

Clinical applications of stem cell transplantation

Clinical application

- Animal model vs. Human
- Experimental vs. therapeutic
- Cell type being used
- Type of disease
- Technique of transplantation

Diabetes

- Mouse embryonic stem cells produced insulin
- However, these cells were not beta cells (of neurological derivation) and insulin secretion was very low and *not* glucose dependent
- Long term insuline-independency was achieved in a trial with autologous hematopoietic stem cell transplantation in newly confirmed type I diabetes

Reference:

S. Sipione et al. 2004. *Diabetologia* 47: 499-508.

Voltarelli et al. 2007. *Jama* 297:1568-1576

Myocardial Infarction

- Rat embryonic stem cells were used in rat model of myocardial infarction to improve ventricular function and repair damaged heart tissue
- Mesenchymal stem cells (MSCs), when introduced into the infarcted heart, prevented deleterious remodeling and improved recovery
- Peripheral blood stem cells + G-CSF improved cardiac function and promoted angiogenesis after myocardial infarction

References:

Hodgson DM, et. al. 2004. *Am. J. Physiol. Heart Circ. Physiol.* 287:H471-H479.

Pittenger MF, Martin BJ. 2004. *Circ. Res.* 95:9-20.

Kang et al. 2004. *Lancet* 363:751-756

Adult Stem Cell Success: New Jaw Bone

- Man's jaw bone was removed due to cancer
- Replacement jaw bone was grown for 7 weeks in his back muscles using his own adult stem cells enclosed in a titanium frame with cow-derived bone mineral blocks and human bone growth factor

Reference:

Warnke PH, et al. 2004. Lancet 364:766-770



Adult Stem Cell Success: Stem Cells for Hearing Loss

- Stem cells in the adult inner ear are capable of differentiating into hair cells

Reference:

Li H, Corrales CE, Edge A, Heller S. 2004. *Trends Mol Med.* 10:309-15.

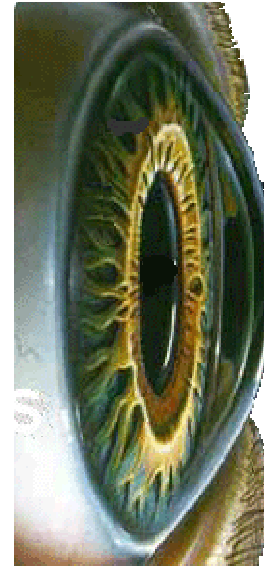


Adult Stem Cell Success: Corneas From Adult MSC

- Adult stem cells taken from patients were grown in culture before transplantation onto the damaged eyes. Sixteen of the 20 patients had improved vision.

Reference:

Schwab, IR et al. 2000. *Cornea* 19: 421-426.



Adult Stem Cell Success: Treatment of Retinitis Pigmentosa

- An injection of adult stem cells saved the sight of mice who would otherwise have gone blind.
- Such treatment could be used for a group of eye diseases called retinitis pigmentosa, in which cells in the retina break down over time, causing gradual loss of vision and sometimes blindness.

Reference:

Otani A., et al. 2004. *J. Clin. Invest.*, 114. 765 - 774.

Adult Stem Cells Work as Well as Embryonic Stem Cells

- Both adult mesenchymal stem cells and embryonic stem cells produced teeth in mice

Reference:

Ohazama A, Modino SA, Miletich I, Sharpe PT. 2004. J. Dent. Res. 83:518-522.



Cancer?

- Cancer is caused by cells of the body multiplying uncontrollably due to genetic mutation or viral infection, in some cases.
- Stem cells would not be useful in therapy.

Mental Health Diseases?

- Since the cause of most mental health diseases is unknown, it is unclear whether stem cells could be useful in therapy.

HIV/AIDS?

- AIDS is caused by an infectious virus (HIV) that attacks the immune system
- Stem cell treatments could improve the function of the immune system, but the effect would be temporary until the new stem cells became infected themselves
- Adult (not embryonic) stem cells would be the preferred treatment

Amyotrophic Lateral Sclerosis

- Mouse model showed that cord blood stem cells are beneficial in reversing spinal cord injury, even when infused 5 days after injury.
- A 2004 review of scientific literature indicated that adult stem cell treatments showed promise for treatment of ALS.
- Since other cell types than motor neurons contribute to cell death (microglia, astrocytes) stem cell replacement strategies have to be developed for those cell types
- iPS – motor neuron cell lines from ALS patients

References:

Garbuzova-Davis, Svitlana, et al. 2003. *Hematotherapy and Stem Cell Research* 12: 255–270.

Silani V et al. 2004. *Lancet* 364:200-2.

Papadeas ST, Maragakis NJ 2009. *Curr Opin Biotechnol* 20:545-551

Multiple Sclerosis

- Adult stem cells (bone marrow or endogenous neural stem cells), but not embryonic stem cells have been used in numerous studies (including some preliminary clinical trials) to treat multiple sclerosis.
- Meanwhile 400 patients are treated worldwide
- Mortality 2-3%

References:

Fassas A + Kimiskidis VK. 2004. *J. Neurol. Sci.* 15:53-58.

Muraro PA et al. 2003. *Curr. Opin. Neurol.* 16:299-305.

Capello E et al. 2009. *Neurol Sci* 30:S175-177

Huntington's disease

- Various clinical trials with fetal tissue transplantation with only limited success
- Demonstration of graft overgrowth in autopsies

Reference

Ramaswami et al. 2007. Cell Transplant 16:301-312

Mb. Parkinson

- Parkinson's disease is a disorder that affects nerve cells in the part of the brain controlling muscle movement
- Disease is progressive – signs/symptoms worsen over time
- Eventually is disabling, but progresses gradually
- Believed to be caused by genetics, environmental factors or a combination of the two

Parkinson's Disease Stats

- First described by James Parkinson in 1817
- Affects ~1 million in the U.S.
- 100-200/100.000 in D
- Onset typically between 50-60 years of age, and slowly progresses with age
- Average onset is 62.4 years of age

Neurological Basis

- “Neurodegenerative Disease” : caused by degeneration (dysfunction and death) of neurons within the brain (nigrastriatal pathway of the basal ganglia)
- **NORMAL BRAIN FUNCTION – Basal Ganglia**
 - Cells in substantia nigra produce/release dopamine
 - Dopamine released by SN neurons lands on neurons of other brain centers, controlling their firing
 - Main targets are caudate nucleus and putamen (striatum)
 - This basal ganglia pathway is involved in regulation of movement

Neurological Basis

- PARKINSON'S BRAIN FUNCTION—Basal Ganglia
 - Cells of substantia nigra degenerate
 - These cells can no longer produce adequate amounts of dopamine
 - Neurons of striatum, etc. are no longer well regulated, thus do not behave in normal manner
 - Results in loss of control of movements – leads to symptoms characteristic of Parkinson's disease

Characteristic Symptoms

- MOTOR
 - tremor
 - bradykinesia
 - rigidity/freezing in place
 - lack of facial expression
 - postural instability
 - stooped, shuffling gait
- NONMOTOR
 - diminished sense of smell
 - low voice volume
 - foot cramps
 - sleep disturbance
 - depression
 - constipation
 - drooling

Conventional Treatments: Medication

- LEVODOPA (L-DOPA)
 - precursor to dopamine, converted to dopamine by nerve cells in the brain
 - Treatment with dopamine not possible, because dopamine can't cross blood-brain barrier
 - Generally combined with carbidopa or benserazide as peripheral decarboxylase inhibitors – helps levodopa get to the brain + reduces some side effects
 - Extended use often produces *dyskinesias* – uncontrolled movements (writhing, twitching, shaking) among other minor side effects

Conventional Treatments: Medication

- **DOPAMINE AGONISTS**
 - not changed into dopamine, but rather act LIKE dopamine at brain synapses where dopamine is usually present (nigrostriatal pathways in Parkinson's patients)
 - Used both as adjuncts to L-Dopa therapy and in younger Parkinson's disease patients
 - Side effects similar to levodopa, but less likely to develop involuntary movements, more likely to cause hallucinations

Conventional Treatments: Medication

- MAO-A Inhibitors
- COMT Inhibitors
- Anticholinergics

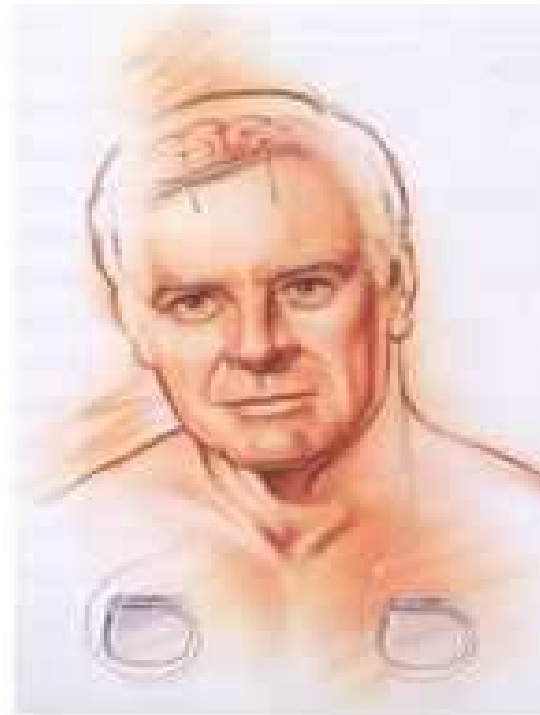
Conventional Treatments:

Surgery

- **Thalamotomy**
 - Involves destruction of small amounts of tissue in the thalamus—major center for relaying messages/transmitting sensations
 - Can cause slurred speech and lack of coordination
- **Pallidotomy**
 - electric current used to destroy small amount of tissue in the pallidum (globus pallidus)
 - May improve tremors, rigidity by interrupting pathway between globus pallidus and thalamus

Conventional Treatments: Surgery

- Deep Brain Stimulation
 - implant device, pacemaker-like unit transmits impulses to electrodes placed in subthalamic nucleus
 - Produces same effects of lesion surgeries, but can be turned on and off



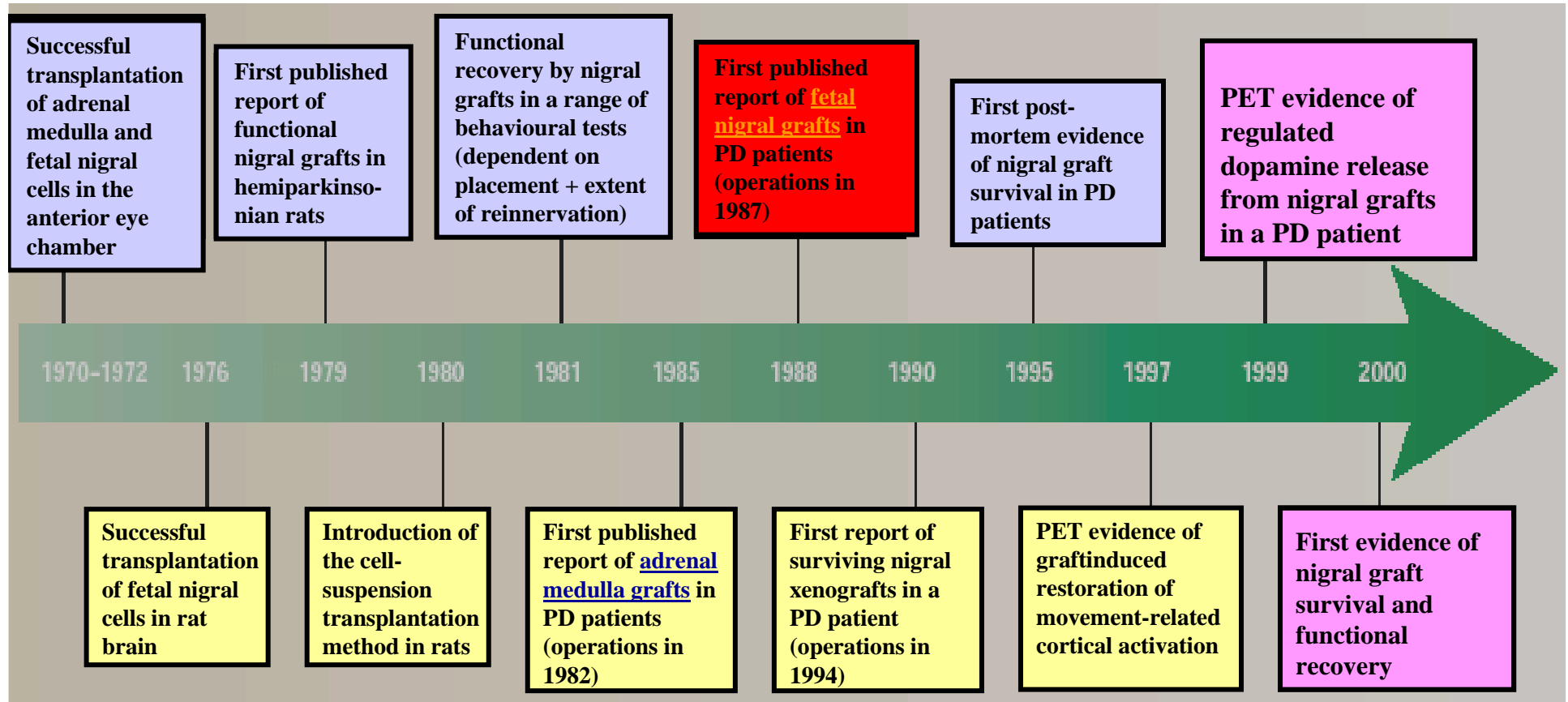
Mb. Parkinson

- Only 50% of rats experienced improvement of symptoms after ES transplantation
- 25% of rats developed brain tumors and died
- Who wants to signup for the first clinical trial?

Reference:

Bjorklund, L. M., R. Sanchez-Pernaute, et al. 2002. *Proceedings of the National Academy of Sciences* 99: 2344-2349.

Cell transplantation in PD – an old story



Modified from S. Dunnett et al., Nature Reviews, 2001

Mb. Parkinson

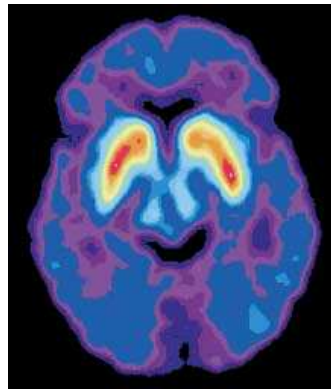
- Madrazo et al.; Fetal homotransplants (ventral mesencephalon and adrenal tissue) to the striatum of parkinsonian subjects. 1990. Arch Neurol. 47:1281-5.
- Improvement esp. in the mesencephalon tissue transplanted group
- Development of severe dyskinesias

Mb. Parkinson

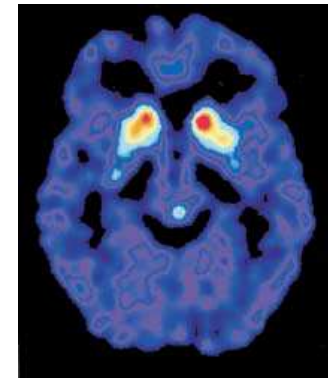
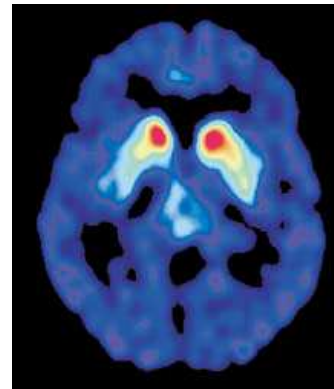
- Freed et al.; Transplantation of embryonic dopamine neurons for severe Parkinson's disease. 2001. NEJM 344:710-719
- 40 Patients with severe Parkinson's disease
- Transplantation of cultured mesencephalic tissue of 4 embryos bilaterally in the putamen (or sham surgery)

Mb. Parkinson

- Transplants survive
- Fiber outgrowth was detected (PET, post mortem)



Before/after transplantation



Before/after sham surgery

Mb. Parkinson

- Clinical improvement in younger patients (< 60y)
- Up to the best effects of L-DOPA on „OFF-symptoms“ preoperatively
- Dyskinesias in 10-15% of transplanted patients even after discontinuation of L-DOPA

Stroke

Outcome of patients with first-ever stroke:

- one fourth die at 30 days
- at 5 years among 30 days survivors:
 - half survive
 - one third are disabled
 - one in seven are institutionalized

SPECIAL CONDITIONS IN NEUROTRANSPLANTATION FOR STROKE

- strokes affect multiple different neuronal phenotypes (cortical, subcortical, white matter)
- strokes disrupt various neuroanatomical pathways (motor, sensory, cerebellar, visual tracts, higher cortical function, etc.) with different clinical manifestations
- neurons are not the only cell type damaged (oligodendrocytes, astrocytes, endothelial cells also affected)

Requirements for Cell Transplantation Research

- Animal model
- Imaging
- Outcome measures including detailed and sensitive behavioral studies
- Histopathology
- Cells

Requirements for Cell Transplantation

- Reliable and readily available source of cells
- Adequate differentiation into desired cell types
- Deliverability of cells at site of injury
- Ability of cells to integrate and function in damaged areas
- No rejection
- No tumor formation

Fetal Stem Cells

- Mixed results in Parkinsons disease
- Transplanted fetal neurons survive, integrate and improve function in animal models
- Difficult to obtain adequate quantities
- Current research potential limited

Bone Marrow Stromal Cells

- Capable of differentiation into multiple cell types including cells similar to NPCs
- Survive, integrate, differentiate and improve function when transplanted into striatum of rats early after ischemic stroke
- Similar results obtained with IV infusion
- Very few transplanted cells show neural markers
- Limited yield - uncertain safety

Umbilical Cord Blood Cells

- Multipotential cells capable of differentiating into neuronal cell types
- IV injection improves functional outcome after ischemia in rats
- Very few surviving cells express neuronal markers
- Early response suggests release of trophic factors
- Distribute to all organs - safety uncertain

Neural Progenitor Cells

- Found in periventricular region in developing brains and adults
- Responds to brain injury including ischemia by proliferation and possibly migration, differentiation
- Capable of differentiating into multiple neuronal cell types
- Survive, proliferate and differentiate when transplanted in rats with ischemic injury
- Source from fetal tissue problematic

Immortalized Cell Lines

- Oncogene transformed or primitive tumors
- Maintained in cell culture and can be cryopreserved
- Not from fetal tissue
- Must undergo some differentiation prior to transplantation - limited cell types
- Concerns about malignant transformation

Expedited Publication

**Transplantation of
cultured human
neuronal cells for
patients with stroke**

NEUROLOGY 2000;55:565-569

D. Kondziolka, MD; L. Wechsler, MD; S. Goldstein, MD; C. Meltzer, MD; K.R. Thulborn, MD, PhD;
J. Gebel, MD; P. Jannetta, MD; S. DeCesare, E.M. Elder, ScD; M. McGrogan, PhD; M.A. Reitman, MD;
and L. Bynum, MD

Neurology, 2000

Origins of LBS (Layton Bioscience Inc.) Neurons

- N-tera 2 cells derived from a lung metastasis of a human testicular germ cell tumor
- Generate post-mitotic neurons with retinoic acid treatment (hNT or LBS neurons)
- Once differentiated, maintain a fetal neuronal phenotype indefinitely in vitro

Characteristics of LBS Neurons

- Permanently post-mitotic
- Resemble immature CNS neurons
- Exhibit cholinergic, glutamatergic, GABAergic and dopaminergic transmitter phenotypes
- Express neurofilament proteins
- Develop functional dendrites and axons
- Form synapses

Advantages of LBS Neurons

- Not from fetal tissue
- Produced in cell culture
- Cryopreserved
- Human cells

LBS Neurons – Preclinical Studies

- Implanted cells survive and integrate in host brain
- Extend processes and form synapses
- Express normal neuronal proteins and markers
- Improve function in rodent stroke models
- Functional improvement proportional to number of surviving cells
- No tumor formation

Phase I: Inclusion Criteria

- Major motor deficit from completed basal ganglia stroke defined on CT or MRI
- Duration of stroke 6 months to 6 years
- Fixed deficit without substantial change for 2 months
- Age 40-75 inclusive
- Men or women
- Ability to provide informed consent

Exclusion Criteria

- Permanent coagulopathy or uncontrolled hypertension
- Concomitant major illness
- Serum creatinine > 2.0 mg/dl
- Participation in other biologics/drug trial
- Pregnancy or intent to become pregnant
- Active malignancy
- Current alcohol or drug abuse
- Inability to understand or cooperate with study

Immunosuppression

- Cyclosporine A: 6 mg/kg
 - Begins one week before surgery and discontinued after two months
 - Dose adjusted by serum levels

Cell Preparation

- Cryopreserved suspension in liquid nitrogen
- Thawed 1 hour before implantation
- Transferred to sterile Isolyte S, pH 7.4
- Cells washed three times
- Cell sample diluted in 0.4% trypan blue, viable and dead cells counted
- Final cell concentration and resuspension calculated
- 3.3×10^7 cells in Isolyte S aliquoted to 120 microL per sterile 1.0 ml vial
- Vials delivered immediately to operating room
- Cells resuspended in OR prior to implantation

Phase I: Cell Numbers

- Patients 1 - 4
 - 2 million cells divided into three 20 microliter implants
- Patients 5 - 12
 - Randomized
 - 2 million or 6 million cells divided into 3 or 9 implants

Phase I: Safety: 35 - 44 Months

No delayed surgical or
cell- related complications
were identified.

Safety: Adverse Events*

- Nausea and vomiting (CS-A)
- Urinary tract infection at one month
- Single seizure at 6 months
- New brainstem stroke at 6 months
- 2 patients died :
 - (1) M.I. At 23 months
 - (2) Pneumonia at 27 mo.
- *none were attributed to cell implantation

Subjective Patient Reports

1. Improved walking
2. Improved walking
3. Less hand/wrist stiffness
4. Stronger proximal arm, better walking, arm and hand no longer “feel dead”
5. Improved arm, wrist and ankle power
10. Improved pinch grip, better memory
11. Clearer speech, better memory
12. Improved sensation and power

Phase II LBS Trial

U of Pittsburgh/Stanford

- Adults with basal ganglia stroke 1-6 years from infarction
- 14 surgical patients and 4 observation controls – all received a stroke rehabilitation program
- Blinded evaluations
- 5 or 10 million neurons divided into 25 implants

Phase II LBS Trial

U of Pittsburgh/Stanford

Safety Results (5-15 months)

- Syncopal episode 1 month after surgery
- Seizure one day after surgery
- Chronic subdural drained at 1 month (plavix/ASA)

U of Pittsburgh Cell Transplantation for Stroke:

Conclusions

- Stereotactic cell implantation was relatively safe with no delayed cell related adverse effects
- PET data, autopsy suggest survival of transplants
- Mixed motor and cognitive improvements in some patients
- Further trials warranted

Mechanisms of Cell Transplantation

- Cellular Reconnection ?
 - repair of lost neuronal connections and conductivity
- Stimulation ?
 - source of neurotrophins, stimulating the regional brain to improved function
 - enhanced host reaction with sprouting of new axons and synapses

Issues with Cell Transplants for Stroke

- Optimal timing of transplant after stroke
- Stroke type and location
- Size of stroke
- Site of cell transplantation
- Number of cells
- Need for immunosuppression
- Long term effects

Cell Transplantation for Stroke:

What comes next ?

Some research is best addressed in humans

- Specific disease model
- Evaluate the factors of patient age, stroke age, brain location, cell concentration, safety, surgical techniques, immunosuppression, cognitive effects, and clinical outcomes

Draw backs

- Problems with ES
- Problems with Adult stem cells

Embryonic Stem Cell Problems: Chromosomal Abnormalities

- Recently, abnormalities in chromosome number and structure were found in three human ESC lines.

References

Draper, J.S., et al., *Nature Biotechnology* December 7, 2003, advance online publication.

C. Cowan et al. 2004. *New England Journal of Medicine* 350: 1353-1356.

Embryonic Stem Cell Problems: Cancer and Tumors

- Rapid growth of embryonic stem cells brings the potential of introducing cancer into patients.
- An embryonic stem cell therapy stroke model in mice found that treated mice developed highly malignant teratocarcinomas at the site of implantation, even when pre-differentiated into neural cells.

Reference:

Erdo F, Trapp T, Buhrle C, Fleischmann B, Hossmann KA. 2004. *Orv. Hetil.* 145:1307-1313.

Embryonic Cloning Problems: Not Enough Human Eggs

“The poor availability of human oocytes (eggs), the low efficiency of the nuclear cell procedure, and the long population-doubling time of human ES cells make it difficult to envision this [therapeutic cloning to obtain stem cells] becoming a routine clinical procedure even if ethical considerations were not a significant point of contention”

Reference:

Odorico JS, Kaufman DS, Thomson JA. 2001. *Stem Cells* 19:193-204.

Embryonic Stem Cell Problems: Tissue Rejection

- Possibility of rejection of stem cell transplants as foreign tissues is very high.
- Hundreds of thousands of stem cell lines would be required to serve the majority of patients

What's Wrong With Adult Stem Cells?

- Can't be patented, since they are derived from the patient
- Can't be marketed and sold at inflated prices due to inability to patent

The future will tell if the job was worth while..

