/resolved</td>
</tr>
</tbody>
</table>
SB623: Improvement in Efficacy Measures From Baseline after 12 Months

ESS

F-M Total Score

NIHSS

F-M Motor Function Total Score

P values represent significance of change versus baseline using the Wilcoxon Signed Rank test (p<0.05).
FLAIR signal may correlate with clinical efficacy (*post-hoc analysis)

- 13/18 patients new T2 FLAIR; DWI negative
- Significant Pearson correlations:
  - ESS total score: 0.818, P<0.001
  - NIHSS total score: −0.688, P<0.01
  - F-M total score: 0.708, P<0.01
  - F-M motor function total score: 0.668, P<0.01
- No correlation with dose
SanBio SB623: Conclusions

- Intraparenchymal transplantation of human modified bone marrow derived stromal cells in chronic stroke patients is safe and feasible
- Clinical improvement observed in multiple functional scales but significance in uncontrolled trial uncertain
- No adverse events related to cells
- Analysis of PET and cognitive testing in progress
- Further phase 2B/3 study planned
ACTIsSIMA Phase 2b Trial – San Bio

- Double blind controlled Phase 2b study of IC SB623 cells in patients with chronic motor deficits from stroke
- 156 patients 18-75 yrs with ischemic stroke 6-90 mo post event.
- mRS 2-4, Motricity Index 30-75 UE, 27-74 LE
- Primary efficacy: FMMS improvement > 10 pts at 6 mo.
- Others: mRS > 1 pt, ARAT > 6pts, Gait velocity, NeuroQol, GRPC (clin meaningful change)
MultiStem: An Adult Adherent Cell Population Distinctive from MSC

Gene Expression Profile

Cell Expansion Profile

Human Cells Isolated From Same Donor

Doublings

Days

MSC / like

MAPC / MultiStem

ESC

MAPC / MultiStem

MSC / like

Violet - MSC
Green - Mesoangioblasts
Red - MAPC (Verfaillie lab)
Blue - MultiStem
Orange - ESC

MultiStem

MSC
Multistem: Mechanisms

**Shifting the Balance in Repair Processes**

- **Immunomodulation**
- **Inflammation Reduction**
- **Neuroprotection, Cytoprotection**
- **Angio- / Vasculogenesis**
- **Cellular Regeneration / Replacement**

*MultiStem® expresses a combination of therapeutic proteins & factors to enhance healing and tissue repair in multiple ways.*
Multistem Prevents Splenic Atrophy after Stroke

Sahota et al. Int J Str 2012
Athersys – MASTERS Trial

- **Design** – Phase II double blind RCT
- **Number of patients** – 126 (65 M, 61 P)
- **Sites** - 33
- **Cell type** – MAPC
- **Administration** – intravenous
- **Timing** – subacute 24-48 hrs
- **Dose** – 1.2 billion
- **Location** – cortical
- **Concomitant therapy** – none
- **Followup** – 12 mo
- **Safety endpoint** – DLT at 7d
- **Efficacy endpoint** – Global recovery (mRS≤2, BI≥95, NIHSS Δ 75%) 90d
- **Other** – MRI, inflammatory biomarkers

Hess et al. Lancet Neurol 2017
Protocol Modifications Implemented to Overcome Early Enrollment Challenges

**Key Eligibility Criteria – Original Design**
- Cortical stroke
- NIHSS 8-20 at baseline (≥24h), stable deficit
- Administration within 24-36h
- tPA or device patients eligible if other criteria met

**Key Changes to Accelerate Enrollment**
- Administration window extended to 48 hours
  - Earlier treatment better
  - But, local cell processing limitations (e.g., open hours) constrained enrollment

- Included patients receiving both tPA-MR
  - Background rates / expectations for this group not well known
  - But, several of our sites treating with tPA + mechanical reperfusion (MR), and such patients seemed to meet criteria
# MASTERS: Baseline Demographics

<table>
<thead>
<tr>
<th>Patient data</th>
<th>MultiStem (n=65)</th>
<th>Placebo (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: mean, range</td>
<td>61.8</td>
<td>62.6</td>
</tr>
<tr>
<td></td>
<td>41-83</td>
<td>37-80</td>
</tr>
<tr>
<td>Sex: % male</td>
<td>53.8%</td>
<td>54.1%</td>
</tr>
<tr>
<td>NIHSS: mean, median</td>
<td>13.4</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>MRI DWI Lesion size in cc³: mean, median</td>
<td>51.6</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>42.3</td>
<td>41.1</td>
</tr>
<tr>
<td>% Administered:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv tPA</td>
<td>44.6%</td>
<td>47.5%</td>
</tr>
<tr>
<td>iv tPA+device</td>
<td>12.3%</td>
<td>14.8%</td>
</tr>
</tbody>
</table>

Hess et al. Lancet Neurol 2017
# MASTERS: Summary of Safety and Efficacy

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global recovery</td>
<td>2.72 (0.76-9.78)</td>
</tr>
<tr>
<td>mRS ≤2</td>
<td>2.28 (0.68-7.61)</td>
</tr>
<tr>
<td>NIHSS ≥75% improvement</td>
<td>1.57 (0.51-4.86)</td>
</tr>
<tr>
<td>BI ≥95%</td>
<td>1.76 (0.59-5.32)</td>
</tr>
<tr>
<td>Infection</td>
<td>0.74 (0.23-2.37)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0.58 (0.17-2.04)</td>
</tr>
<tr>
<td>Respiratory AEs</td>
<td>0.52 (0.16-1.66)</td>
</tr>
<tr>
<td>Mortality</td>
<td>0.34 (0.05-2.24)</td>
</tr>
</tbody>
</table>
MASTERS Trial: Improvement in Excellent Outcome Following Treatment with MultiStem Therapy

Excellent Outcome = mRS ≤1, NIHSS ≤1, and BI ≥95

Note: Early-treated means <36 hour administration, representing 31 MultiStem subjects
Impact on Inflammatory Cytokines

### Intent-to-Treat Population

**Difference in Fold Change at Day 7**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Day 7, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.01</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.03</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.03</td>
</tr>
<tr>
<td>IL-12</td>
<td>0.12</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.03</td>
</tr>
<tr>
<td>IL-2</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**Aggregate Fold Change from Baseline Through Day 30**

- MultiStem <36hrs
- Placebo
- Multistem

* p < 0.01

Note: Evaluating available data from ITT population; controlling for differences in baseline values, and missing values

Hess et al. Lancet Neurol 2017
### MASTERS: Impact on Healthcare Measures:
Early MultiStem Treatment (< 36 hrs)

<table>
<thead>
<tr>
<th>Category</th>
<th>MultiStem (n=31)</th>
<th>Placebo (n=61)</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial hospitalization days, mean</td>
<td>6.8***</td>
<td>9.8</td>
<td>-3.0 days</td>
</tr>
<tr>
<td>ICU days, mean</td>
<td>3.0</td>
<td>4.3</td>
<td>-1.3 days</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>16.1%**</td>
<td>47.5%</td>
<td>-31.4%</td>
</tr>
</tbody>
</table>

At day 30,

- % subjects in Inpatient Rehabilitation: 51.6% vs. 61.4% (9.8% difference)
- % subjects in Outpatient Rehabilitation: 38.7% vs. 28.1% (10.6% increase)
- Rehabilitation hours per week, median: 10.0 vs. 11.3 (1.3 hours difference)

At 90-day interim

- % subjects with Hospital Readmission: 19.4% vs. 24.6% (5.2% difference)

* 31 MultiStem patients (≤36 hours administration) v. 61 placebo patients  

**p-value ≤ 0.02

Wechsler et al. ISC 2016, Los Angeles, CA
Reneuron
CTX Clonal Human Neural Stem Cells

- Conditionally immortalized fetal neural stem cells immortalized with retroviral c-mycER
- Multipotent neural stem cell line
- Increases angiogenesis, neurogenesis, immune modulation
Reneuron – PISCES II

- **Design** – Phase II open label
- **Number of patients** – 21
- **Sites** – 8 in UK
- **Cell type** – modified NSC
- **Administration** – intracerebral
- **Timing** – subacute/chronic 2-13 mo
- **Dose** – 20 million
- **Location** – subcortical
- **Concomitant therapy** – physiotherapy
- **Followup** – 12 mo
- **Safety endpoint** – Adverse events
- **Efficacy endpoint** – 2 pt improvement in ARAT grasping and lifting test at 3 mo
PISCES II

- 21 pts 2-13 mo post stroke
- No adverse events related to cells
- Did not reach primary endpoint of 2 pts improving 2 points at 3 mo
- 2 achieved at 6 mo, 3 at 12 mo.
- 15/21 pts responded to one or more of 4 efficacy measures
- Phase III study planned

Source: ReNeuron website accessed May 2017
Upcoming Cell Therapy Trials – MASTERS 2

- Double blind randomized placebo controlled phase 3 trial of subacute stroke
- 300 patients randomized 1:1 Multistem v. placebo
- Ischemic stroke involving cerebral cortex
- Intravenous infusion of 1.2 billion cells
- 18-36 hrs post stroke
- NIHSS 8-20
- IV tPA and MT included
- Primary outcome Rankin distribution at 90 days; Followup to 1 year
Upcoming Cell Therapy Trials – PISCES III

- Randomized placebo controlled Phase IIb trial for chronic stroke
- 110 subjects, 1:1 cells v. placebo
- Age 35-75
- Stereotactic implantation of CTX0E03 cells
- Sham surgery and partial burr hole in placebo group
- Hub (surgery) and spoke (followup) sites to maintain blind
- 6-12 mo post stroke
- mRS 3-4, some residual upper limb movement
- Primary outcome mRS at 6 mo – improvement of 1 or more
- Standardized physical therapy for 12 weeks post entry
Cell Replacement Strategies

Dihne et al. Stroke 2011
Summary

- Few therapies available for stroke recovery
- Cell therapy is a viable approach and shows promising results in animal models
- Paracrine functions more likely than cell replacement
- Multiple potential mechanisms including immune modulation
- Phase I and II clinical trials show adequate safety
- Early efficacy results promising