# Mesenchymal Stem Cells: Clinical Application and outlook The CWRU experience

May 8, 2013

Stanton L. Gerson, MD

Asa & Patricia Shiverick and Jane Shiverick (Tripp)
Professor of Hematological Oncology
Director, Case Comprehensive Cancer Center
Director, Seidman Cancer Center

Director, National Center for Regenerative Medicine

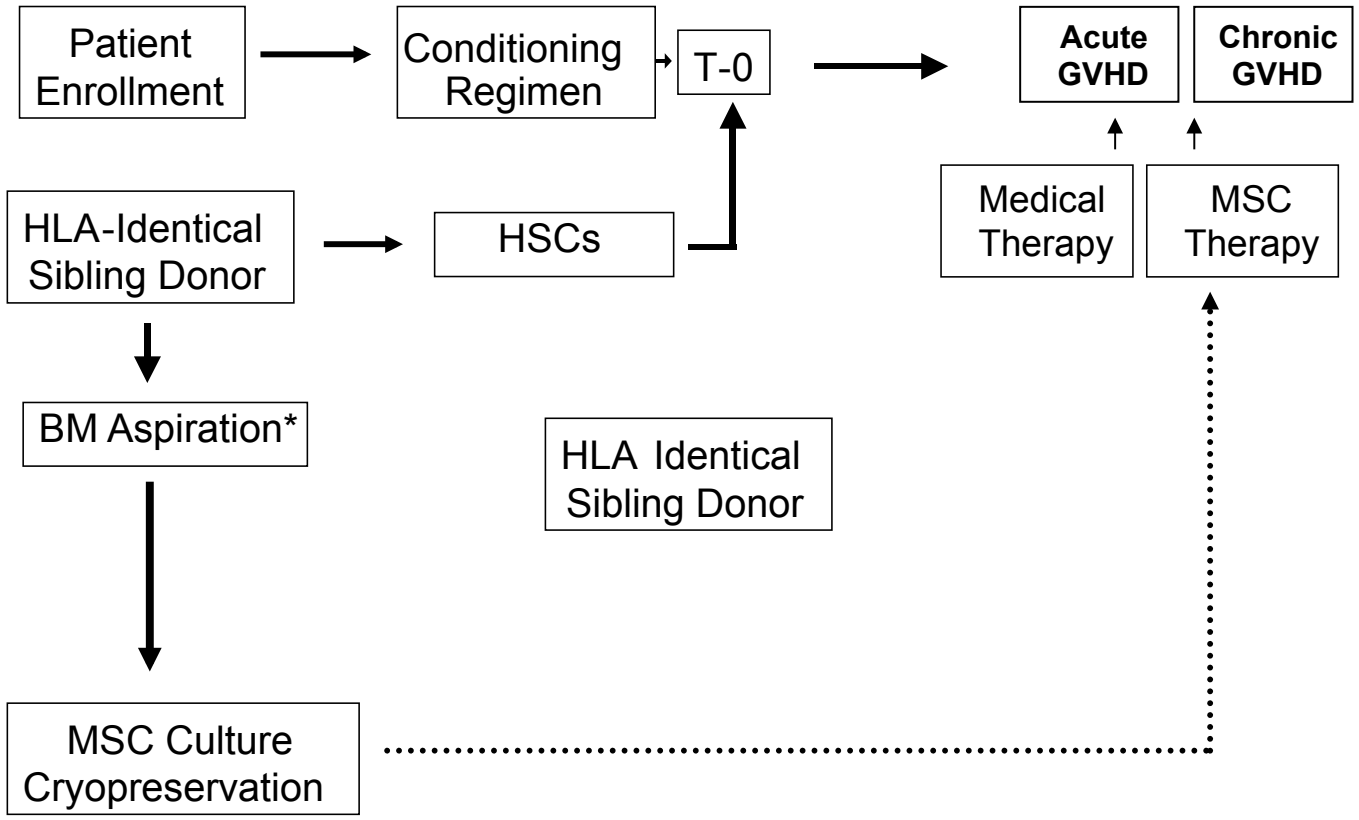
# Phase I trials with MSCs
# 1992-2013

- Phase 1 infusion after autologous hematopoietic stem cell transplantation
- Phase 1 infusion during autologous stem cell transplantation for advanced breast cancer
- Phase 1 infusion after allotransplantation for congenital disorders
- Phase 1 infusion with allograft for AML and NHL

Bone Marrow Transplantation 16(4):557-64, 10/1995. , J Hematother 6(5):447-55, 10/1997;
Exp Hematol 27(11):1675-81 11/1999 ; Bone Marrow Transpl 30(4):215-222 8/2002 ;
Biol Blood Marrow Transplant 11(5):389-98, May 2005.

# Phase I trial for steroid refractory GVHD

CWRU 3Y03




*Occurs before HSC collection in acute and chronic GVHD and after HSC collection in chronic GVHD only

# Case 3Y03: Matched related donor MSC culture expansion characteristics

| **Culture medium** | Standard media | FGF supplemented |
|---|---|---|
| **# cultures** | 18 | 6 |
| **age of donors** | 52 (38 - 67) | 49 (39-58) |
| **volume of marrow aspirate (median)** | 29 ml | 37 ml |
| **Infusion cell dose/kg** | 0.5 - 2.4 x $10^6$ | 1.7 - 2.4 x $10^6$ |
| **MSCs at harvest** | 161.0 ± 0.5 x $10^6$ | 158 ± 0.5 x $10^6$ |
| **days to harvest*** | 41 (23 - 66) | 24.5 (20-41) |

# MSC release criteria

- >95% Viability
- Negative bacterial/fungal cultures
- >90% positive for MSC surface markers CD105, CD73, CD90
- <5% positive for hematopoietic surface markers CD14 and CD45
- No detectable mycoplasma or endotoxin

# Lab Correlates

- Immune Studies (MLR and Elispot)
- Serum Cytokine Measurement (ELISA)
  IL-2, IFγ, IL-10, IL-4, TNF-α, IL-1
- MSC Chimerism in patients with skin GVHD (FISH)
- Circulating MSCs (15 min, and 1,2,3 days post infusion)

# Factors influencing MSC expansion capacity: univariate analysis

| Factor | coefficient | Odds ratio | p-value |
|---|---|---|---|
| Age (per year increase) | -0.077 | 0.926 | 0.227 |
| Number of cells at P1 (per $10^6$ increase) | -0.015 | 0.985 | 0.761 |
| Marrow volume (per unit increase) | 0.0265 | 1.027 | 0.646 |
| Days to first passage (per day increase) | -0.333 | 0.717 | 0.098 |
| Number of MNCs in harvest (per $10^6$ increase) | -0.00073 | 0.999 | 0.875 |
| Donor's sex (Male vs. Female) | -0.406 | 0.667 | 0.697 |

# Evaluation of an FBS replacement for culture expansion of human MSCs

| DMEM LG+ 10% FBS | Xenofree media |
|---|---|
| Isolate MNCs on Percoll gradient (1.073 g/ml) | Isolate MNCs on Ficoll gradient (1.078g/ml) |
| No surface coating of culture vessel | Collagen/fibronectin coating of surface vessel |
| Primary plating density $1.7x10^5$ MNCs/$cm^2$ | Primary plating density $5x10^4$ MNCs/$cm^2$ |
| Passage cells weekly at 95% confluence | Passage cells every 3-4 days at 70% confluence |
| Cell detachment agent: porcine trypsin | Cell detachment agent: accutase |
| Harvest cells at Passage 4 to characterize and assess purity | Harvest cells at Passage 4 to characterize and assess purity |

# Xenofree vs FBS: Culture Endpoints at Passage 3

| | Xenofree | FBS |
|---|---|---|
| Immunophenotyping | CD105/CD73<br>99.07% n=4 | CD105/CD73<br>98.49% n=4 |
| Cell yield per 10cc starting marrow | 297 x $10^8$ MSCs | 2.85 x $10^8$ MSCs |

Conclusion:

- Preliminary studies show that MSC culture in xenofree medium results in a **100x greater yield** per volume bone marrow compared to standard FBS containing medium.
- This results in less passages and less media to reach target dose and thus a shorter culture period

# UTILIZING MSC TRANSPLANTATION TO TREAT MS: UPDATE ON AN ONGOING PHASE I TRIAL

Workshop on Transplant and Cellular Therapy
for Autoimmune Diseases – CIBMTR 2013

Jeffrey A. Cohen, MD
Mellen Center for MS Treatment and Research
Neurological Institute
Cleveland Clinic

Cleveland Clinic

University Hospitals

Case Western Reserve University

National Center for Regenerative Medicine

# MSC Treatment of Multiple Sclerosis

| Reference | Indication | Patients | MSC Source |
|---|---|---|---|
| Connick 2012 | SPMS | 10 | Autologous culture-expanded BM MSCs administered IV |
| Karussis 2010 | RR, SP, PP MS | 15 | Autologous culture-expanded BM MSCs administered IV and IT |
| Liang 2009 | PP MS | 1 | Allogeneic umbilical cord MSCs administered IV and IT after CTX |
| Mohyeddin Bonad 2007 | Treatment-refractory MS | 10 | Autologous culture-expanded BM MSCs administered IT |
| Rice 2010 | Chronic MS | 6 | Fresh BM cells enriched for MSCs |
| Riordan 2009 | Treatment-refractory MS | 3 | Autologous non-expanded adipose MSCs |
| Yamout 2010 | SPMS | 10 | Autologous culture-expanded BM MSCs administered IT |

# Safety Considerations with MSC Transplantation

- Infusion-related adverse effects
  - Embolic phenomena
  - Immunogenicity including anti-FBS Ab
  - Bradycardia, MI, encephalopathy, stroke from DMSO
- Infection
  - Contamination
  - Immunosuppression
- Neoplasia
- Ectopic tissue formation
- MS related: allergic phenomena, autoimmunity, disease activation

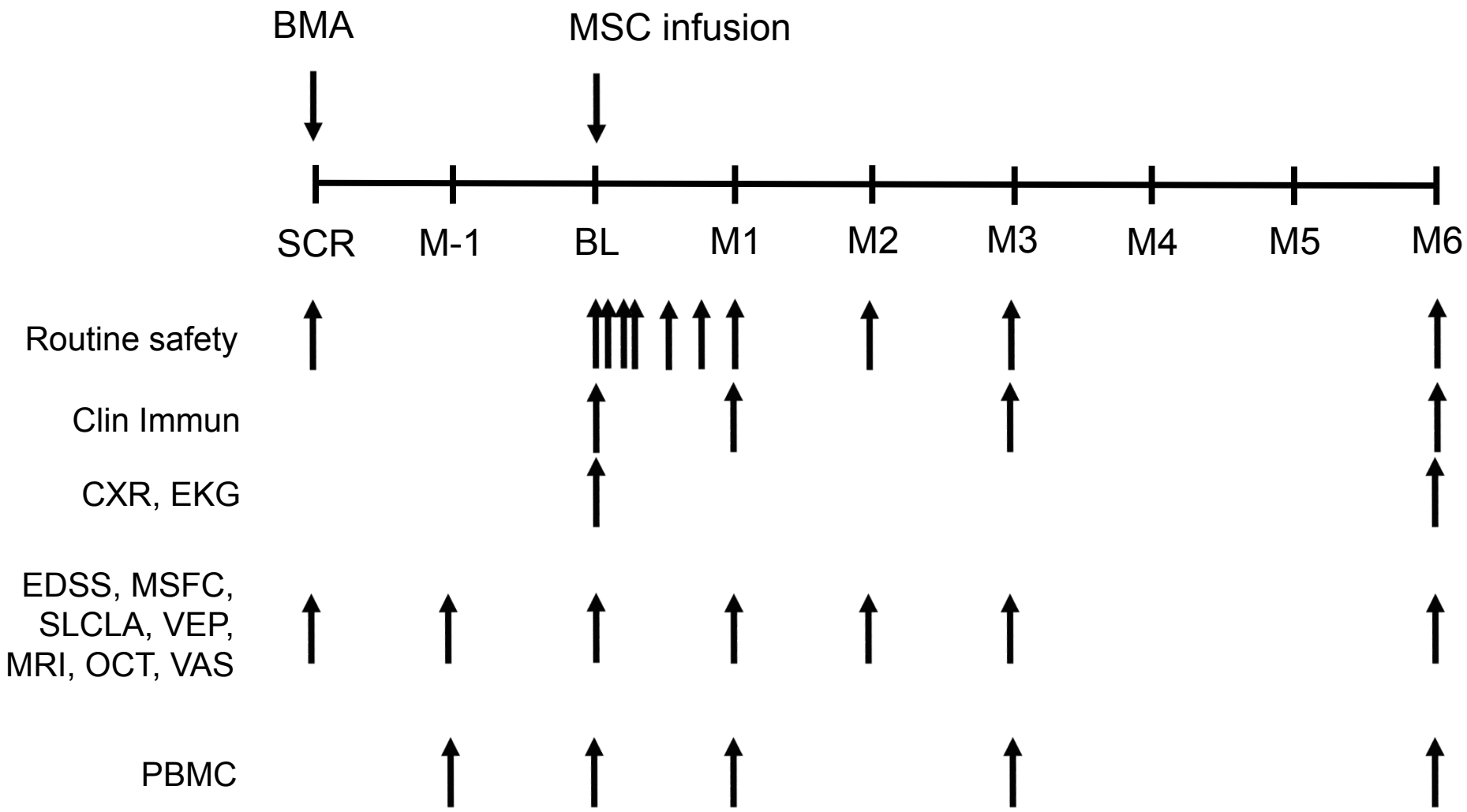
# Phase 1 Trial of Autologous MSC Transplantation in MS

| | |
|---|---|
| IND | BB-13917 |
| Clinicaltrials.gov | NCT00813969 |
| Study Population | 24 participants<br>RR or SP/PR MS (~12 each)<br>EDSS 3.0-6.5<br>Active disease in prior 24 months<br>Afferent visual system involvement |
| Treatment | Single IV infusion of 1-2 x$10^6$/kg autologous culture-expanded bone-marrow-derived MSCs |
| Follow-up | 2 months pre- and 6 months post-treatment |
| Correlates | immunoreactivity studies of MSC |

# Phase 1 Trial of Autologous MSC Transplantation in MS

| | |
|---|---|
| Primary outcome | Feasibility, and infusion-related safety and tolerability over 1 month |
| Secondary outcomes | Safety and tolerability over 6 months |
| | GdE MRI lesions at Month 1 |
| Exploratory outcomes | Pre- vs. post transplant: |
| | Self-reported overall status |
| | Relapses, EDSS, MSFC |
| | MRI: GdE, N/E T2, T2-vol, T1-vol, whole brain and GM atrophy, DTI, MTI |
| | Visual pathways: SLCLA, VEP, OCT |
| | Immunologic mechanistic studies |

# MS-MSC-001: Study Summary



# Study Status

- Enrollment and Treatment
  - 24 of planned 24 participants enrolled
  - 21 participants infused
  - 1 culture failure; participant was replaced
  - All the other participants received the target dose of cells

Updated: 29 MAR 2013

# Study Status

- Follow-up
  - 15 participants have completed the study
  - All planned assessments have been performed
- Safety
  - No treatment-related severe (CTCAE grade 3+) or serious adverse events
  - No evidence of paradoxical disease activation
- Efficacy
  - Exploratory efficacy analyses are in progress

Updated: 05 APR 2013

# Culture Kinetics and Yield

| | Percoll Yield ($x10^6$) | Final Yield ($x10^6$) | Culture Duration (days) | Dose ($x10^6$/kg) |
|---|---|---|---|---|
| N | 21 | 21 | 21 | 21 |
| Mean ± SD | 481.5 ± 268.3 | 275.9 ± 129.1 | 29.6 ± 11.8 | 1.9 ± 0.2 |
| Minimum | 151.8 | 110.0 | 16 | 1.3 |
| Maximum | 1127.0 | 586.0 | 62 | 2.0 |

- 1 culture terminated at 28 days
- All other cultures were successful and cell products fulfilled the stringent release criteria
- Variable BMA yield of nucleated cells, MSC growth rate, and final yield
- No obvious correlation with demographics, other medical diagnoses, MS clinical features, or medications

Updated 25 MAR 2013

# Evaluation of an FBS Replacement for Clinical Grade Culture-Expansion of Human MSCs

| Current Method (DMEM LG + 10% FBS) | Mosaic Media-Becton Dickenson |
|---|---|
| Isolate MNCs on Percoll gradient (1.073 g/ml) | Isolate MNCs on Ficoll gradient (1.078g/ml) |
| No surface coating of culture vessel | Collagen/fibronectin coating of surface vessel |
| Primary plating density $1.7x10^5$ MNCs/$cm^2$ | Primary plating density $5x10^4$ MNCs/$cm^2$ |
| Passage cells weekly at 95% confluence with media changes every 3 days | Passage cells every 3-4 days at 70% confluence |
| Cell detachment agent: porcine trypsin | Cell detachment agent: accutase |
| Harvest cells at Passage 4 to characterize and assess purity | |

# Culture Endpoints at Passage 4

| | Mosaic | FBS |
|---|---|---|
| MSC purity (immunophenotyping) | CD105/CD73 98.5% n=3 | CD105/CD73 99.1% n=3 |
| MSC yield per 10cc of starting marrow | $2.85 \times 10^8$ | $297 \times 10^8$ |

Conclusion:

- Preliminary studies show that MSC culture in Mosaic medium results in a greater yield per volume bone marrow compared to standard FBS containing medium
- This results in fewer passages, less media, and a shorter culture period to reach the target dose

# Mechanistic Studies
# Amit Bar-Or (Montreal NI)

- Assessments
  - Immune cell phenotyping by flow cytometry
  - Myelin-antigen-specific proliferation and IFN$\gamma$ production
  - $T_h1$, $T_h2$, $T_h17$ effector responses of $CD4^+$ and $CD8^+$ T-cells
  - B-cell proliferation and cytokine production
  - Regulatory functions of $T_{reg}$, NK, and B-cells

  - Longitudinal assessment, comparing pre vs. post transplant:
  - Months -1, 0, 1, 3, 6

# Comparative Studies of MSCs from MS vs. normal donors

- MSC growth kinetics, differentiation
- Expression of signaling,immunoregulatory, & neurotrophic molecules
- Functional in vitro and in vivo immunologic and neurobiologic effects of MSCs and soluble products

# MSC clinical trials in development at CWRU/UHCMC under our IND

- Autologous BM-derived MSC for intervertebral disk repair: IND and IRB approved protocol, preparing to open for accrual

- Autologous adipose-derived MSC for urinary incontinence: pre-IND

- Allogeneic BM-derived MSC for wet AMD: in development

- Allogeneic BM-derived MSC for cystic fibrosis: in development

# MSC clinical development issues

- MSC source and release
- Optimizing culture expansion with potency
- Optimal cell transfer for multi-site use
- Validated potency, efficacy, characterization
- In vivo distribution, destiny, homing and longevity

# Scenarios for clinical MSC manufacture to support multicenter trials

| Centralized site | Site specific | Commercial |
|---|---|---|
| Heavy workload; personnel and space constraints | Manageable workload | Release tested product is infusion ready |
| One set of procedures | Site specific procedures | Best for “off the shelf” indications |
| Minimal variability | Variability in expertise between sites | Sponsor needs to manage inventory at all sites |
| Logistics and cost of shipping final product to clinical site | Requires a centralized QA to ensure quality of final product | Logistics and cost of shipping final product to clinical site |