This presentation has been prepared by the NSW Stem Cell Network, Stem Cells Australia and The Royal Australasian College of Physicians with the assistance of the National Stem Cell Foundation of Australia for the information of health practitioners and the public. Permission is granted for non-commercial use for educational purposes.

The images show examples of stem cell differentiation.
Overview

• What are stem cells?
• Stem cells in the clinic
• Unproven stem cell treatments
• Legal, ethical and social considerations

This slide introduces the topics in the presentation.
What is a stem cell?

A stem cell is a primitive cell capable of:
- self-renewal
- conversion to more differentiated cells
- proliferation
- leading to regeneration of tissues

Basics of what makes a stem cell a stem cell.
Note the use of ‘tissue’ rather than ‘adult’ stem cells. Tissue is a more accurate description as the category also includes fetal and cord blood stem cells.

Points you may wish to raise if presenting these slides are:
- tissue stem cells are multipotent, they only give rise to a limited number of different tissues
- under the right conditions tissue stem cells can be grown in the laboratory
- now thought to be present in most tissues.

This slide is a good opportunity to introduce haematopoietic stem cells (HSCs) and their long use and history in medicine.

This is also a good opportunity to introduce mesenchymal stem cells (MSCs) which are currently being investigated in many clinical trials for several indications.

Mesenchymal stem cells:
- Promote angiogenesis
- Anti-inflammatory properties
- Non-immunologically reactive
- May act to stimulate new endogenous cell formation rather than differentiating into desired cells
- Usually prepared from bone marrow but also increasingly from adipose tissue

For more information on MSCs, see http://www.nature.com/nrm/posters/mscs/mscsposter.pdf
Human embryonic stem cells were first reported in 1998. The images on this slide are:
1 - blastocyst showing inner cell mass (National Stem Cell Foundation of Australia).
2 - colony of human embryonic stem cells (Diabetes Transplant Unit, Prince of Wales Hospital).
3 - multiple colonies of human embryonic stem cells growing on fibroblasts (Diabetes Transplant Unit, Prince of Wales Hospital).
Use photo of blastocyst to identify inner cell mass and state that this is isolated and cultured to form ESC.

Other points you may wish to raise are:
• At the completion of their infertility treatment, IVF couples must decide what happens to any embryos they may still have frozen. Not all couples will have excess embryos. Their options are to discard the embryo (disposed of in biological waste), donate the embryo to research, or donate their embryo/s to another couple.
• Human ESC are immortal in the right conditions and can differentiate into all three germ layers and theoretically all cell types
• Transplantation of undifferentiated ESC carries a high risk of teratoma formation. However transplantation of differentiated cells reduces this risk considerably
• It has been legal to use human embryos in research in Australia since 2002 when specific legislation was passed - Research Involving Human Embryos Act 2002. Any researcher wanting to use embryos in their research must obtain a licence from the government specifically for their project.
In vitro fertilisation

Fertilisation and early embryonic development occurs in the laboratory.

This slide recapitulates the process of in vitro fertilisation, which often produces more human embryos than are required to produce a baby for the parents.
This slide shows schematically how human ESC are created from excess IVF and SCNT embryos.

At this point mention that stem cells can also be derived from parthenogenetic activated eggs.

When introducing SCNT state that human embryonic stem cells were first created with this technique only recently, in 2013. (Tachibana N, et al. *Cell* 2013; 153: 1228-1238.)

It has been legal to use excess, donated human embryos for research in Australia since 2002 provided researchers receive ethics approval and obtain a licence from the NHMRC. SCNT has been legal in research in Australia (except Western Australia where the State legislation differs from the federal legislation) since 2006 when a modification was made to the original legislation. Researchers involved in SCNT experiments must also apply for a licence from the NHMRC and seek ethics approval.

Reproductive cloning remains banned.
Induced pluripotent stem cells (iPSC)

- Can create stem cells directly from a patient
- Can be maintained indefinitely in the laboratory
- Pluripotent with similar properties to ESCs


Consider discussing that:
Induced pluripotent stem cells are a new discovery - human (Takahashi K et al. Cell 2007; 131: 861–872) and mouse (Takahashi K, Yamanaka S. Cell 2006; 126: 663-676).

Initial techniques employed viral induction but techniques have evolved to replace with non-viral means.

Properties not exactly the same as human ESCs and do not obviate the need for ESCs in research.

iPSC valuable to study ‘disease in a dish’ where stem cells are made directly from a patient with a particular condition and for drug screening and discovery – around the world many disease specific iPSC lines have now been made.

iPSC are a source of patient-specific stem cells for transplant, with first human transplant carried out in 2014, with cells differentiated from iPSC (www.ipscell.com/2014/09/stem-cell-landmark-patient-receives-first-ever-ips-cell-based-transplant/). There is a risk of tumour formation, as with human ESC, because of pluripotency.
Different attributes

<table>
<thead>
<tr>
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<th>Pluripotent stem cells</th>
<th>Tissue stem cells (multipotent)</th>
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<tr>
<td></td>
<td>ESCs</td>
<td>iPSCs</td>
</tr>
<tr>
<td>Source</td>
<td>Embryo</td>
<td>Somatic cell</td>
</tr>
<tr>
<td>Rate of proliferation</td>
<td>High</td>
<td>High</td>
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<tr>
<td>Availability</td>
<td>High</td>
<td>High</td>
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<tr>
<td>Spontaneous</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td>differentiation</td>
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<td></td>
<td>capacity to produce</td>
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<td>diverse cell types</td>
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Pluripotent stem cells, both hESC and iPSC, when undifferentiated, will form teratomas when transplanted (Takahashi K et al. Cell 2007; 131: 861–872).
While the focus is on the much hoped for revolution in cellular replacement therapies, much of the value especially in the immediate future is in research and in knowledge gained from their use in the laboratory and as an adjunct in the development of new drugs (screening and toxicology).

Pluripotent stem cells and their derivatives provide an unlimited source of normal and disease cell lines for screening.


Graph is Australian data showing increasing use of cord blood for haematopoietic stem cell transplants.
The blood cancers (haematologic malignancies) for which patients in Australia received an unrelated bone marrow or cord blood unit transplant were:

- Acute Myelogenous Leukaemia (AML)
- Acute Lymphoblastic Leukaemia (ALL)
- Myelodysplastic Syndromes (MDS)
- Non-Hodgkin Lymphoma (NHL)
- Other Leukaemias include Juvenile Leukaemias and Chronic Lymphocytic Leukaemia
- Chronic Myelogenous Leukaemia (CML)
- Hodgkin Lymphoma (HL)

The number of transplants continues to increase, with the largest areas of growth in AML, ALL, MDS and NHL.
Many clinical trials are underway involving stem cells
- majority involve HSCs (CD34+) for improvements for into blood disorders and immune deficiencies
- growing number using MSC (CD34-) for other conditions
- trials also using fetal stem cells and more recently cells derived from HESC
- approved trials listed on www.clinicaltrials.gov and www.anzctr.org.au

MSC = mesenchymal stem cell

iPSC clinical trials commenced in Japan, 2014 – fast track approval in that country

This graph shows time trends in 4749 stem cell clinical trials, globally. The proportion of clinical trials classified as novel applications of stem cell therapies increased from 2004 to 2011. Within novel clinical trials (n = 1058), most of the increase since 2006 was due to trials using MSCs. The decrease in clinical trials in 2012 may be due to a lag in trial registration.

Novel clinical trials most commonly had a regenerative therapeutic goal (87%), and this subset of clinical trials primarily targeted tissue injury from degeneration and ischaemia.
Cochrane review of stem cells for heart disease. Analysing the literature, the Cochrane reviewers found “some evidence that stem cell treatment may be of benefit.” But, they noted, “the quality of the evidence is relatively low because there were few deaths and hospital readmissions in the studies, and individual study results varied. Further research involving a large number of participants is required to confirm these results.”

(http://www.cochrane.org/CD007888/VASC_stem-cell-treatment-for-chronic-ischaemic-heart-disease-and-congestive-heart-failure)

Recent study published in BMJ (2014 Apr 28; 348: g2688. doi: 10.1136/bmj.g2688) found that many of the most promising results in the field are illusory and that the potential benefits of stem cells to treat heart disease are probably far more modest than we have been led to believe.
Stem cell trials for cardiac disease: 2

Acute myocardial infarction

- number of trials underway involving bone marrow and adipose derived stem cells
- one randomised double blind trial showed ongoing benefit up to 18 months
  - better mean reduction in infarct size
  - better improvement in LV perfusion
  - no major adverse events
- larger, randomised trial now underway in Europe

One randomised double blind trial showed ongoing benefit up to 18 months — see: Gao LR et al. *BMC Medicine* 2015; 13: 162.)

This trial took many years and dollars to get approval as first in human using hESCs.

Five patients with neurologically complete, thoracic spinal cord injury as classified by the American Spinal Association Impairment Scale, were administered a relatively low dose of two million AST-OPC1 cells at the spinal cord injury site 7-14 days post-injury. The subjects received low levels immunosuppression for the next 60 days. The patients have been followed to date for 2-3 years through numerous clinical visits, MRIs, and neurological assessments.

Chronic thoracic spinal cord injury

- Numerous trials using MSCs and olfactory ensheathing cells from nose
  - Limited benefit, with one report of tumour formation from olfactory cells

- StemCells Inc are conducting Phase I/II trial in Switzerland
  - fetal-derived neural stem cells
  - 12 patients (both complete & incomplete) enrolled
  - monitoring for safety and have demonstrated improvements in neurological function below injury site
  - Results were expected to be announced in mid-2015
  - 1st patient enrolled in phase II trial to study cervical SCI


First patient enrolled in phase II trial to study cervical SCI (www.stemcellsinc.com/About-Us/CEO-Corner).

This approach for corneal disease was recently approved by the European Medicines Agency (Dolgin E. *Nat Biotechnol* 2015; 33: 224-225).

In trial undertaken at University of New South Wales (UNSW), 16 people treated, with 10 achieving a clinically stable corneal epithelium at a median of 2.5 year follow up. Half of these had some improvement in visual acuity (Bobba S et al. *Stem Cell Res Ther* 2015; 6: 23).

Pictures of eyes are from A/Professor S Watson showing before and after treatment of aniridia. Picture of cells are human corneal epithelial cells growing on a CIBA Vision contact lens, and was provided by A/Prof N Di Girolamo, School of Medical Sciences, UNSW.
Stem cell trials for blindness: 2

Macular degeneration

- Several trials underway or about to begin; one completed
- US company Advanced Cell Technology (now trading as Ocata Therapeutics) started two clinical trials in 2011
  - Dry age related macular degeneration
  - Stargardt’s macular degeneration
  - Phase I/II trials use retinal pigment epithelial (RPE) cells derived from human ESC
- Stem Cells Inc has completed phase 1 study in Stargardt’s macular degeneration using allogeneic human neural stem cells
- London Project to Cure Blindness with Pfizer planning trial using sheets of RPE for acute macular degeneration
- RPE derived from iPSC transplanted in Japan, October 2014

The trials by Advanced Cell Technology (now trading as Ocata Therapeutics) were the second and third trials to begin worldwide to use cells made from hESC.

More information about StemCells Inc trials is available at www.stemcellsinc.com/Clinical-Programs/AMD.
Stem cell trials for cerebral palsy

- Substantial speculation about use of umbilical cord blood
- Phase II clinical trial at Duke University
  - re-infuse autologous cord blood in children <6yrs and seeking to treat 120 children
  - assumes infused cells will home to areas of ischaemic damage
  - results yet to be published but has attracted a lot of attention based on anecdotal reports
- Korean study
  - 96 treated (31 with erythropoietin & donor cord blood)
  - Improvement in motor skills and cognitive function

Korean study (Min K et al. *Stem Cells* 2013; 31: 581-591)

Monash University (MIMR-PHI) together with Cell Care Australia (private cord blood bank) are planning a similar autologous cord blood transfusion study in children with cerebral palsy.
Stem cell trials for stroke

- Although improvement demonstrated in animal models, not yet shown in patients
- UK based company ReNeuron have commenced a trial
  - Phase I study – 12 patients using genetically engineered fetal neural stem cells
  - involves intracerebral injection into patients with significant disability 6 to 24 months after stroke
  - proposed action though known anti-inflammatory, trophic and pro-angiogenic properties
  - Interim data (9 patients) no adverse events
While not restricted to stem cells, medical tourism is a growing concern. Issue with stem cell tourism is the prematurity of the technology being offered as ‘treatment’ now with no or little justification/safe or follow-up. Desperate patients are paying large sums of money for such therapies. Companies offering treatments usually do not have animal data or clinical trial data to back up their claims. Clinics often claim that their treatments are completely risk free. Several adverse events have been reported in the literature either as a direct result of the stem cell therapy or from the procedure:

- An Israeli boy with ataxia telangiectasia who had been to Russia three times for intracranial and intrathecal injections of fetal neural stem cells was subsequently diagnosed with a multifocal brain tumour. Tests found that the tumours were of non-host origin and had been derived from at least two of the donor fetuses. (Amariglio N et al. PLoS Med 2009; 6: e1000029)
- A clinic in Germany was shut down following the death of an 18 month old child who suffered internal bleeding as a result of injections into the brain with stem cells (www.telegraph.co.uk/news/worldnews/europe/germany/8500233/Europes-largest-stem-cell-clinic-shut-down-after-death-of-baby.html).

Stem Cells Australia, the National Stem Cell Foundation of Australia and the NSW Stem Cell Network offer individualised responses to patient queries. See their websites, which are listed on the final slide.

Unproven autologous stem cell interventions in Australia

Increasing number of clinicians are offering unproven interventions using cellular extracts derived from liposuction

• No clinical trial evidence of efficacy
• Practitioners claim benefit by anecdotal patient statements
• Usually priced around $10,000 per “treatment”
• Offered for a variety of disorders, including neurodegenerative, but mostly for osteoarthritis and other musculoskeletal disorders
• Cells are delivered into joints and sometimes by intravenous, intramuscular or intrathecal injection
• Therapeutic Goods Administration is reviewing regulation – currently exempt
• Medical Board of Australia and AHPRA has jurisdiction over advertising and investigation of complaints but no direct involvement to date
• Self-regulated Code of Conduct is being promoted by the newly formed Australian Cell Therapy Society, including independent oversight

Self-regulated Code of Conduct is being promoted by the newly formed Australian Cell Therapy Society (www.acts.org.au), including independent oversight. The independent oversight is intended to be provided by the External Advisory Board and at least some members of the Code Committee (page 46 of Code of Conduct).

Some members of the Australian Cell Therapy Society have clinical trials registered with the Australian Clinical Trial Register, underway for treatment of osteoarthritis – numbers are 12615000260527, 12615000257561, 12615000258550, (uncontrolled) and 12614000814673 (controlled).

For more reading on the topic of unproven therapies, please see:

• Munsie M, Pera M. Stem Cells Development 2014; 23 Suppl 1: 34-38.
The Australian Stem Cell Centre Patient Handbook, a free resource, is available from www.stemcellsaustralia.edu.au (website of Stem Cells Australia).

The Patient Handbook contains a checklist of questions that patients should be asking and strong recommendations that patients discuss this information with their regular physician.

The Patient Handbook aims to empower patients to make a fully informed decision.

The International Society for Stem Cell Research also has a website - www.closerlookatstemcells.org which provides information about stem cells and advice for patients.
Australia is fortunate to have a regulatory framework that allows stem cell research but only when strict criteria are met:

- legal since 2002 to use donated IVF embryos for licensed research projects
- passage of legislation only occurred following extensive public and parliamentary debate
- key criteria that embryos are no longer required for infertility treatment and donors cannot be paid
- legal since 2006 to create an embryo for licensed research using SCNT but under no other circumstances
- cloning for reproduction is specifically banned in the legislation.
## Regulatory frameworks elsewhere

Allow procurement of hESC from spare embryos:
19 countries including Australia

Allow creation of human embryos for research purposes: Australia, Belgium, Israel, Japan, Singapore, South Korea, Sweden, UK, USA (6 States and privately)

Prohibit procurement of hESC from spare embryos but allow import of hESC lines: Germany, France

Prohibit procurement of hESC: Austria, Italy, Norway, Poland, US State of Nebraska

Prohibit human cloning: 51 countries

For more information on the different policy approaches adopted around the globe visit [http://www.stemgen.org](http://www.stemgen.org)
Regulatory framework in the clinic

Stem cell research has ethical issues and these are governed by guidelines released by NHMRC, with approval from an institutional ethics committee required.

Clinical translation and manufacturing are covered by specific legislation, although there is an exemption for autologous cell applications. This is under review.

Ensuring appropriate consent is obtained from the donors is essential no matter what type of stem cell research will be conducted.
Advice about stem cells?

- Stem Cells Australia
  www.stemcellsaustralia.edu.au

- National Stem Cell Foundation
  www.stemcellfoundation.net.au

- NSW Stem Cell Network
  www.stemcellnetwork.org.au

- International Society for Stem Cell Research
  www.closerlookatstemcells.org

- National Health & Medical Research Council