Professor Richard Boyd

Monash University

Australia

Stem Cells - Hopes Hurdles and Clinical Horizons - Main Session (Workshop options scheduled)

Friday, 21 June 2013 Start 4:30pm Duration: 20mins Baytrust

Stem Cell Therapies:
"hurdles, hopes and applications"

Stem cells to the rescue – are they for you or your grandchildren or both?

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MAGELLAN STEM CELLS

GENESIS STEMCELL 领航干细胞 Life is Valuble

miscl monash immunology and stem cell laboratories

Australian Stem Cell Centre

norwood immunology

ProStemCell 保信幹細胞

Hong Kong Stem Cell bank and clinic

Why the need for stem cells?

- Children develop debilitating diseases that effect them for life
- *AND*
- The older we get, the sicker we get !
- Average age has gone from ~30 in 1800's to now over 80
- Living longer but often carrying disease or degenerative conditions
  - Quality of life is suffering….

HOPES: Diseases for stem cell treatment..

- **Osteoarthritis "wear and tear" from aging and sport**
- **Neurotrauma – Alzheimers, Parkinson's, spinal cord injury, motor neurone disease, stroke**
- **Cardiovascular**
- **Repair of Immune system**
  - **Immune deficiency**
    - **Cancer – chemotherapy, radiation induced immunodeficiency**
    - **Aging**
    - **Chronic infection**
  - **Autoimmune disease (type 1 diabetes, MS, rheumatoid arthritis)**
  - **Transplantation tolerance**
- **Skin - burns, wounds**
- **Lung (COPD, asthma, allergies)**
- **Kidney disease**
- **Diabetes……**
- **Healthy start to life – cerebral palsy, lung, kidney**

Our Goals

To establish:

- **"State of the Art" stem cell treatment clinic(s) in Australia, New Zealand, China, USA …**
- **prestigious international partners,**
- **rigorous pre-clinical studies *"Evidence based medicine"***
- **Isolating, analysing and banking a range of stem cells**
  - **Newborn stem cells *"Nature's once in a life-time whole body repair kit"***
  - **Adult stem cells (eg adipose tissue, bone marrow, blood)**
- **Innovative, cutting-edge clinical research laboratories**

" creating a new medicine"

HURDLES

- **Ethical**
  - **Strict Government regulations now in place**
- **Safety**
  - **Must have pre-clinical animal data, preferably large animals (more realistic models for human disease)**
- **Practical**
  - **Which ones to use?**
  - **Commercial IP – who owns it?**
- **Funding – this is a *BIG* problem**

Stem cells are very exciting but
The BIGGEST PRACTICAL PROBLEM….

- **Unless they are your own, stem cell therapies will be considered as foreign transplants and will be rejected by the patients immune system!**

- *Even MSC's are immunogenic and they can up-regulate MHC Class II in an inflammatory environment*

- Induced pluripotential stem cells (iPS)? – safety issues and potential rejection still problems

Overcoming the problem of immune rejection:

Re-educate immune system to accept foreign "stem cell-based" grafts as "Self"

Thymus based tolerance:

- Thymus produces all T cells and
- deletes self reactive T cells
- (only ~5% make it to blood)
- ***Also produces Tregulatory cells***
  - ***inhibit anti self-responses,***
  - ***contributes to peripheral tolerance***
- no general immunosuppression,
- long term - "permanent"

Three major problems with thymus-based tolerance:

1. Need to get "donor" cells into thymus

Donor-host chimeric BM, thymus and immune system:

- present "donor" antigens to developing T cells;
- eliminate any that are potentially "donor" reactive;

2. The thymus rapidly degenerates with age from puberty

- ~5 -10% of its maximal capacity by ~30 years of age

3. Thymus also requires constant input of HSC from BM

- BM also deteriorates with age and badly damaged by chemotherapy/radiotherapy

"Thymus biology problem…"

The thymus

- Early in life produces all mainstream T cells required for immune competence ….

BUT atrophies with age from ~ puberty:

- Fewer naïve T cells, more memory; defective effectors?

- ***Loss of thymus function*:**
  - ***increased opportunistic infections in aged;***
  - ***Increase incidence and burden of cancer;***
  - ***poor recovery from chemotherapy;***
  - ***poor vaccine responses; autoimmunity;***

The key to long term tolerance of therapeutic transplants including stem cells is the Thymus

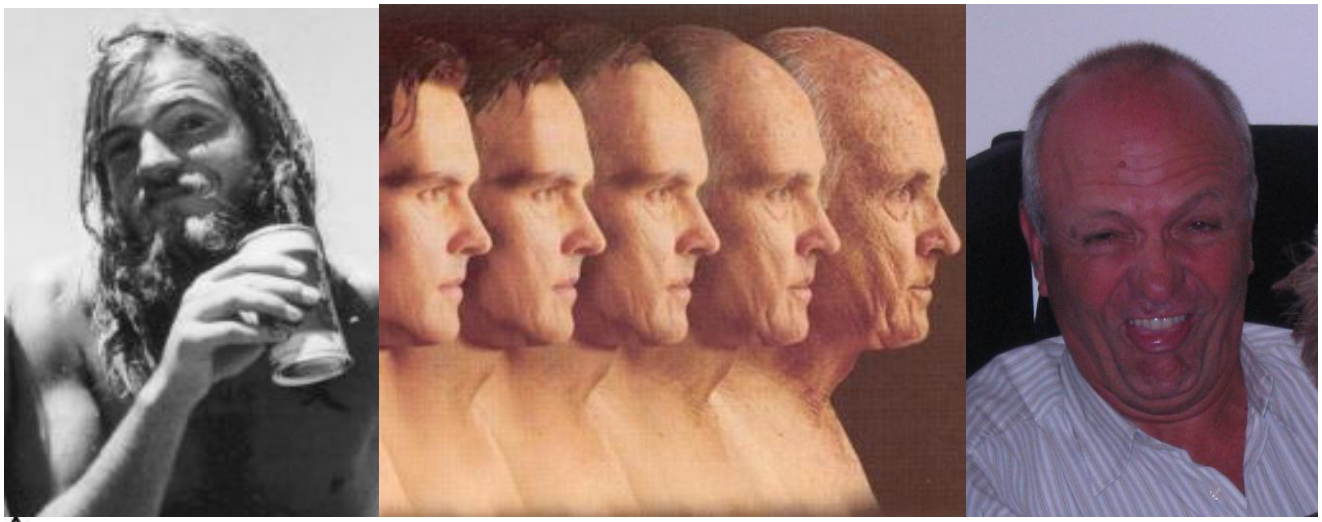
Most diseases needing therapy are in the aged…

BUT the thymus degenerates with age?!

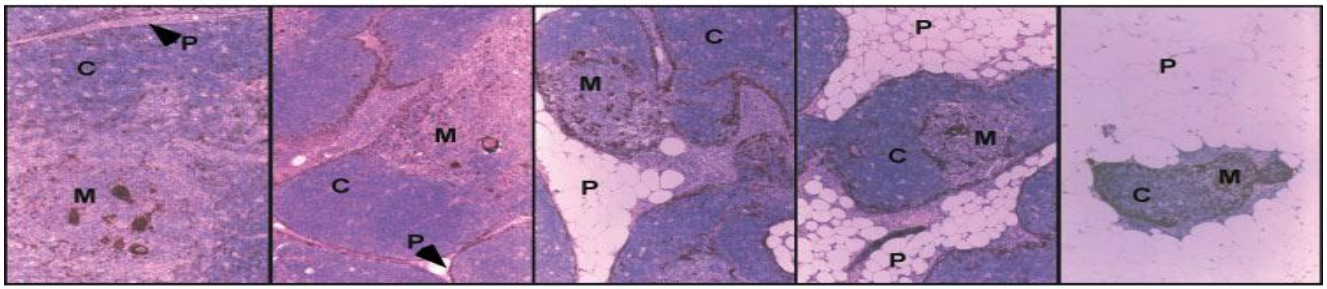
Can we restore the old thymus and immune system ?

Do we even have a thymus as adults?

The several ages of thymic atrophy



A



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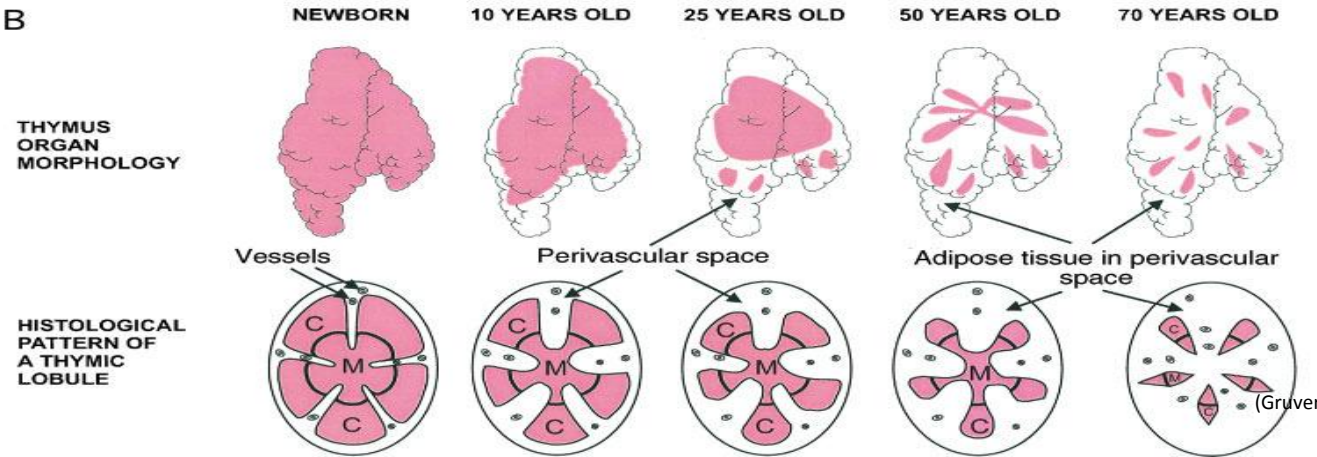


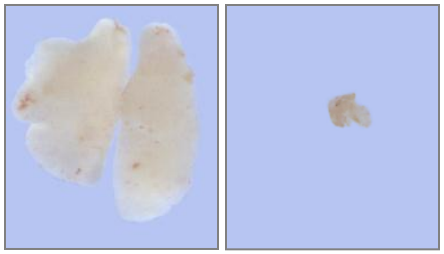
(Gruver, 2007)

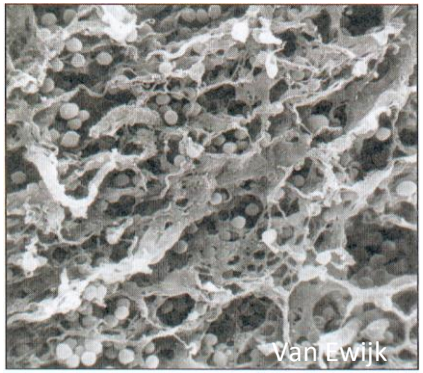
mouse

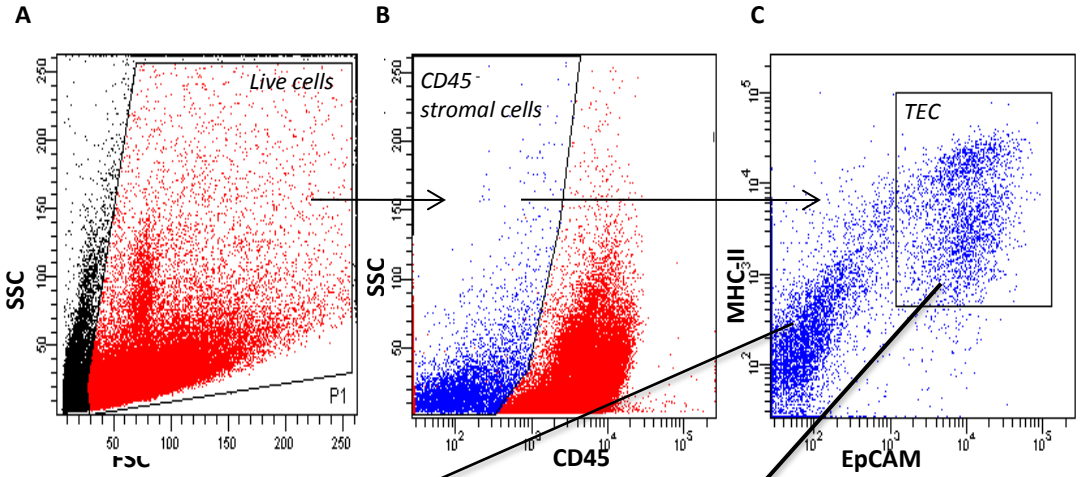
1 mth

24 mth

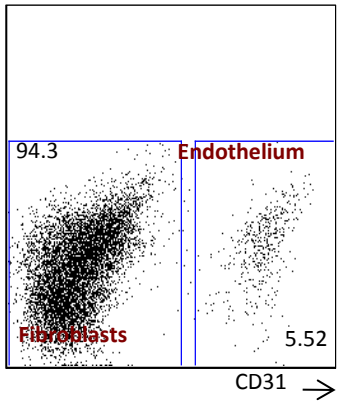
Thymic stromal cell microenvironment: cellular analysis as the basis for rejuvenation

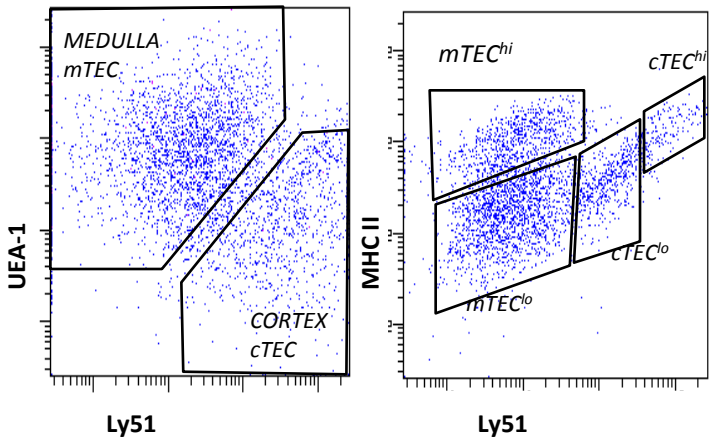






Non-TECs





Hammett, Seach, Chidgey

Can we reverse thymic aging?

A hint from the clinic…

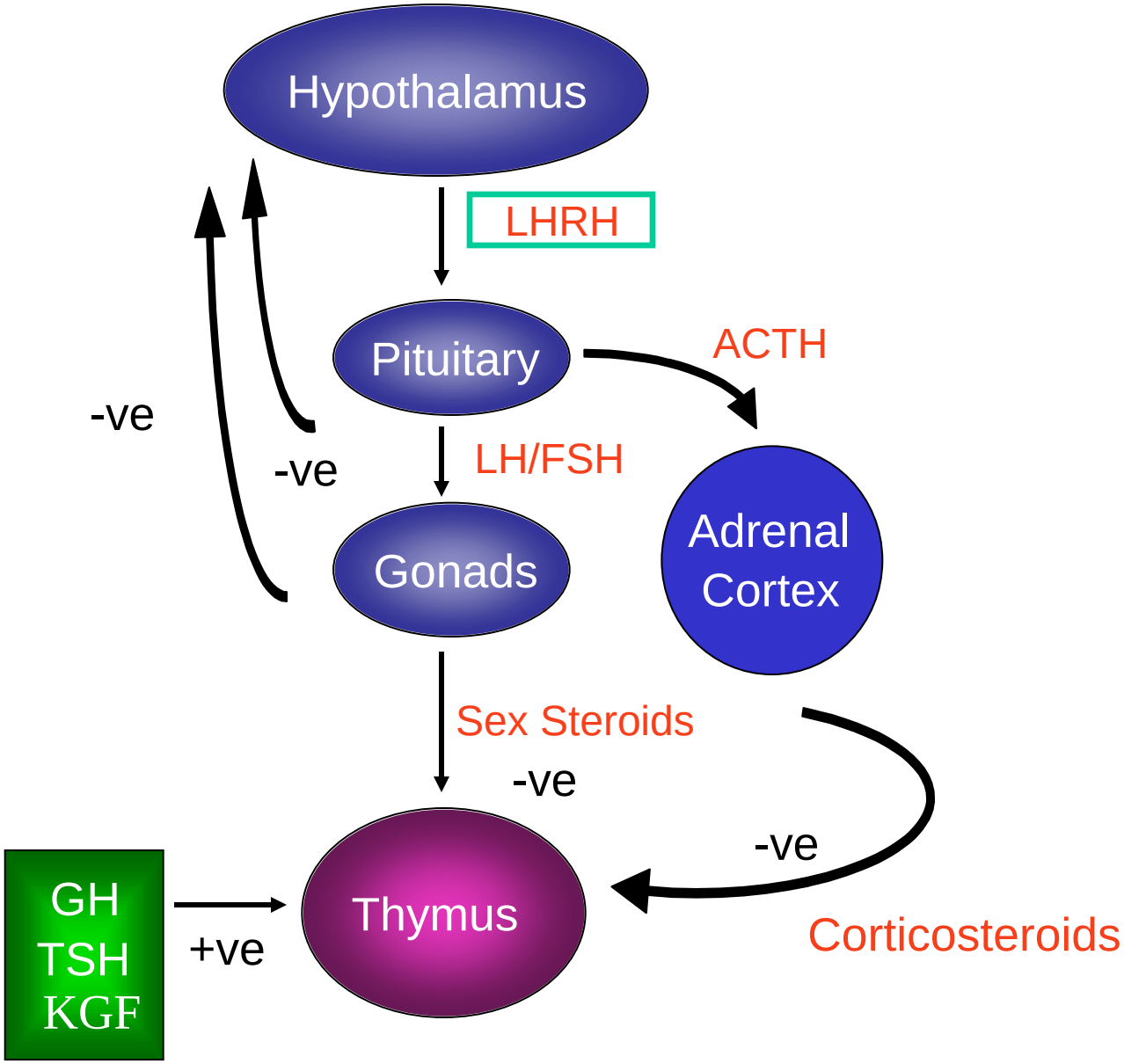
- Children recover immunity post-chemotherapy/HSCT within 6 months;
  - why? - *active thymus*
  - (adults >2 years and then only scant naïve T cells)

- And the major difference between children and adults is…??

Sex steroid levels

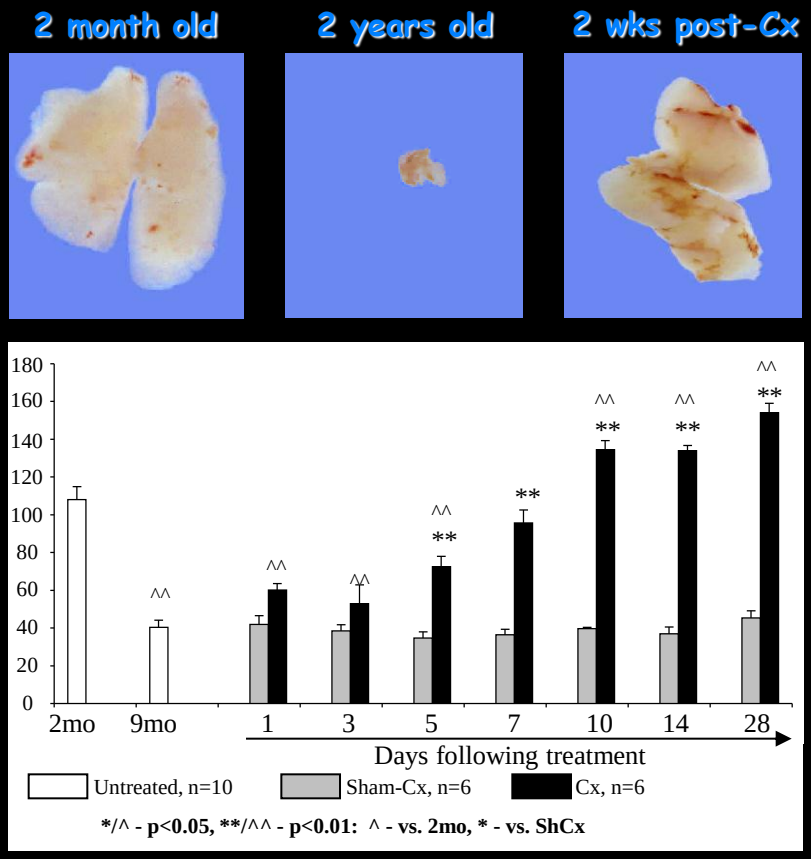
– so how can we politely reduce them?

Neuroendocrine Immune Axis



Can LHRH (GnRH) reverse age-induced thymic atrophy?

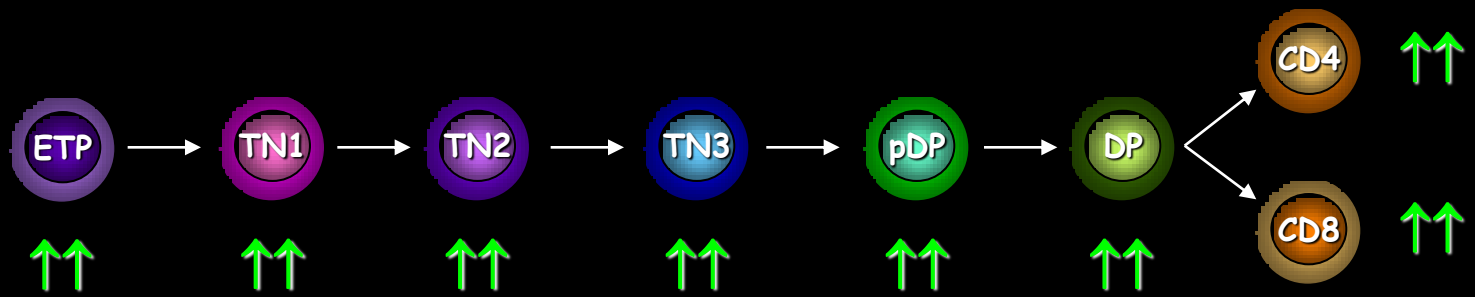
Withdrawal of sex steroids reverses age-related thymic atrophy



- Increased thymic cellularity
- Enhanced production of T cells and export into periphery
- Restoration of thymic architecture
- Improved function of peripheral T cells
- Reduced damage and enhance recovery from chemotherapy
- Improved BM function

Goldberg et al JI 2009, 2010
Dudakov et al, Trends Immunol 2010; JI 2009 (a,b)

Sutherland *et al.* (2005) *J. Imm.* 175:2741-2753
Heng *et al.* (2005) *J. Imm.* 175:2982-2993
Gray *et al.* (2005). *Curr. Opin. Imm.* 17:137-143



Increase in all developing thymocyte subsets

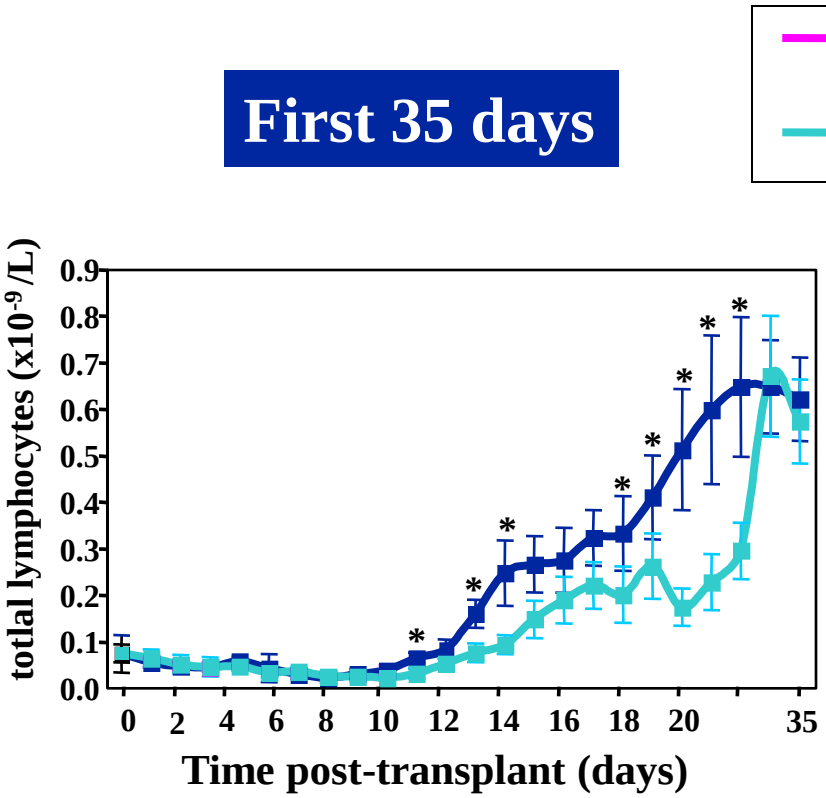
Can we restore thymic function clinically?
Does LHRH work in humans??

- What happens to prostate cancer patients taking LHRH as standard of care?
- -> increase naïve CD4+ T cells
- *(Sutherland et al 2005)*

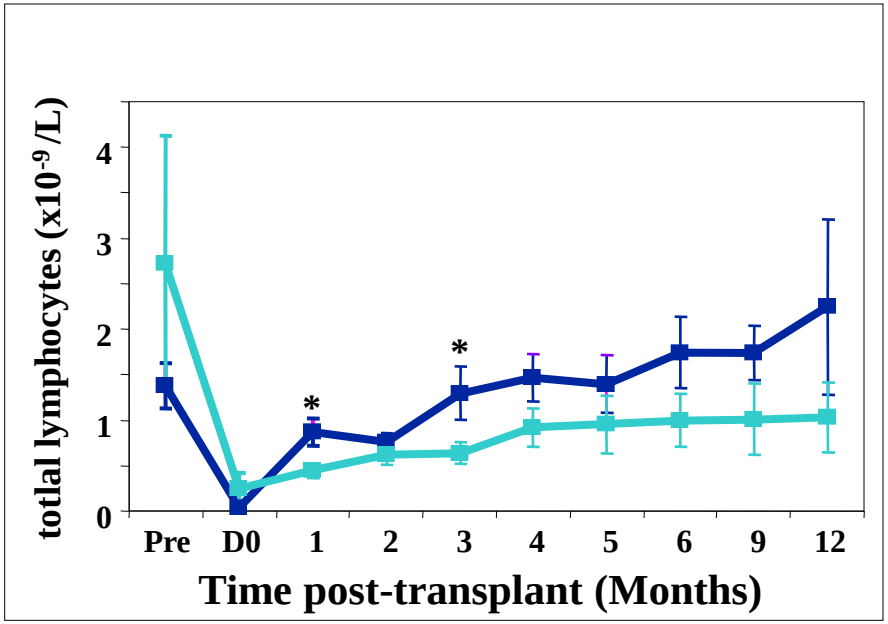
- What about leukaemia/lymphoma patients, high dose chemotherapy, HSC transplants
- Can LHRH restore naïve CD4+ T cells?

LHRH increases Total Lymphocyte Count post-chemotherapy and allo-HSCT

First 35 days

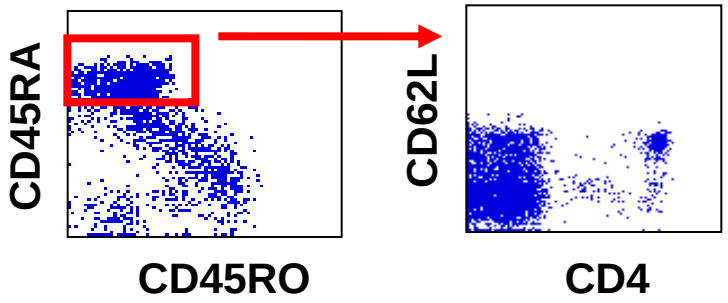


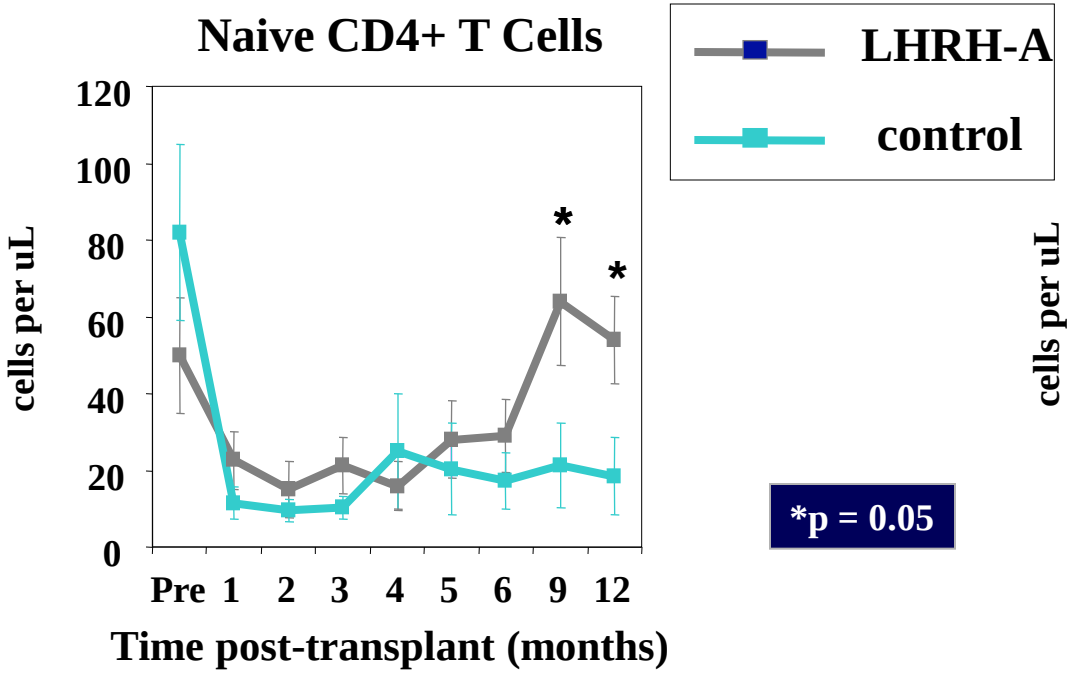
First 12 months

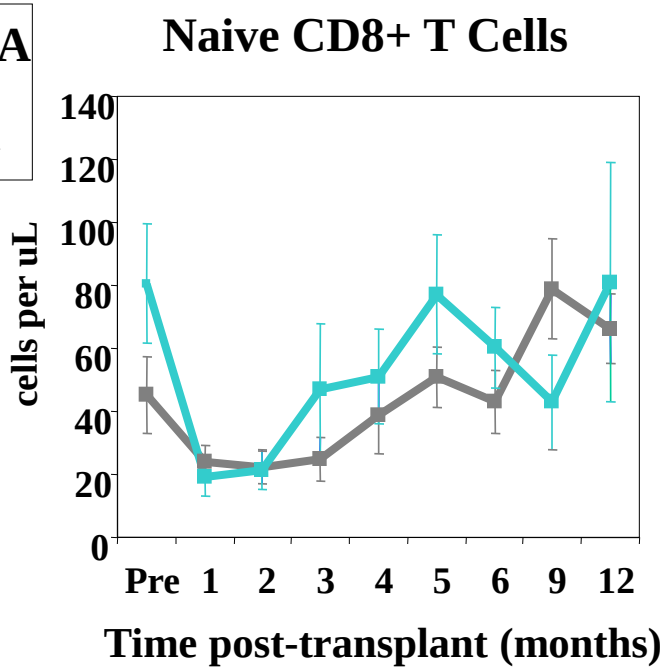


*p = 0.05

LHRH enhances Naïve CD4+ T cell recovery post-chemotherapy and allo-HSCT







LHRH increases IgM and IgG responses to neoantigen (KLH) in double-blinded, randomised, placebo controlled, multi-centre trial in cancer-BMT

Table 2. Comparison of the Differences Between Pre- and Post-Vaccine Measurements

	Placebo				Leuprolide Acetate 3M Depot 11.25 mg			
Outcome measure	N	Mean (SD) Post-Pre Difference	Min-Max	*P* value[a]	N	Mean (SD) Post-Pre Difference	Min-Max	*P* value[a]
IgM	5	0.57 (0.83)	-0.16-1.84	0.31	8	2.91 (2.07)	0.99-7.38	0.008
IgG1	5	16.76 (28.15	-0.42-65.87	0.19	8	46.16 (44.01)	12.83-147.16	0.008
IgG2	2	0.23 (0.47)	-0.01-0.56	1.00	4	19.05 (24.91)	-1.95-54.38	0.25
IgG3	1	0.07	0.07	1.00	3	2.09 (0.71)	1.31-2.70	0.25
IgG4	0	NA	NA	NA	3	0.45 (0.17)	0.31-0.64	0.25
IFNγ	5	10.25 (11.41)	1.47-30.26	0.06	6	2.09 (4.85)	-2.60-9.97	0.44

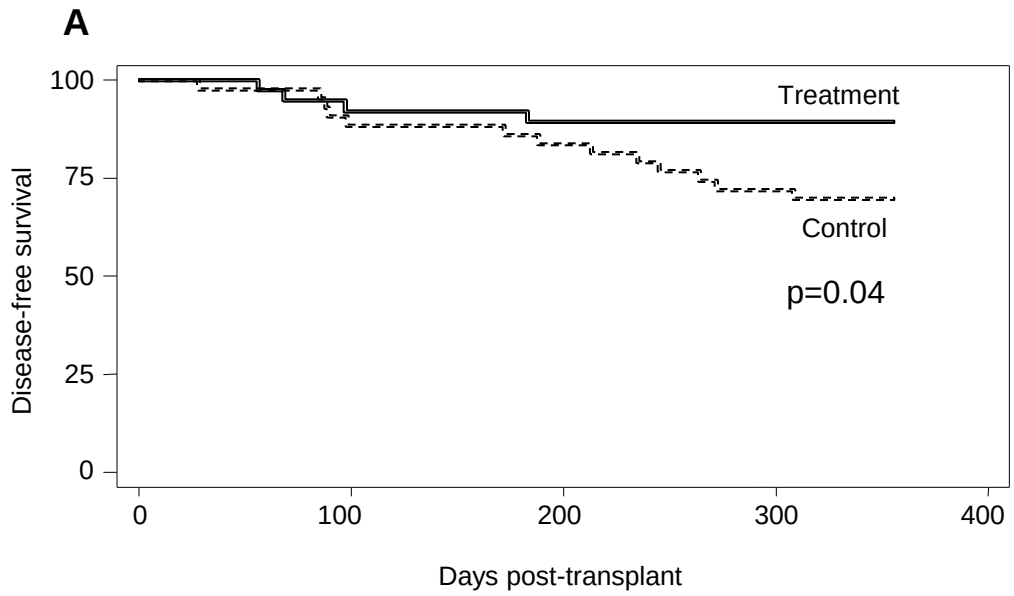
[a]2-sided *P* value from Wilcoxon signed rank test. IgG indicates immunoglobulin G antibody; IgM, immunoglobulin M antibody; IFNγ interferon gamma; NA, not available. Pre-KLH vaccination assessment conducted at 6 months and post-vaccination at 7 months. Values below lower limit of quantification were not included in the table

Champlin et al 2011

The return of thymus function means:

- More rapid recovery from chemotherapy and radiation
- Boost in new T cells exiting to the blood
- Better responses to infections
- Better responses to vaccines
- Lower incidence of cancer?

Clinical: LHRH Enhances disease free survival in autologous HSCT patients…..*but early days..*



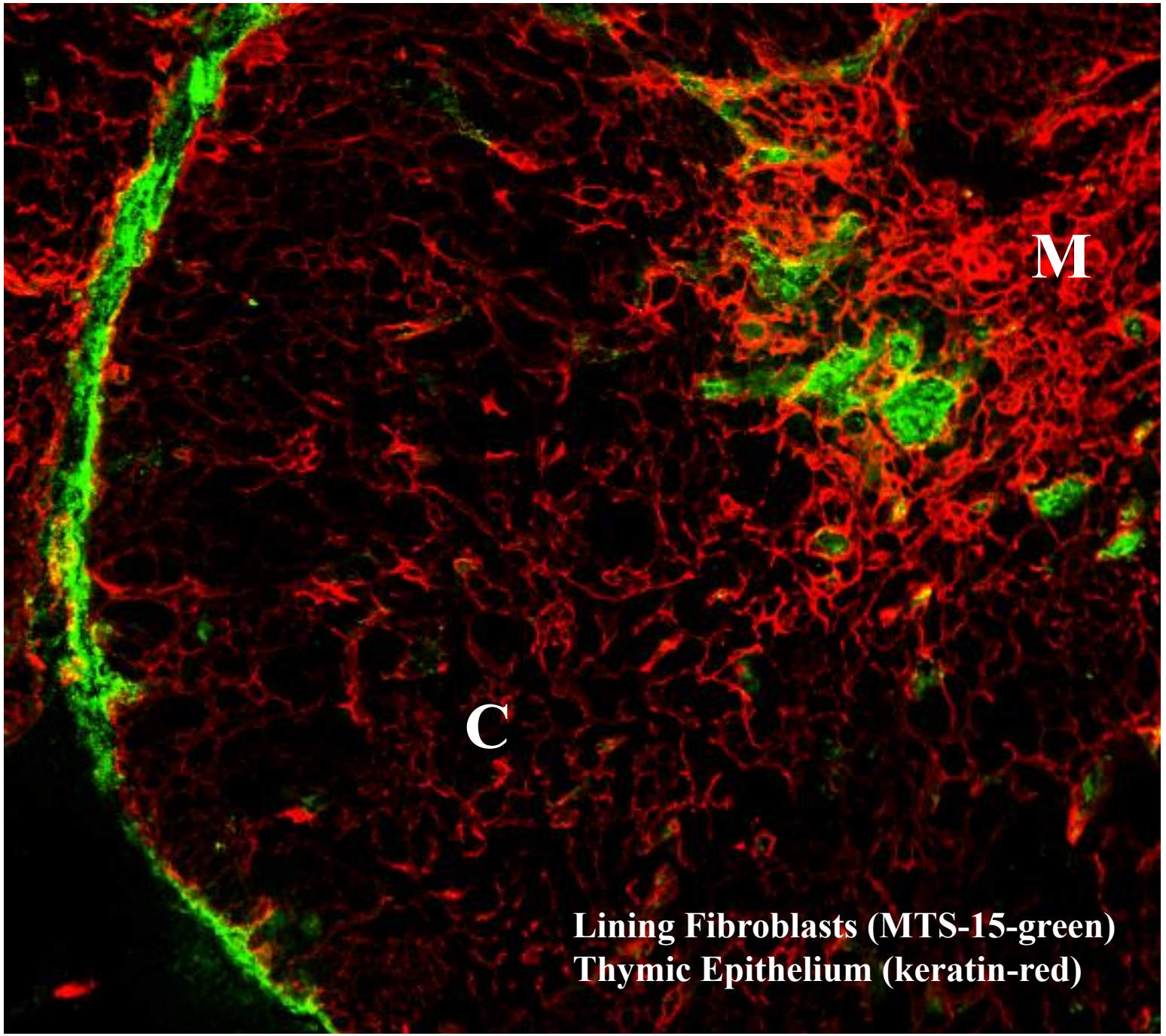
Thymus Regeneration with *DONOR BLOOD STEM CELLS (HSC)* creates a blood cell "chimera" = donor tolerance!

- Donor HSC engraft in bone marrow
- Migrate to thymus
- Convert to "donor" T cells and Dendritic cells
- *Present donor MHC to developing T cells*
- *Induce tolerance to donor*
  - *Clonal deletion; T Regulatory cells*
- Any graft from that donor should be accepted
- *We have now proven that this works*
  - *Donor skin graft retained for over a year*
  - *Non-donor graft rejected.*
  - ***PROBLEM OF STEM CELL REJECTION SOLVED???***

LHRH protects young mice before increase in sex steroids – how?

A Clue: the thymus produces its own LHRH

- This is increased in castrated mice
- The thymus has receptors for LHRH
  - Expressed in low levels in cortical and medullary epithelial cells,
  - highest in thymic mesenchymal fibroblasts


M
C
Lining Fibroblasts (MTS-15-green)
Thymic Epithelium (keratin-red)

Clinical LHRH is "agonist"
Stimulation of endogenous stem cells?

- may act directly by stimulating fibroblasts

- MTS 15+ fibroblasts express high levels of **growth factors** for thymic epithelium – **stem cells?**
  - Increased levels when thymus damaged eg chemotherapy, eg FGF 1, 7, 10, IL6
    - Gray et al J.Immunol 2007

Not all patient's thymuses respond to rejuvenation

Thymus Regeneration : stem cells to the rescue??

- What are the targets of aging?
  - HSC/ lymphoid progenitors in BM - source of T cells
  - *(Danika Khong, Jaz Dudakov, Ann Chidgey)*

  - ***Thymus epithelial cells*** are absolutely essential for T cells
    - Is there a **thymus stem cell**?

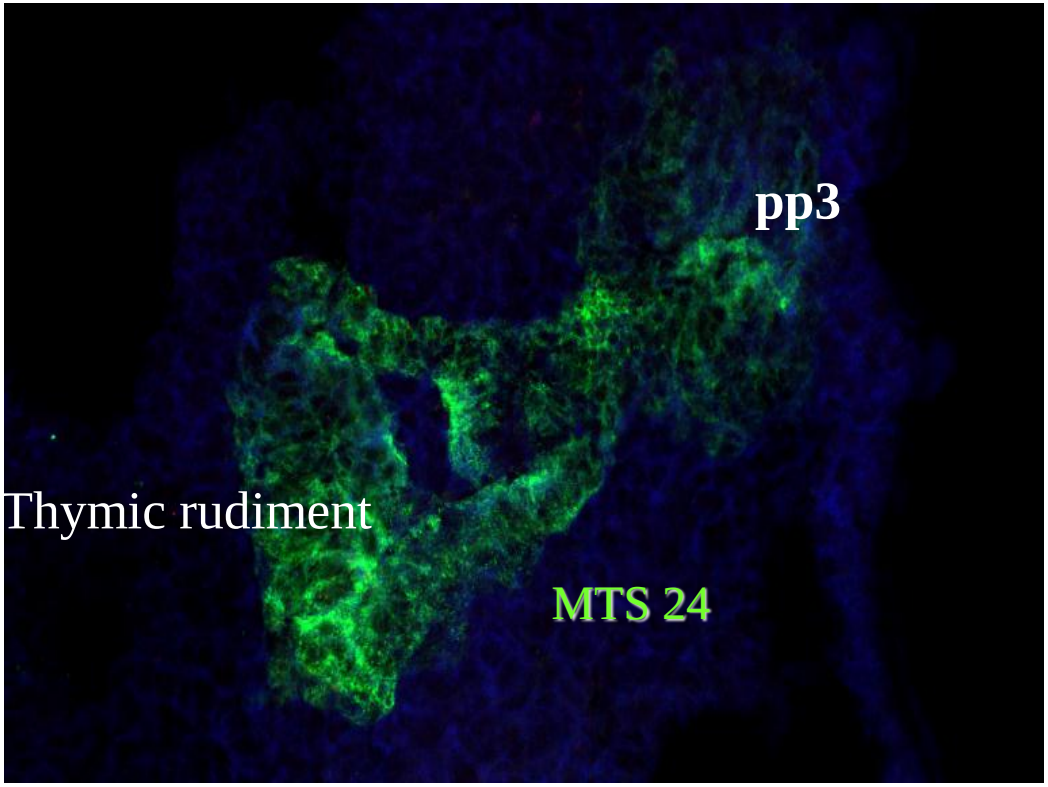
Thymic development

MTS 24 identifies thymic epithelial stem cell

At E11 thymus begins from the 3 pharyngeal pouch

Ù HSC migrate in from E12

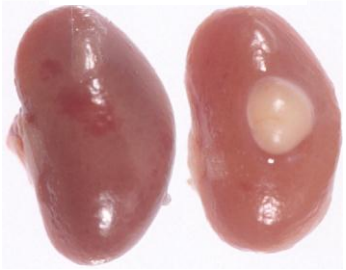
Ù MTS 24 labels thymic primordial epithelium



Can these stem cells make a thymus?

MTS24^{-} **MTS24^{+}**

R1 **R2**

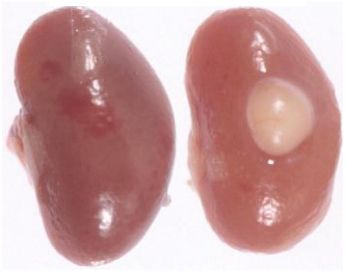


8 wks post transplantation

CD45^{-}MHCII^{+}MTS24^{+} thymic epithelial cells: develop into a complete thymus

MTS24^{-} **MTS24^{+}**

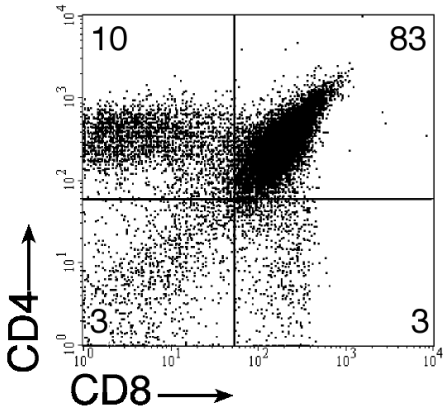
R1 **R2**



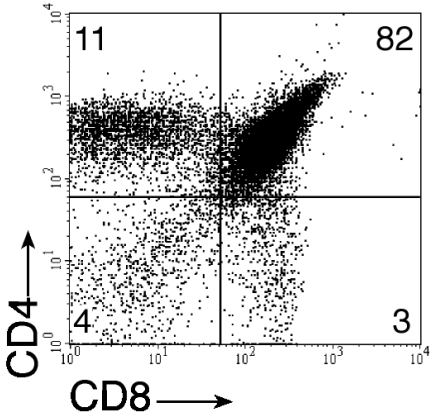
8 wks post transplantation

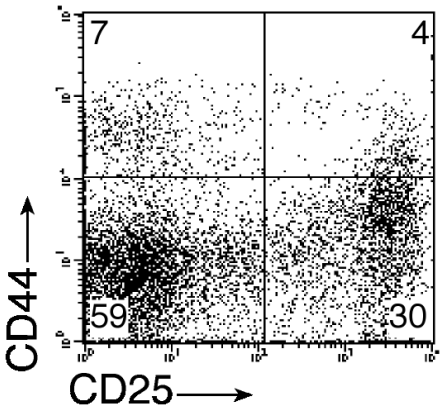
(Gill et al, Nature Imm. 2002)

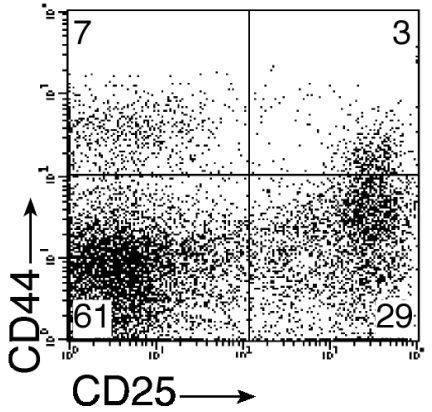
MTS24^{+} derived



E15.5 control



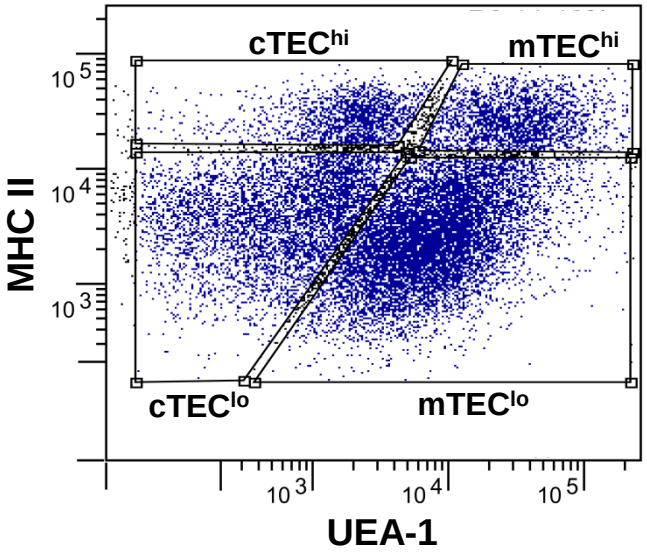


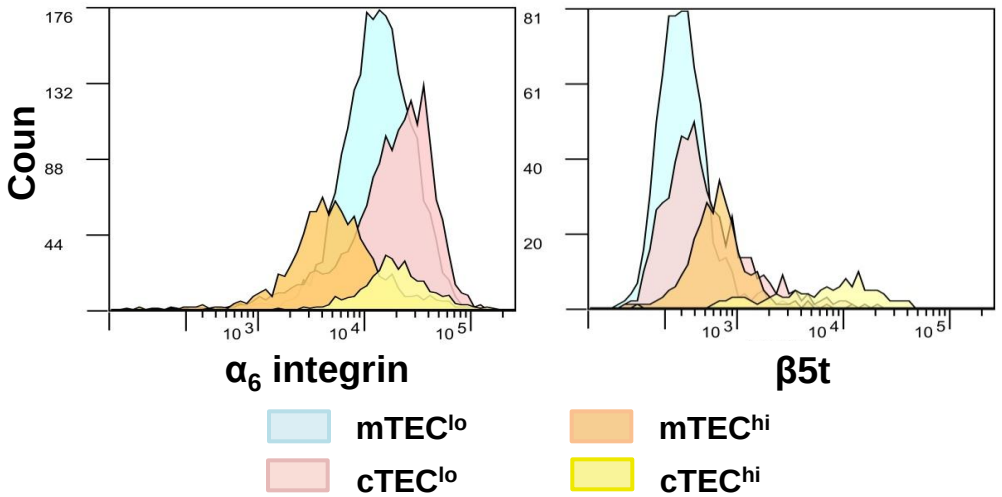


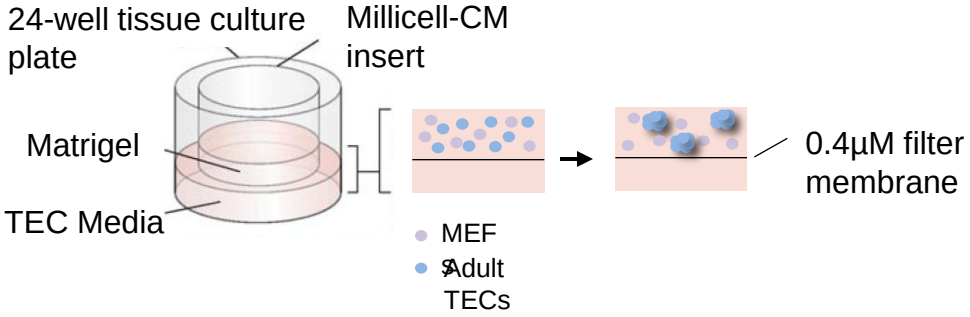
Sadly…

- MTS 24 + cells lose their capacity to form a thymus by the end of gestation!
- Why? Technical problem? Change in function?
- Is there an adult thymus stem cell equivalent?

Searching for *ADULT* thymic epithelial stem cells: phenotype and function

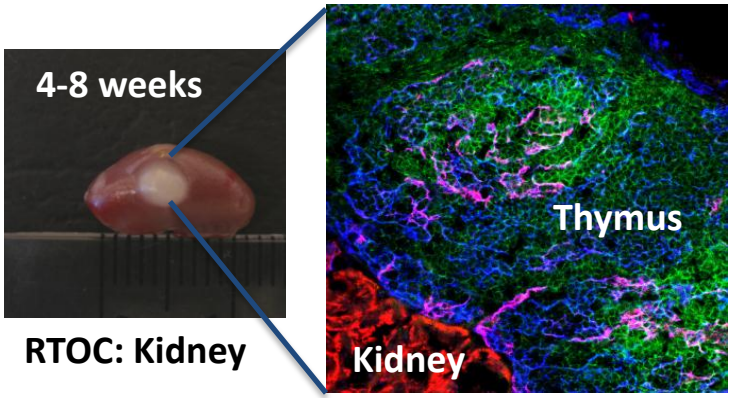






Modified from (*Bertoncello and McQualter, 2011)*

E15+adult TEC



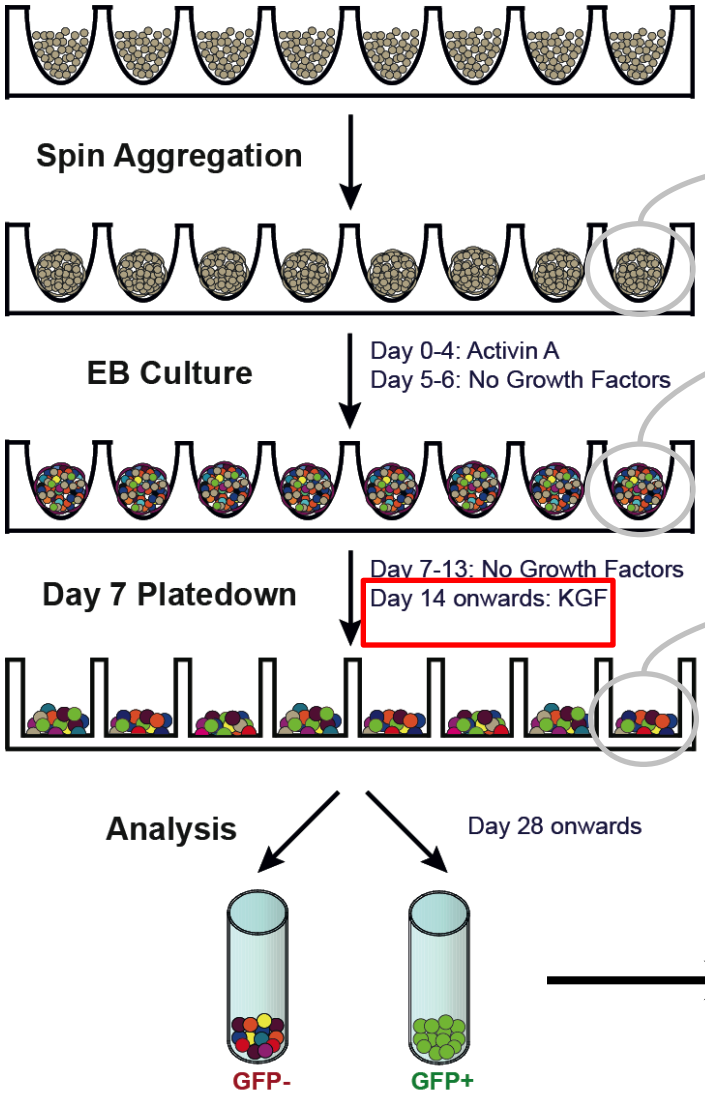
Wong, Seach, Chidgey

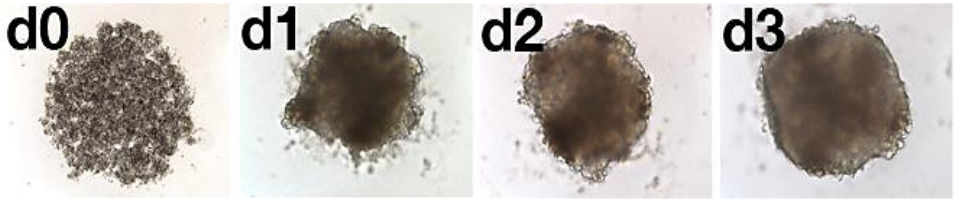
Can we create thymic stem cells?

Derivation of thymus from ES cells:

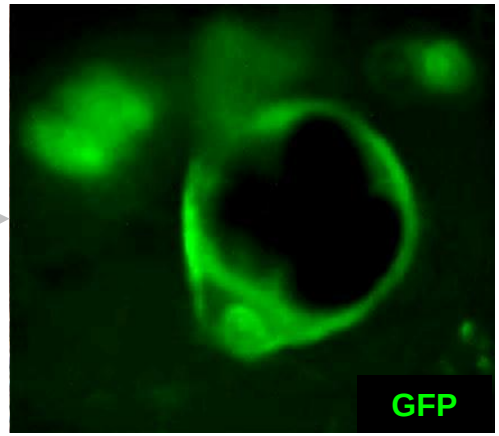
- GFP targeted “Fox N1” (mouse, human)

Differentiation of FOXN1$^{GFP/w}$ hESCs





(Ng et al, 2008, Nature Protocols)

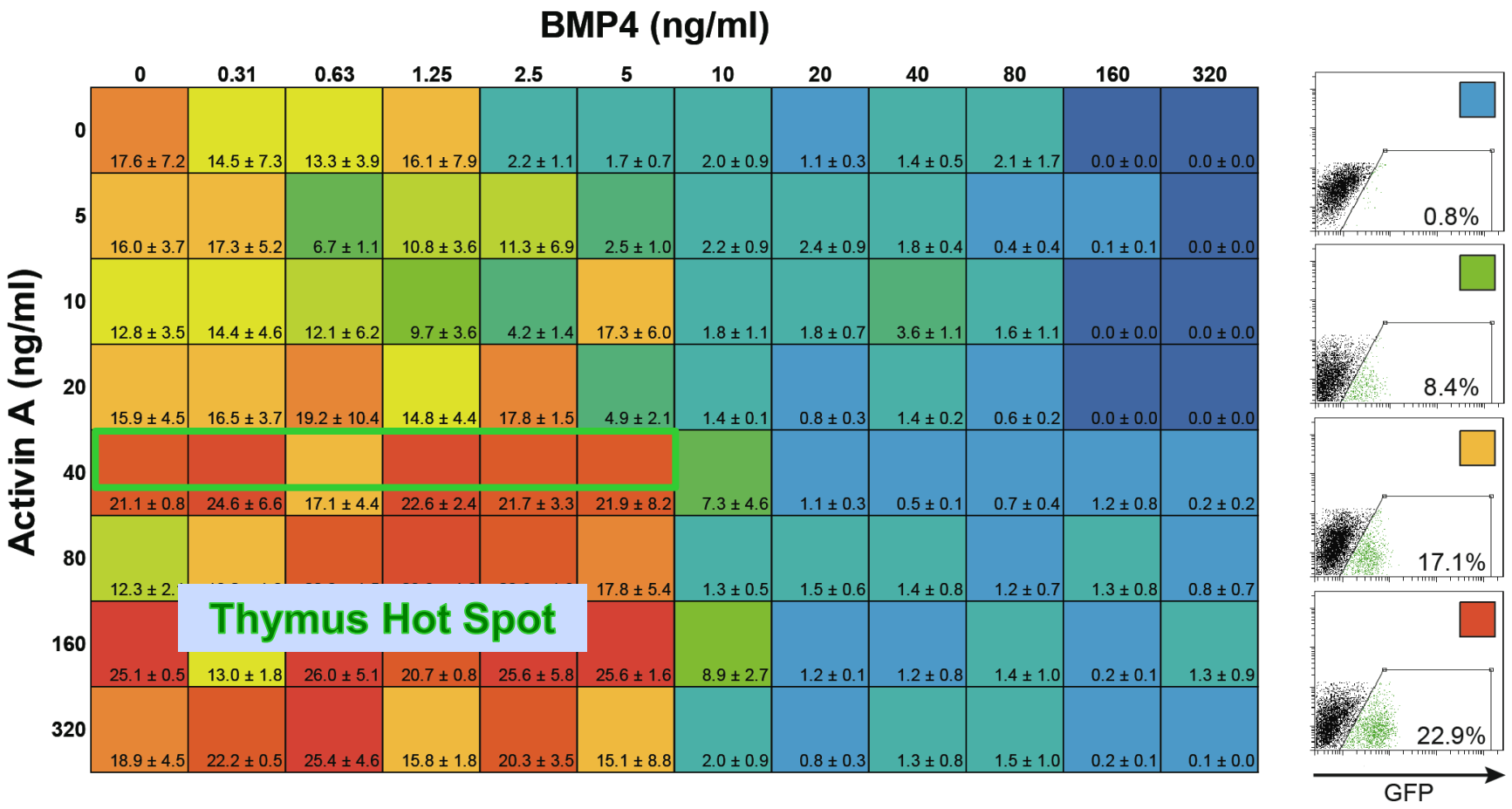


FACS analysis, qPCRs

Chew-Li Soh, Ed Stanley

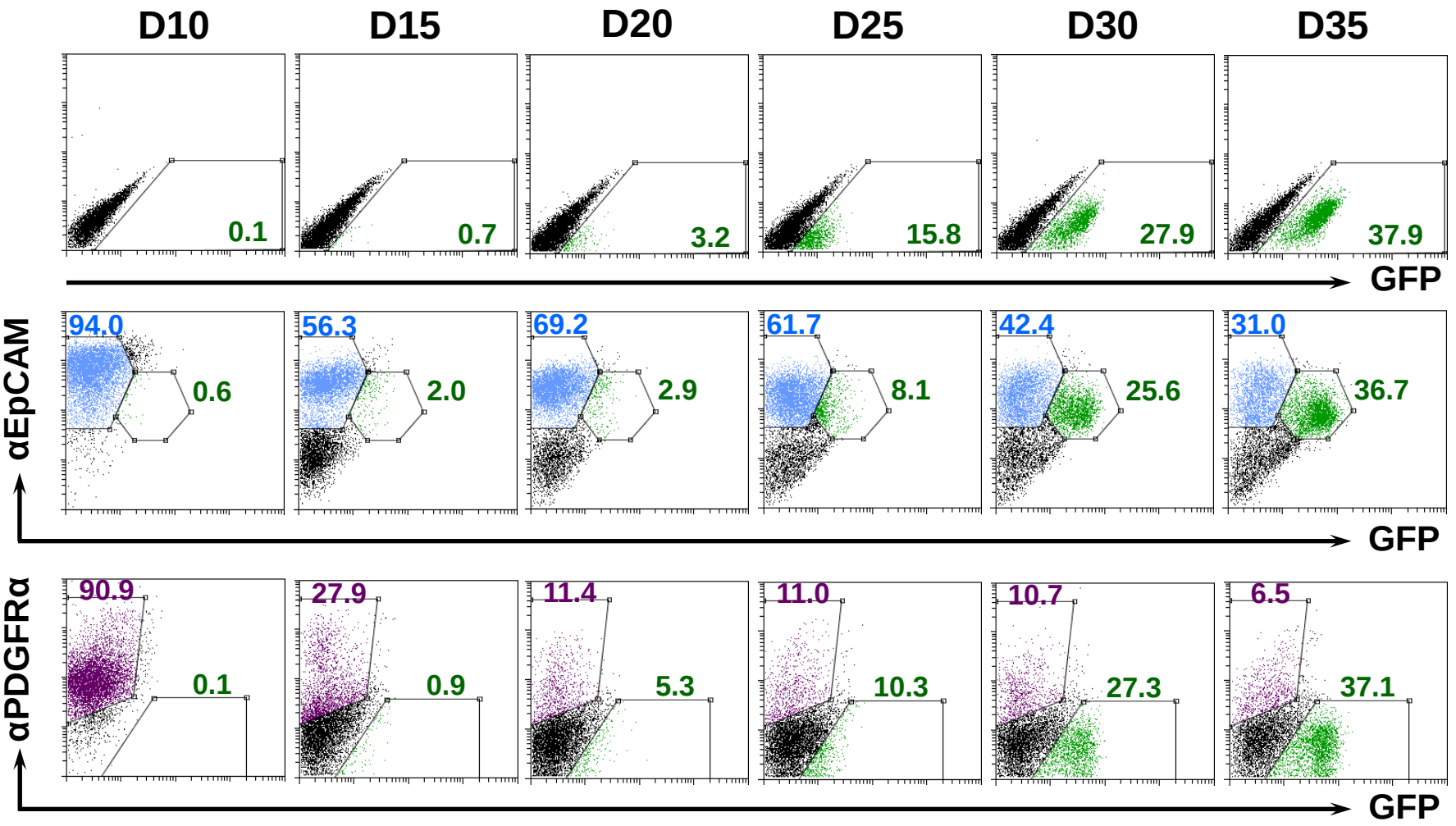
Differentiation of FOXN1$^{GFP/w}$ hESCs

FOXN1$^{GFP/w}$ APEL Medium 'Heat Map':



Chew-Li Soh, Ed Stanley

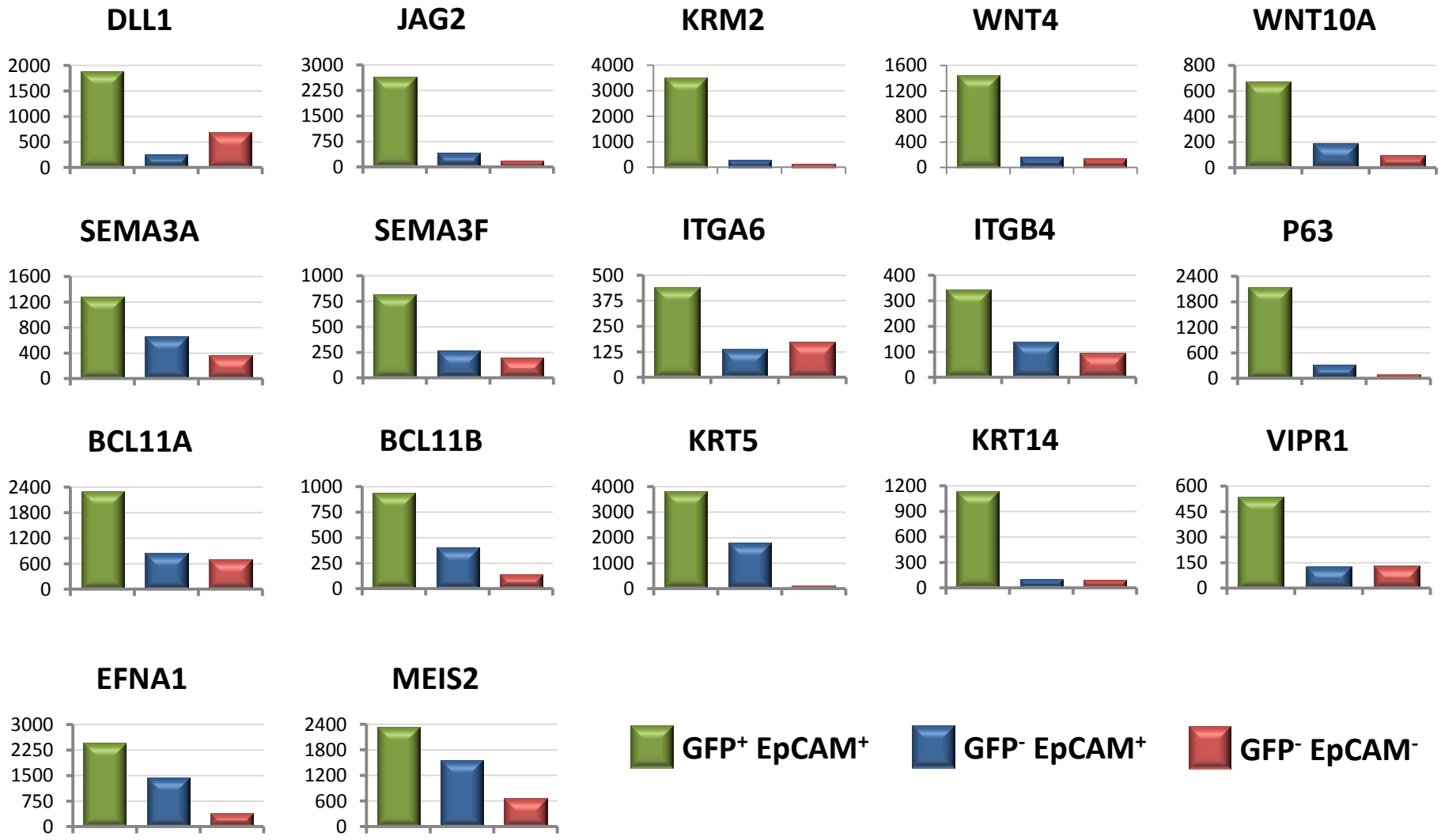
Differentiation to FOXN1-GFP⁺ Cells



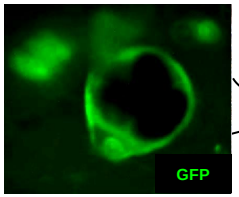
FOXN1-GFP⁺ cells emerge at ~D20 of differentiation,
and co-express the TEC marker **EpCAM**

FOXN1-GFP⁺ cells *do not* co-express the mesodermal marker **PDGFRα**

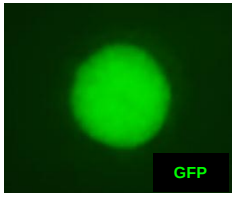
TEC-Associated Genes Expressed in GFP$^+$ EpCAM$^+$ Cells



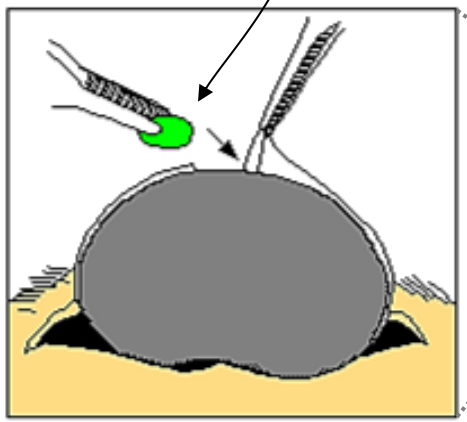
GFP+ Cells Form Kidney Grafts



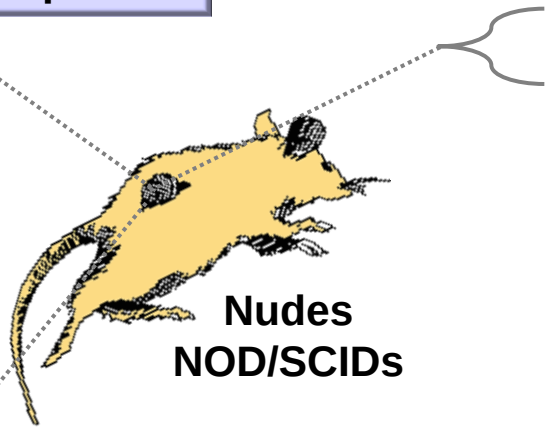
Whole EBs



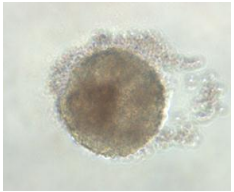
GFP+ EpCAM+



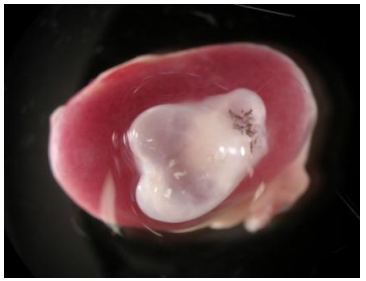
Kidney Capsule Grafts

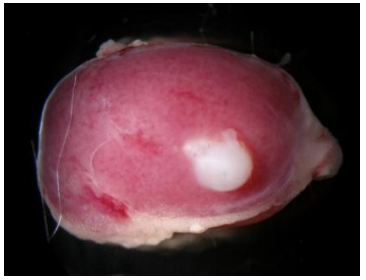


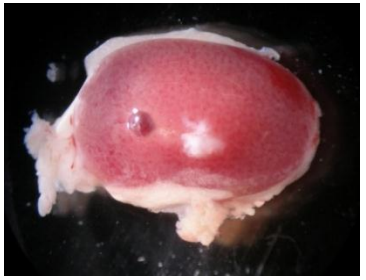
Nudes NOD/SCIDs

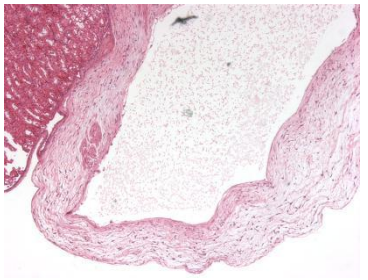


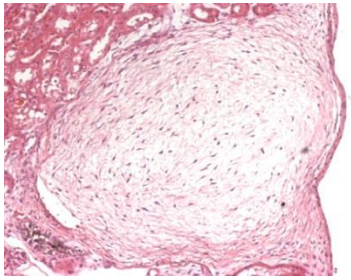
GFP- EpCAM-

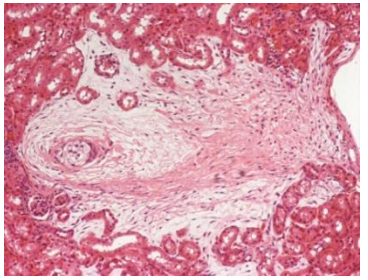


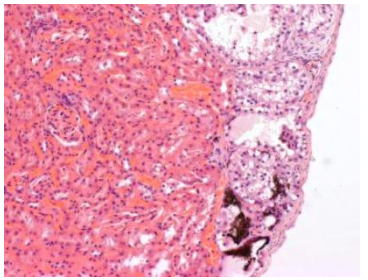












A HURDLE

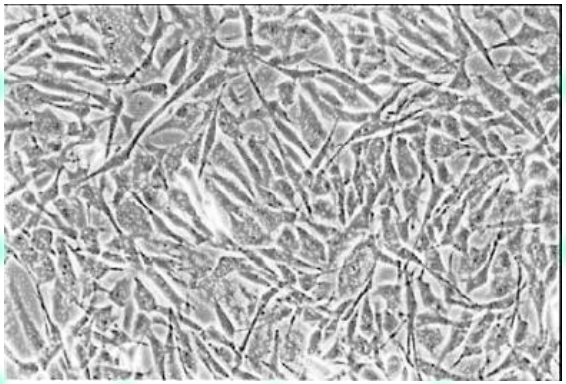
Stem Cell are very exciting but

back to the BIGGEST PRACTICAL PROBLEM….

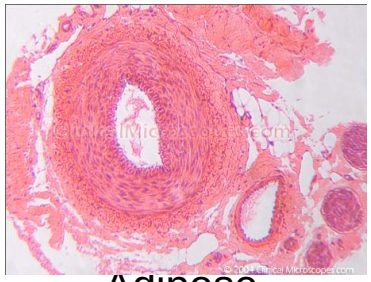
- **How can we find a source of our own multipotential/pluripotential stem cells, that are safe, ethical and effective in treating a wide range of diseases ?**

Capabilities of MSC

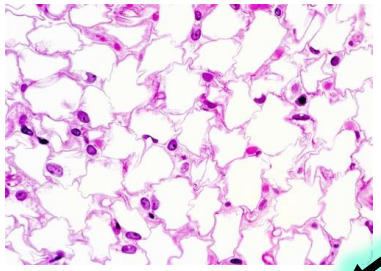
MESENCHYMAL STEM CELLS



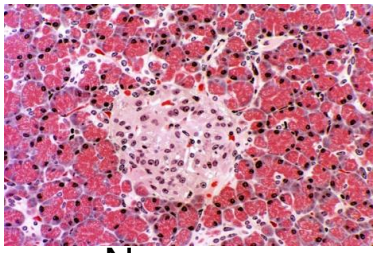
Endothelium



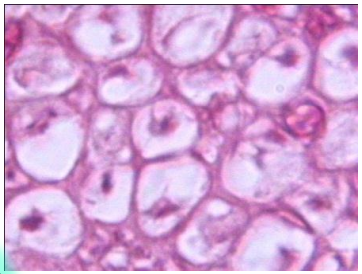
Adipose

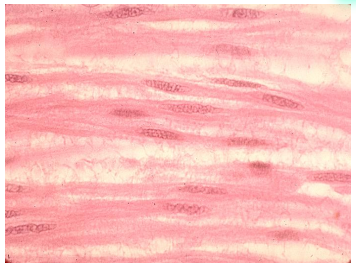


Pancreas

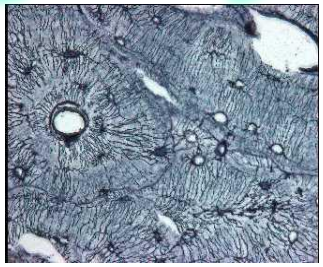


Neuron

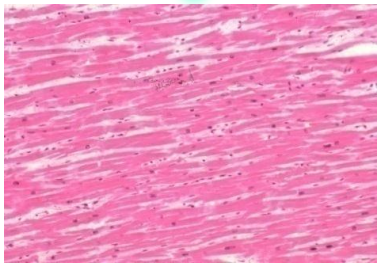




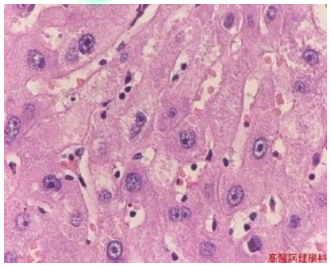
Muscle



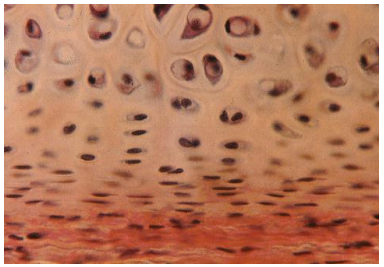
Bone



Myocardium



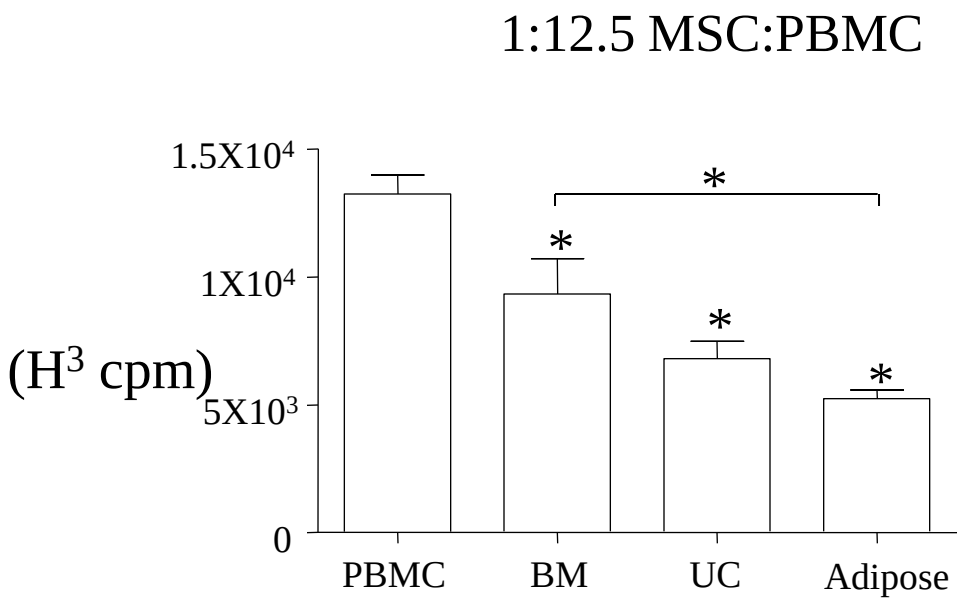
Liver



Cartilage

MSC are anti-inflammatory/immunosuppressive

Inhibition of T lymphocyte MLR proliferation assay



Adipose MSCs may be more suppressive than bone marrow or umbilical cord……

Veterinary applications

- to improve animal health; pre-clinical model for humans
- **Adipose-derived cells:**

  (A) Stromal vascular fraction (SVF)

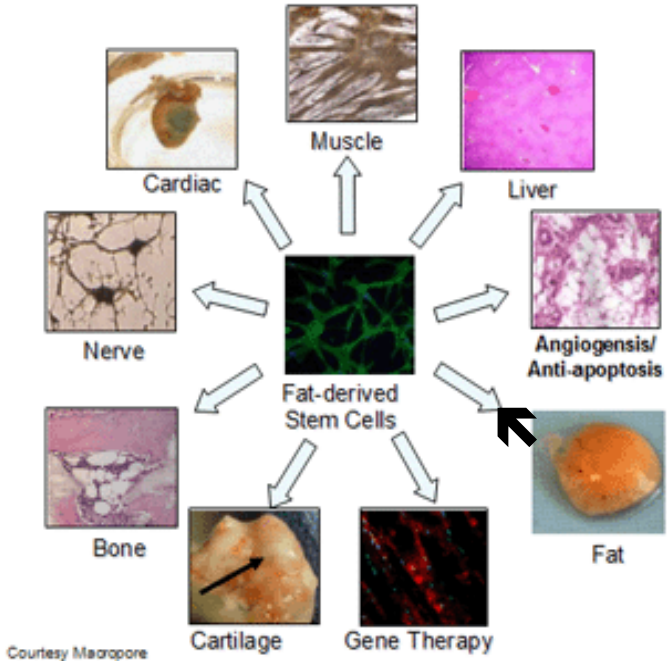
  – MSC, Tregs, type II Macrophages

  – Pre-adipocytes, fibroblasts, endothelium

  (B) In vitro expanded MSC

  – Immunosuppressive; pro-repairing
- Horses with bowed tendons, ligament injuries, and fractures,
- Dogs with osteoarthritis
- More than 5,000 horses treated since 2003
- More than 4,500 dogs treated since 2006
- More than 350 dogs, cats, horses in Australia

  (Australian Veterinary Stem Cells , Vet Stem)



Veterinary applications -

SVF

- *>70% of all animals responded*
- *No serious adverse effects*
- *Strong evidence of pain reduction and return of capacity*

Expanded MSC

- *>70% of all animals responded*
- *100% of younger dogs*

Osteoarthritis (most)

Chronic kidney failure

Cardiovascular disease (dilated cardiomyopathy)

(Australian Veterinary Stem Cells , Vet Stem)

MSC's are clinic ready…

- TGA approved
- Easily obtained
- Isolation with "minimal manipulation"
- Can be expanded in culture; GMP requirements
- Autologous (no risk of rejection); "here and now"
- Allogeneic (off the shelf for acute conditions; next phase)
- Excellent pre-clinical safety and efficacy data

Human Evidence – MSC Safety

Author	Patients	Disease	Injection	Follow-up	Study	Symptom Improvement
Wakitani 2010	41	Knee Osteoarthritis	IA	11 years	Safety Study	No Cancer, No infections
Centeno 2010/2011	339	Knee, Back, Hips	IA, Paraspinal	2 years	Safety study	No Cancer, No infections
Rodrigeuz 2012	13	Rheumatoid Arthritis	IV and IA	13 months	Safety study	One episode of fever & myalgia
Guadalajada 2012	50	Perianal fistulas	Local	3 years	Safety and efficacy	No Cancer No injection

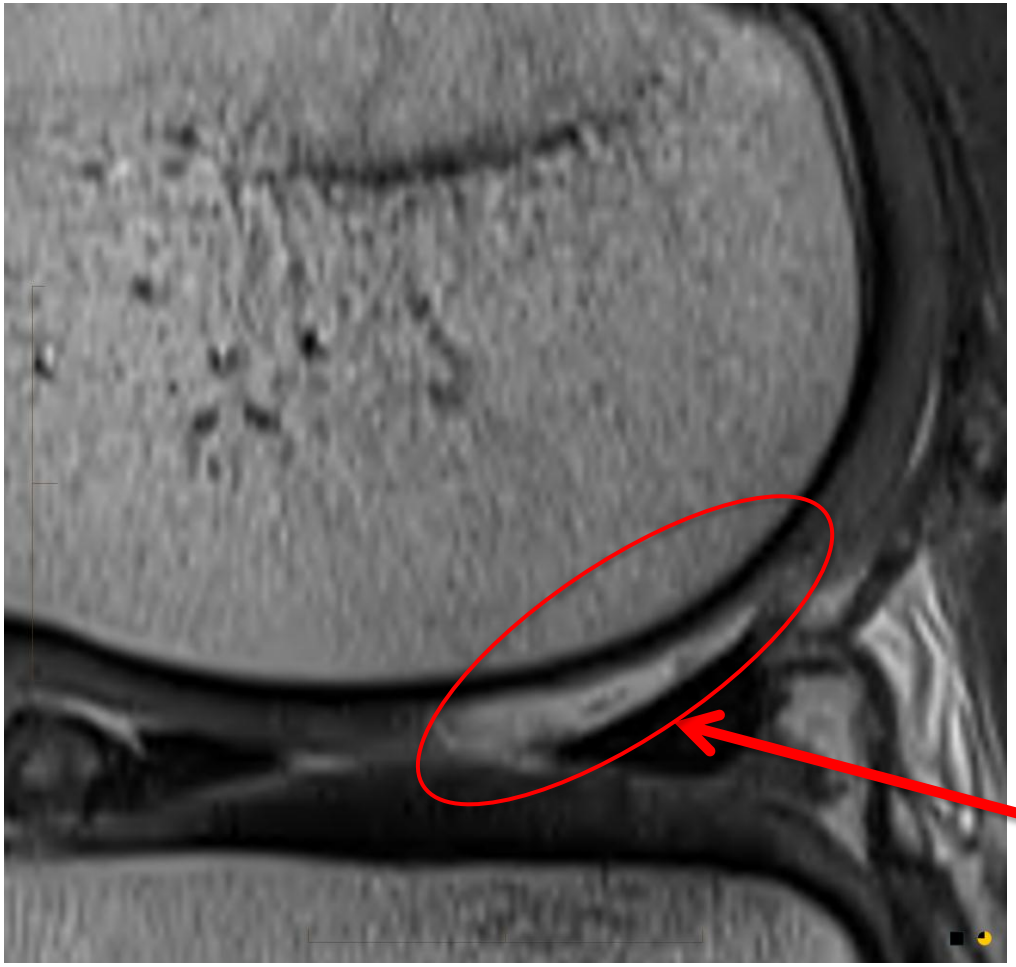
LAKESIDE
SPORTS MEDICINE CENTRE

Human Chondral Defects, OA

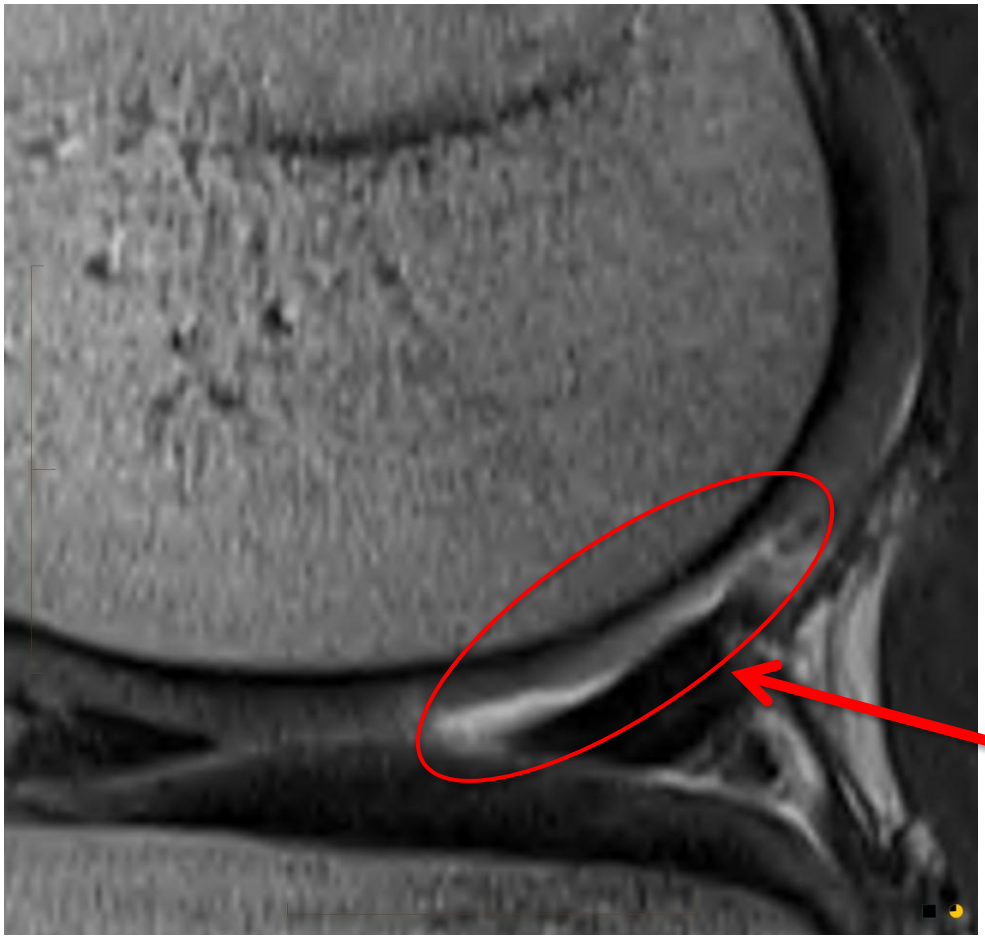
Dr DAN BATES

- 12% of the population will suffer chondral lesions
- These progress to osteoarthritis
- 15% of the Australian population suffer Osteoarthritis
- Costs 23 billion dollars per year to Australian economy

Left Lateral Femoral Condyle – Pre-treatment



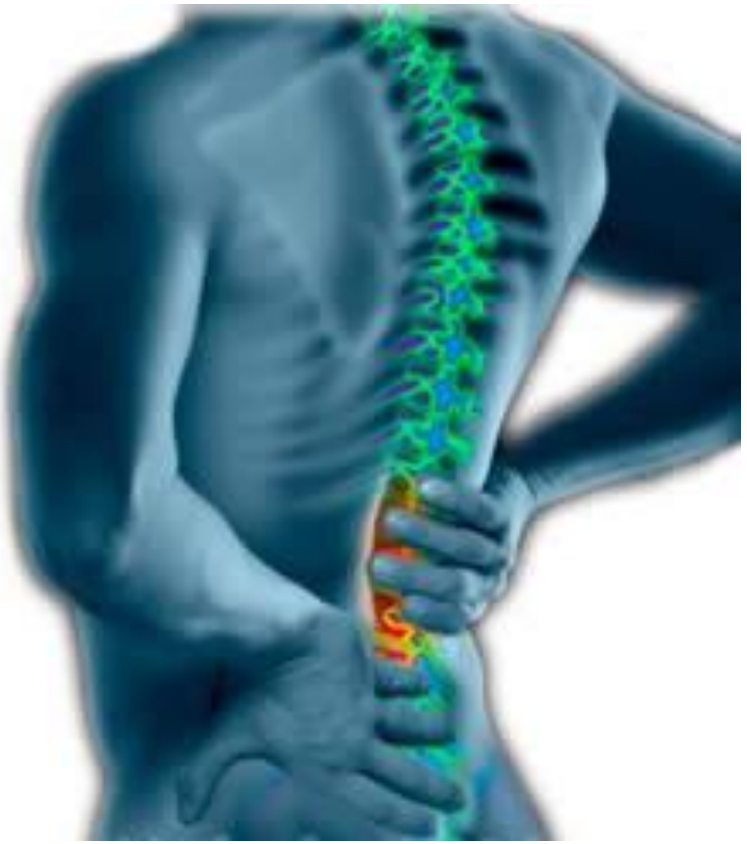
12 Months post treatment



Defect Coverage at 12 months (Grey Line)

Spinal disc injury
– repair with mesenchymal stem cells!

- 80% of population back or neck pain
- 30% of asymptomatic individuals have **degenerative** change on MRI
- 90% of asymptomatic individuals over 60 years of age have **degenerative** change on MRI
- Rebuild disc with mesenchymal stem cells Tony Goldschlager (Mesoblast)



SUMMARY: Immediate Clinical Trial Opportunities for MSC's : degenerative and inflammatory conditions

Utilise the anti-inflammatory, pro-repairing properties of MSC from AUTOLOGOUS adipose (SVF; expanded MSC) or Umbilical Cord (allogeneic or autologous)or bone marrow

Over 300 clinical trials with MSC registered on NIH Clinical Trials register

- Osteoarthritis
- Autoimmune disease
- Inflammatory bowel disease
- Type 2 Diabetes
- Respiratory failure (COPD, asthma)
- Cardiovascular disease
- Liver cirrhosis
- Spinal disc repair

Acknowledgements

All MISCL Staff and students

Associate Prof Ann Chidgey,

Professors Zhicheng Xiao, Claude Bernard, Nick Birrell

Mark "every lab should have one" Malin

Thymus regrowth and stem cells

Nat Seach, Kahlia Wong, Chew-Li Soh, Ed Stanley, Marco Barsanti, Maree Hammett, Lisa Spyroglou, Jarrod Dudakov, Gabby Goldberg, Jayne Sutherland (Jade Homan, Jade Barbuto)

Neonatal stem cells

Immune Regeneration

MSC & AEC in Wound Repair

Standard Operating Procedures for isolation of AECs

Adrienne Calder, Anthony Park, Abdulaziz Alsharif

Asthma

Tracy Heng

Sacha Khong

Tolerance

Tracy Heng

Jess Morison

FRC

Anne Fletcher

AVSC – Magellan – CMA Dr Dan Bates, David Connell

Peter Hansen, Peter Britton, Geoff Barnard

Peking Union Medical School

"Robert" Zhao

ProStemCell

Louis Chan, Robert Chin,

Anthony Wong, Kenneth Chan

MONASH University

miscl monash immunology and stem cell laboratories

Stem Cells and Immune Regeneration Lab – gotta love ‘em!

Cheers