Lecture #20:
Stem Cell Therapies
Embryonic and Adult Stem Cells

THE SUPPLY CHAIN
Embryonic and adult stem cells as a source of new tissue.

Egg → Zygote → Blastocyst → Gastrula → Embryonic stem cells → Self renewal → Differentiation signals → Endoderm, Mesoderm, Ectoderm

Sperm → Ectoderm, Mesoderm, Endoderm → Somatic stem cells → Self renewal → Differentiation signals → Cell types restricted to organ source, Any cell type?
## Adult Stem Cells

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Stem cell location</th>
<th>Niche components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissues with constant turnover</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematopoietic system</td>
<td>Bone marrow</td>
<td>Macrophages*, T\textsubscript{Reg} cells*, osteoblasts, adipocytes, nestin* MSCs, CAR cells, glia</td>
</tr>
<tr>
<td>Intestine</td>
<td>Fast-cycling: base of crypt</td>
<td>Paneth cells*, mesenchymal cells</td>
</tr>
<tr>
<td>Interfollicular epidermis</td>
<td>Basal layer of epidermis</td>
<td>Dermal fibroblasts</td>
</tr>
<tr>
<td>Hair follicle</td>
<td>Bulge</td>
<td>K6* bulge*, dermal papilla, adipocyte precursor cells, subcutaneous fat, dermal fibroblasts</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tissues with low or no turnover</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Subventricular zone, subgranular zone</td>
<td>Ependymal cells, vasculature</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Between the basement membrane and the muscle fibres</td>
<td>Myofibres* (?)</td>
</tr>
</tbody>
</table>

*Hsu and Fuchs, Nature Rev Mol Cell Biol, 2012*
Adult Stem Cell Therapies

- Bone marrow transplants to rebuild immune system (HSC)
- Skin grafts for burns or other injuries (skin stem cells)
* There are examples of intertissue plasticity- but likely happens at very low frequency under normal physiologic conditions
Cell Fusion as Possible Mechanism after Transfer

Umbilical-Cord Blood Transplantation

- Less restrictive HLA-compatibility requirements, can be provided quickly
- Variable volume, can have fewer cells than typical BM
- Public vs. private banking
Mesenchymal Stem Cells

MSC Therapies
Example: Myocardial Infarction

Ranganath et al, Cell Stem Cell, 2012
### MSC Therapies

**Example: Myocardial Infarction**

#### Table 1. Ongoing MSC-Based Clinical Trials for Cardiovascular Diseases Registered at clinicaltrials.gov

<table>
<thead>
<tr>
<th>Clinical Trial ID</th>
<th>Phase</th>
<th>Condition</th>
<th>No. of Patients</th>
<th>Outcome Measure</th>
<th>Cell Delivery Route</th>
<th>Basis of Trial Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01394432</td>
<td>III</td>
<td>AMI</td>
<td>50</td>
<td>LVSV</td>
<td>endocardial</td>
<td>reduction in scar formation and increased reverse remodeling</td>
</tr>
<tr>
<td>NCT00877903</td>
<td>II</td>
<td>MI</td>
<td>220</td>
<td>ESV, LVEF, infarct size</td>
<td>intravenous</td>
<td>improvement in myocardial remodeling and reduction in incidence of CHF</td>
</tr>
<tr>
<td>NCT00790764</td>
<td>II</td>
<td>SCI</td>
<td>60</td>
<td>safety</td>
<td>intracoronary and transendocardial</td>
<td>development of mature and stable vessels and improved cardiac function via combinatorial effect of BM-MNCs and MSCs</td>
</tr>
<tr>
<td>NCT00555828</td>
<td>I/II</td>
<td>MI</td>
<td>25</td>
<td>safety, feasibility</td>
<td>transendocardial</td>
<td>transdifferentiation of mesenchymal precursor cells (MPCs) into cardiomyocytes</td>
</tr>
<tr>
<td>NCT00672222</td>
<td>I</td>
<td>AMI</td>
<td>28</td>
<td>safety, efficacy</td>
<td>space surrounding target vessel (perivascular)</td>
<td>improvement in cardiac function via MSC paracrine actions</td>
</tr>
<tr>
<td>NCT01291329</td>
<td>II</td>
<td>AMI</td>
<td>160</td>
<td>myocardial metabolism, perfusion, LVEF</td>
<td>intracoronary</td>
<td>transdifferentiation of MSCs into cardiomyocytes</td>
</tr>
<tr>
<td>NCT00768066</td>
<td>I/II</td>
<td>IHF</td>
<td>60</td>
<td>safety</td>
<td>transendocardial</td>
<td>stimulation of endogenous cardiac stem cells by the transplanted MSCs</td>
</tr>
<tr>
<td>NCT00644440</td>
<td>I/II</td>
<td>CHF</td>
<td>60</td>
<td>LVEF</td>
<td>intramyocardial</td>
<td>development of new myocardium and blood vessels</td>
</tr>
<tr>
<td>NCT00587990</td>
<td>I/II</td>
<td>LVD</td>
<td>45</td>
<td>safety, LVEF, infarct size, ESV</td>
<td>intramyocardial</td>
<td>combinatorial effects of bypass surgery and MSC transplantation</td>
</tr>
<tr>
<td>NCT00721045</td>
<td>II</td>
<td>HF</td>
<td>60</td>
<td>safety, efficacy</td>
<td>transendocardial</td>
<td>MPC-induced large blood vessel formation and cardiac repair</td>
</tr>
<tr>
<td>NCT00418418</td>
<td>II</td>
<td>MI</td>
<td>60</td>
<td>LVEF, safety</td>
<td>intramyocardial</td>
<td>combinatorial effects of bypass surgery and MSC transplantation</td>
</tr>
<tr>
<td>NCT00883727</td>
<td>I/II</td>
<td>MI</td>
<td>20</td>
<td>myocardial perfusion, infarct size</td>
<td>intravenous</td>
<td>transdifferentiation of MSCs into cardiomyocytes and production of new blood vessels</td>
</tr>
<tr>
<td>NCT01087996</td>
<td>I/II</td>
<td>LVD, MI</td>
<td>30</td>
<td>safety, efficacy</td>
<td>transendocardial</td>
<td>neo-myogenesis induced by transplanted allogenic and autologous MSCs</td>
</tr>
<tr>
<td>NCT01076820</td>
<td>I/II</td>
<td>MI, LVD</td>
<td>10</td>
<td>safety, efficacy</td>
<td>transendocardial</td>
<td>transdifferentiation of MSCs to produce new blood vessels</td>
</tr>
<tr>
<td>NCT01449032</td>
<td>II</td>
<td>CMI</td>
<td>60</td>
<td>safety, efficacy</td>
<td>not specified</td>
<td>angiogenesis</td>
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<tr>
<td>NCT01442129</td>
<td>II</td>
<td>HF</td>
<td>30</td>
<td>safety, efficacy</td>
<td>intramyocardial</td>
<td>MPC-induced angiogenesis via paracrine signaling combined with LVAD implantation</td>
</tr>
<tr>
<td>NCT01392625</td>
<td>I/II</td>
<td>NDC</td>
<td>36</td>
<td>safety, efficacy</td>
<td>transendocardial</td>
<td>neomyogenesis via MSC-CSC interaction</td>
</tr>
<tr>
<td>NCT01270139</td>
<td>I/II</td>
<td>CAD</td>
<td>180</td>
<td>plaque volume stenting</td>
<td>functional restoration of blood vessels via nanoburning and MSC paracrine effects</td>
<td></td>
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<tr>
<td>NCT01436123</td>
<td>I</td>
<td>CAD</td>
<td>120</td>
<td>plaque volume stenting</td>
<td>reduction of plaque via paracrine signaling in combination with burning effects from Si-Fe NPs</td>
<td></td>
</tr>
</tbody>
</table>

LVSV, left ventricular systolic volume; SCI, severe coronary ischemia; IHF, ischemic heart failure; CHF, congestive heart failure; LVD, left ventricular dysfunction; ESV, end systolic volume; LVEF, left ventricular ejection fraction; CMI, chronic myocardial ischemia; LVAD, left ventricular assist device; NDC, nonischemic dilated cardiomyopathy; CAD, coronary artery disease.

Ranganath et al, Cell Stem Cell, 2012
Question:
Should Stem Cell Treatments Be Regulated As Drugs?
Directed Differentiation- $\beta$ cells

ES/iPS $\rightarrow$ endoderm $\rightarrow$ pancreas $\rightarrow$ endocrine $\rightarrow$ insulin

Sox17 $\uparrow$ Pdx1 $\uparrow$ Ngn3

IDEs $\quad$ indolactam V

Melton D A Phil. Trans. R. Soc. B 2011;366:2307-2311
How can you obtain pluripotent stem cells (ES cells) from a particular patient?
Reprogramming of Somatic Cells

- Somatic Cell Nuclear Transfer, i.e. Cloning

Reprogramming of Somatic Cells

- Somatic Cell Nuclear Transfer, i.e. Cloning

http://www.pbs.org/wgbh/nova/body/cloning-process.html
Reprogramming of Somatic Cells

• Induced Pluripotent Stem Cells (iPS)

  Skin fibroblasts

  Retroviral transfection of 4 transcription factors
  Oct-4, Sox2 (pluripotency)
  c-Myc, Klf-4 (proliferation)

  Select Nanog+ cells

  iPS cells
<table>
<thead>
<tr>
<th>Feature</th>
<th>ES</th>
<th>ES/SCNT</th>
<th>iPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune Compatibility</td>
<td></td>
<td></td>
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<tr>
<td>Yield</td>
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<td>Expansion</td>
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<td>Affordability</td>
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<tr>
<td>Tumors</td>
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<td></td>
<td></td>
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<tr>
<td>Ethics</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ES</td>
<td>ES/SCNT</td>
<td>iPS</td>
</tr>
<tr>
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<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Immune Compatibility</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Yield</td>
<td>+</td>
<td>--</td>
<td>-</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Expansion</td>
<td>+</td>
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<td>Affordability</td>
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<td></td>
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</tr>
<tr>
<td>Tumors</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(After Differentiation OK- All will Form Teratomas if Not Differentiated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethics</td>
<td>-</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>

Approx. $5,000, But decreasing
iPS- Disease Models

Melton D A Phil. Trans. R. Soc. B 2011;366:2307-2311
Direct Cell Reprogramming

Nicolas & Kriegstein, Nature, 2010
A Screen For Neuronal-Fate-Inducing Factors

Transfect with a panel of 19 neuronal genes

Vierbuchen et al, Nature 2010
Defining a Minimum Pool for Neuronal Induction

A, Ascl1; B, Brn2; M, Myt1l; O, Olig2; Z, Zic1

Vierbuchen et al, Nature 2010
Direct Conversion of Cardiofibroblasts to Cardiomyocytes

Ieda et al, Cell 2010
In vivo reprogramming of adult pancreatic exocrine cells to β-cells

Qiao Zhou¹, Juliana Brown², Andrew Kanarek¹, Jayaraj Rajagopal¹ & Douglas A. Melton¹

One goal of regenerative medicine is to instructively convert adult cells into other cell types for tissue repair and regeneration. Although isolated examples of adult cell reprogramming are known, there is no general understanding of how to turn one cell type into another in a controlled manner. Here, using a strategy of re-expressing key developmental regulators in vivo, we identify a specific combination of three transcription factors (Ngn3 [also known as Neurog3], Pdx1 and Mafa) that reprograms differentiated pancreatic exocrine cells in adult mice into cells that closely resemble β-cells. The induced β-cells are indistinguishable from endogenous islet β-cells in size, shape and ultrastructure. They express genes essential for β-cell function and can ameliorate hyperglycaemia by remodelling local vasculature and secreting insulin. This study provides an example of cellular reprogramming using defined factors in an adult organ and suggests a general paradigm for directing cell reprogramming without reversion to a pluripotent stem cell state.
ES/iPS Clinical Therapies

- Advanced Cell Technology
  Macular degeneration- less rejection (BBB)
  Clinical Assessment tools, animal models
ES/iPS Clinical Therapies

• Advanced Cell Technology
  Macular degeneration- less rejection (BBB)
  Clinical Assessment tools, animal models

• Geron
  Clinical trial- human ES-derived oligodendrocytes to treat spinal cord injuries
ES/iPS Clinical Therapies

• Advanced Cell Technology
  Macular degeneration - less rejection (BBB)
  Clinical Assessment tools, animal models

• Geron
  Clinical trial - human ES-derived oligodendrocytes to treat spinal cord injuries

But....
Geron Study- Nov ‘11

IN FOCUS NEWS

REGENERATING MEDICINE

Stem-cell pioneer bows out

BY MONYA BAKER

The first company to test a human embryonic stem-cell product in patients has become the first big player to bail out of the field. Last week’s move by investor confidence and raised questions about whether the company had overreached itself, as well as understanding just how difficult new therapies are to develop.

Geron, based in Menlo Park, California, announced 14 November that it would cease work on its stem-cell therapy programme to focus on its anti-cancer portfolio. “It’s kind of a heartbreak,” says Melissa Carpenter, principal of Carpenter Group Consulting in Seattle, Washington, who served as Geron’s director of stem-cell biology in the company’s early days. “It is truly unfortunate for the field that the first possible product didn’t get to go out of the gate.”

Human embryonic stem (ES) cells have the potential to turn into any of the body’s cell types, and so could replace defective tissues in myriad diseases. Geron chose to pursue a treatment for spinal-cord injury — an ambitious goal, but at least because spinal damage involves many cell types. Some in the field speculate that Geron went forward with the programme, at least in part, because the neural-cell promise it was testing were relatively easy to derive from human ES cells, and because dramatic results in animal studies impressed investors when the company needed funding. However, human trials are often the only way to test such unprecedented therapies.

Only four of a planned eight patients in Geron’s phase I trial have received injections of specialized cells derived from human ES cells, John Scardino, who became Geron’s chief executive in September (see ‘Rough ride’), says that the company will continue to monitor enrolled patients, but will not recruit more. None of the four patients receiving stem-cell injections suffered serious adverse events, but there were no limits that the therapy was working (although phase I trials are not designed to test for efficacy). The price of Geron’s stock fell by more than 30% at the news, from US$2.35 to $1.50 per share as Nature went to press. But the company estimates that discontinuing stem-cell research will save $20 million a year, allowing it to continue a dozen phase II clinical trials of its two cancer products in the next two years without raising additional funds. Cell therapy studies would have taken longer and cost more.

Advanced Cell Technology (ACT) of Santa Monica, California, is now the only company conducting regulatory-approved clinical trials involving human ES cells: these aim to treat degenerative eye diseases using specialized retinal cells.

Investors and patients are eager for reassurance that human ES cells have commercial and therapeutic potential, says Robert Lanza, chief scientific officer of ACT. “The field at this early point desperately needed a big success,” he says. “It certainly puts a lot of pressure on us to deliver.”

Geron’s decision comes shortly after a ruling that products and processes involving human ES cells are not patentable in Europe (see Nature, http://dx.doi.org/10.1038/news.2011.997; 2011), but analyst Tensi Benjak of investment bank Rodman & Renshaw in New York believes that Geron’s decision was unrelated. It was probably already in the works when long-time chief executive Thomas Okarma left the company abruptly in February, and reflects a change in business strategy rather than a verdict on cell therapies in general, he says. “Just because Geron is out, it doesn’t mean that other trials will slow down.”

Geron had invested heavily to bring human ES cells to clinical trials. It funded the studies leading to the cell’s derivation in 1998 (J. A. Thomson et al. Science 282, 1145–1147; 1998) and burned through cash while devising ways to manufacture specialized cells from the stem cells, as well as running extensive animal tests to show that the cells were safe enough to use in humans. These efforts paved the way for others trying to move stem cells into the clinic. “It’s exponentially easier,” says Lanza. “We know exactly what (regulatory) wants.”

There may be other benefits for the field. Geron controls extensive intellectual property relevant to human ES-cell therapy, says Ken Taylor, a stem-cell patent expert at the University of California, Berkeley, and it may now be more willing to license this portfolio to help others pursuing similar therapies.

Asked what other companies can learn from Geron’s decision, Michael West, who ran Geron from 1990 to 1998 and is currently chief executive of biotech company BioTime of Alameda, California, simply suggests: “Don’t be the first one out the door. The first one out the door gets all the arrows in his back.”

ROUGH RIDE

Geron led the field in bringing cell therapy to clinical trial, but paid the price of being first.

NOVEMBER 1998

Geron founded by Michael West.

NOVEMBER 1998

Human embryonic stem cells (pictured below) derived and cultured for the first time, funded by Geron.

MAY 2000

Despite a 23,000-page submission from Geron, the US Food and Drug Administration decides that its clinical trial of human embryonic stem cells should not proceed.

JANUARY 2009

Clinical trial approved, just three days after US President Barack Obama’s inauguration.

AUGUST 2009

Clinical trial put on hold again.

JULY 2010

Hold lifted, clinical trial proceeds.

OCTOBER 2010

Geron announces that those proven to patient in clinical trial.

FEBRUARY 2011

Long-term chief executive Tom Okarma resigns.

SEPTEMBER 2011

Geron appoints John Scardino as chief executive.

NOVEMBER 2011

Geron quits stem-cell research and development.
Important Current Questions and Research Areas

- Engraftment (what happens to transplanted stem cells?)
- Safety (ensure that stem cell treatments do not cause cancer or other diseases)
- iPS vs. ES (can iPS be used instead of ES)
- Immunological tolerance
- Adult stem cells in other tissues? (optimize expansion and function)
Regulation
Should Stem Cell Treatments Be Regulated As Drugs?
Regulation
Should Stem Cell Treatments Be Regulated As Drugs?

THIS WEEK

The darker side of stem cells
An investigation by Nature has found that patients in Texas are receiving unproven stem-cell treatments. The state and the company involved need to ensure that they follow FDA guidelines.

Stem cells offer the hope that one day they will be able to cure a huge range of disorders. But too many people are promising those cures to patients now, long before there is any evidence that they work. These claims are potentially misleading at best, and at worst could be downright harmful.

This week, Nature raises important questions about one company that works with adult stem cells: Celltex Therapeutics in Houston, Texas. Nature’s investigation, reported on page 13, suggests that the company has supplied adult stem cells to Texas doctors who offer unproven treatments to patients, and that the company is involved in these treatments. One doctor claims that the treatments are part of a clinical study run by Celltex and that the company pays him US$300 a time to inject the cells into patients, who are charged up to $25,000 for a course. The US Food and Drug Administration (FDA) considers it to be a crime to inject unapproved adult stem cells into patients. David Eller, chief executive of Celltex, denies that the company is involved in treatment procedures, but would not comment on Nature’s findings about how its cells are used or answer questions about them.

Celltex has the backing of state governor Rick Perry, who has tried adult-stem-cell treatments himself. And the company recently recruited Glenn McGee, editor-in-chief of the American Journal of Bioethics, to be its president of ethics and strategic initiatives. McGee, whose move they are akin to simple skin grafts from one part of the body to another, which do not require validation in an FDA-approved clinical trial.

The FDA can help to clarify these matters. A sensible first step in the new state regulations would be a requirement for any firm that plans to inject processed stem cells into patients to contact the FDA, which can advise on whether federal rules — the same federal rules that have already been used to arrest stem-cell practitioners and to stop a company pushing unapproved treatments elsewhere — apply to what they are doing. Once past that step, Texas could move on to develop its own safety regulations.

If the Texas Medical Board were to act according to its stated pledge to protect patients, then it would make clear the need for clinical validation of adult stem cells before use and would rescind the medical licences of any doctors in breach of rules on using unapproved treatments. If Celltex truly wants to help patients, then it should refuse to supply stem cells for medical procedures until those procedures are properly proven to be effective. And if the company is serious about demonstrating clinical effectiveness itself, then it should start by contacting the FDA about what needs to be done.

http://www.nature.com/nature/journal/v483/n7387/pdf/483005a.pdf
Controversial stem-cell company moves treatment out of the United States

Celltex to send patients to Mexico.

David Cyranoski
30 January 2013

US citizens who had pinned their hopes on a company being able to offer stem-cell treatments close to home will now need to travel a little farther. Celltex Therapeutics of Houston, Texas, stopped treating patients in the United States last year following a warning from regulators. A 25 January e-mail to Celltex customers indicates that the firm will now follow in the footsteps of many other companies offering unproven stem-cell therapies and send its patients abroad for treatment — but only to Mexico.

The stem-cell treatments offered by Celltex involved extracting adult stem cells from a patient, culturing them and then reinjecting them in a bid to replenish damaged tissue. It had been offering the treatment for more than a year — with one of its high-profile customers being Texas governor, Rick Perry — when the US Food and Drug Administration (FDA) wrote to the company on 24 September 2012 advising it that the stem cells it harvested and grew were more than "minimally manipulated" during Celltex's procedures. As such, the FDA regarded the cells as
Regulation
Should Stem Cell Treatments Be Regulated As Drugs?

ISSCR: Report

Patients Beware: Commercialized Stem Cell Treatments on the Web

A report by the International Society for Stem Cell Research (ISSCR)'s Task Force on Unproven Stem Cell Treatments outlines development of resources for patients, their families, and physicians seeking information on stem cell treatments.

Cell Stem Cell 7, 43–49, July 2, 2010 ©2010 ISSCR