Pathology Summer School 2016

“Towards a cure: the role of pathologists in regenerative medicine”

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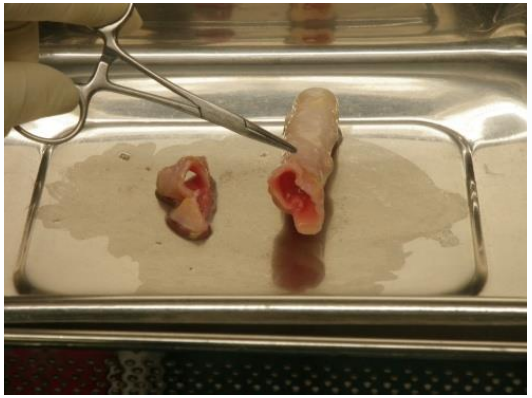
&
University College London

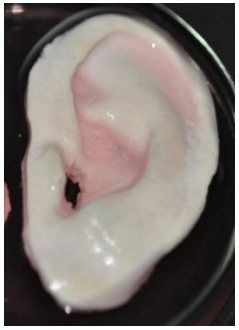
- Thanks to Irvine Scientific for the use of their cartoon

What is an ATMP?

An ATMP is a medicinal product as defined in Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (the Directive). Specifically, an ATMP is a biological medicinal product which is either:

- a gene therapy medicinal product as defined in Part IV of Annex I to Directive 2001/83/EC;
- a somatic cell therapy medicinal product as defined in Part IV of Annex I to Directive 2001/83/EC; or
- a tissue engineered product as defined in Article 2 1 (b) of the ATMP Regulation.





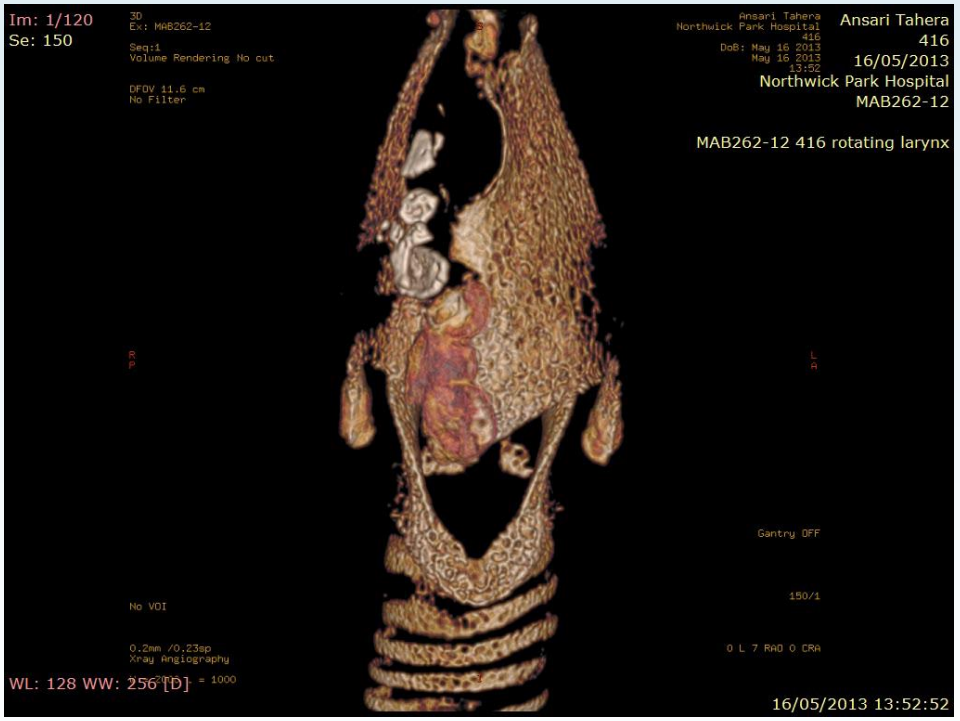
What are the specific regulations covering cell therapies?

- 2001 - 2
  - 2001-83-EC Medicines Directive
    - Substance includes human blood and blood products
    - Implicit application to human somatic cells
  - 2002-98-EC Blood Directive
    - Procurement / labelling /traceability of blood and blood products
- 2004
  - Clinical trials directives enacted – include “substantially modified somatic cells” as IMP for the first time. GMP manufacture required plus MA (IMP) and Qualified Person.
- 2006
  - Tissues & Cells Directives enacted in UK and regulated by HTA
- 2009
  - ATMP Regulations published and regulated in UK by MHRA
  - Procurement of starting material licensed and “with traceability to standard of 2001-83-EC”
  - “nonsubstantial” defined
  - Inclusion of HEC for one-off, non-trial products
  - 2009-120-EC amended 2001-83-EC to included ATMP

ATMP Cell & Tissue therapy trials at UCLP

- "Studies" – not trials
  - CMV-specific immune regeneration post HSCT –Ph I/II and Ph III
  - Allogeneic MSC infusions for severe GvHD – Ph I/II
  - ProT4 DLI for relapse post allow HSCT – Ph III multicentre
- Clinical Trial
  - Autologous tumour lysate pulsed DC in paediatric glioma – Ph I
  - Retinal pigmeted epithelia (RPE) derived from hESCs in Startgardts – Ph I/II
  - Allogeneic primed NK cell therapy for AML – PhI/II
  - ASCAT – PhII
  - MSC-Trail – PhI & PhII
  - **Autologous stem cell seeded cadaveric tracheal transplant**
  - **Autologous stem cell-derived cell seeded biocompatible tissue structure for tracheal transplant**
  - **Autologous stem cell-derived cell seeded biocompatible tissue structure for nasal reconstruction**

3-D Tissue-Engineered ATMPs at UCLP



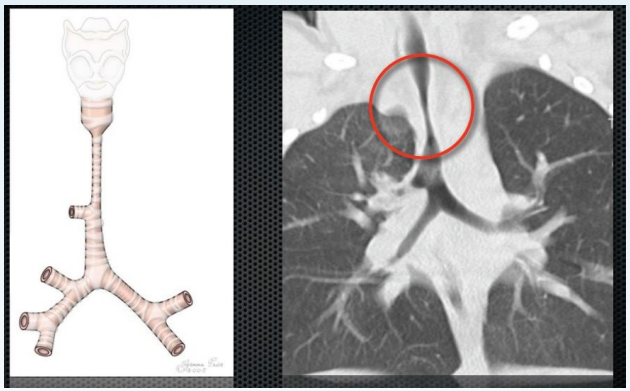
What are the roles for pathologists in ATMP development and delivery?

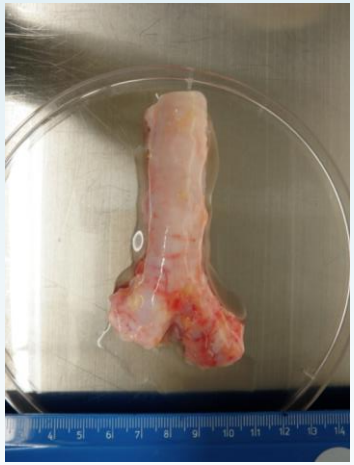
- Cytogeneticist – karyotyping donor cells
- Haematologist – procurement and end user
- Histopathologist – tissue QC, tumour QC and provision
- Immunopathologist – cell QC and potency assays
- Immunogeneticist – cell and tissue identity QC
- Microbiologist – donor and product screening and EM
- Veterinary pathologist – pre-clinical development
- Virologist – donor screening

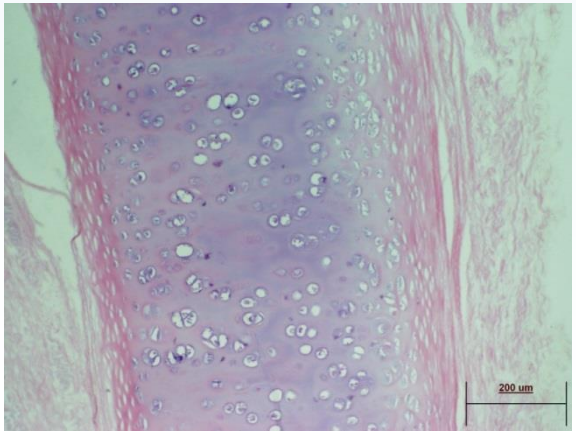
Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study

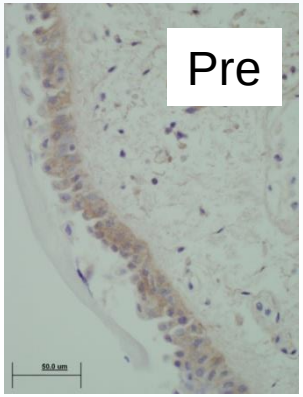
Martin J Elliott, Paolo De Coppi, Simone Speggiorin, Derek Roebuck, Colin R Butler, Edward Samuel, Claire Crowley, Clare McLaren, Anja Fierens, David Vondrys, Lesley Cochrane, Christopher Jephson, Samuel Janes, Nicholas J Beaumont, Tristan Cogan, Augustinus Bader, Alexander M Seifalian, J Justin Hsuan, Mark W Lowdell, Martin A Birchall

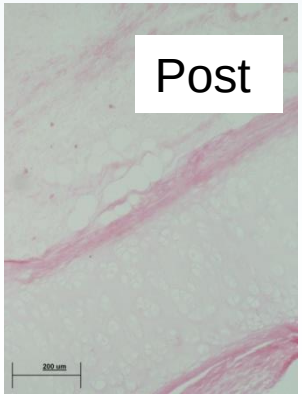
www.thelancet.com Published online July 26, 2012











αMHC class I

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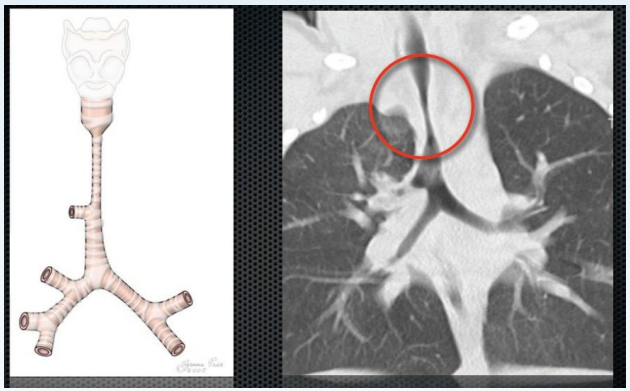

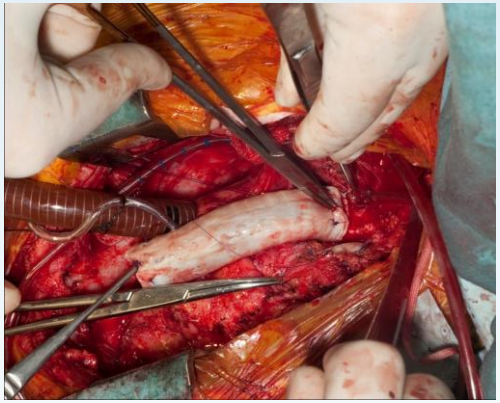

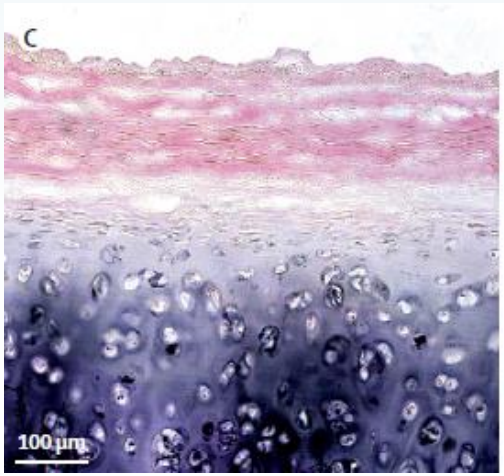

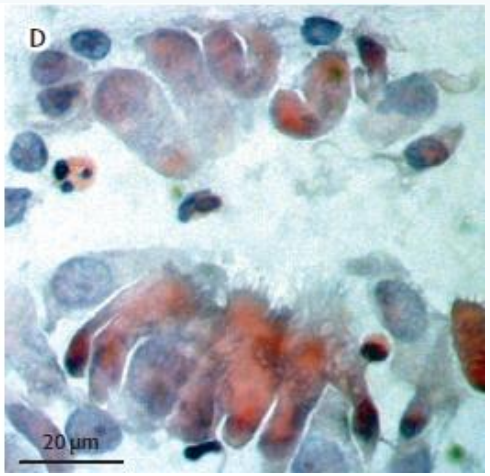

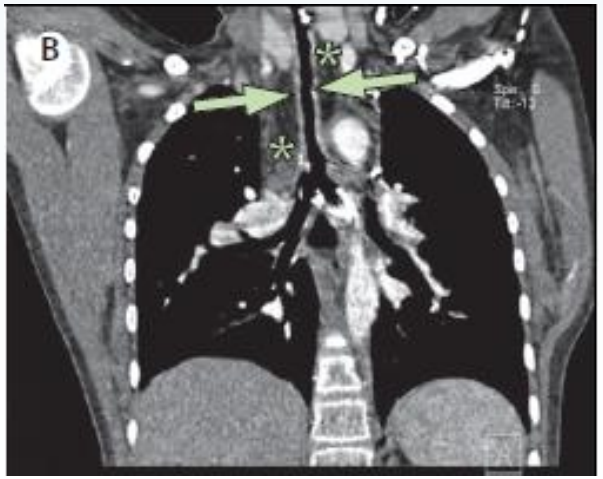

Immunomodulatory effect of a decellularized skeletal muscle scaffold in a discordant xenotransplantation model

www.pnas.org/cgi/doi/10.1073/pnas.1213228110

Jonathan M. Fishman[a,b,c,d], Mark W. Lowdell[b], Luca Urbani[a], Tahera Ansari[c], Alan J. Burns[e], Mark Turmaine[f], Janet North[b], Paul Sibbons[c], Alexander M. Seifalian[g], Kathryn J. Wood[h], Martin A. Birchall[d], and Paolo De Coppi[a,i,1]

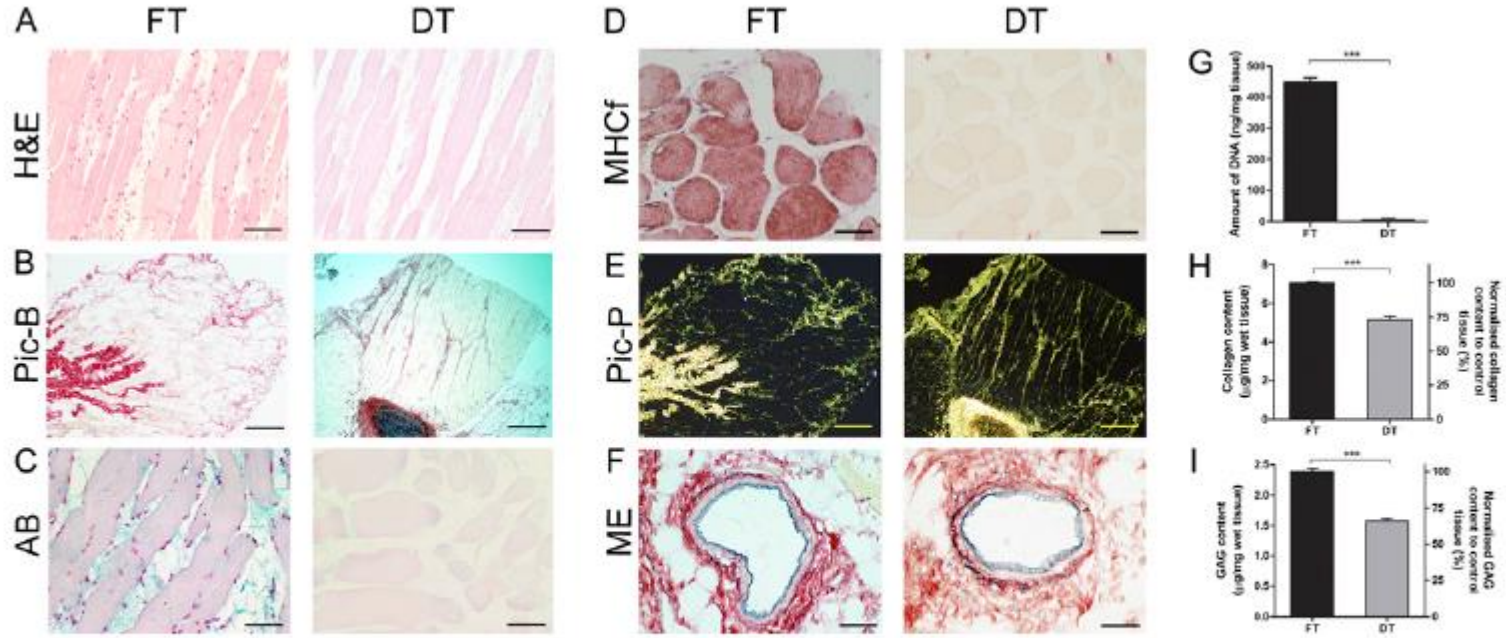

Fig. S1. Characterization of a decellularized skeletal muscle scaffold for tissue engineering. (*A*) H&E staining demonstrating loss of nuclei and preservation of morphology in decellularized tissue (DT) compared with fresh tissue (FT). Collagen staining with Picrosirius-red stain under brightfield microscopy (*B*) and plane polarized light (*E*) of FT and DT, respectively. Glycosaminoglycan (GAG) staining with Alcian blue (*C*) and elastin staining with Miller's elastin (*F*). (*D*) Immunohistochemistry of myosin heavy chain (fast fibers). (*G*) DNA quantification. (*H*) Collagen quantification. (*I*) Sulfated-GAGs quantification of FT and DT, respectively. (Scale bars: *A*, 100 μm; *C*, *D*, and *F*, 50 μm; *B* and *E*, 500 μm.) $n = 12$ in each group. Statistical significance is indicated by the asterisks where $***P < 0.001$.

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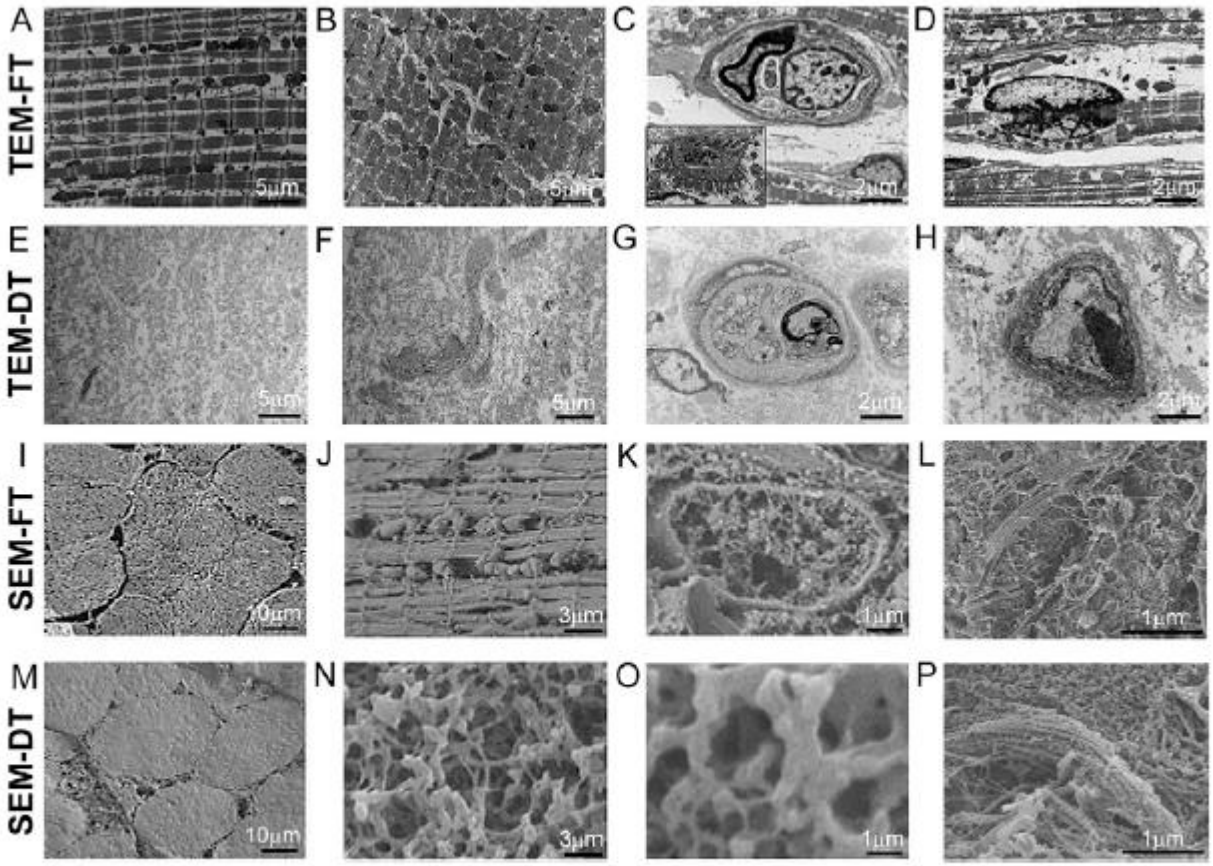

Fig. S2. Transmission electron microscopy (TEM) (*A–H*) and scanning electron microscopy (SEM) (*I–P*) of FT and DT, respectively. DT results in loss of the actin-myosin filaments and nuclei (*E* and *N*) compared with FT (*A–D* and *I–L*), with preservation of overall muscle fiber morphology (*M*) and collagen fibers in DT (*F* and *P*). Decellularized axonal and blood vessel structures are seen in DT (*G* and *H*). *C Inset* represents a neuromuscular junction seen at higher power magnification in FT. Decellularization leads to the generation of a porous matrix (*N* and *O*). Scale bars as shown in the figure.

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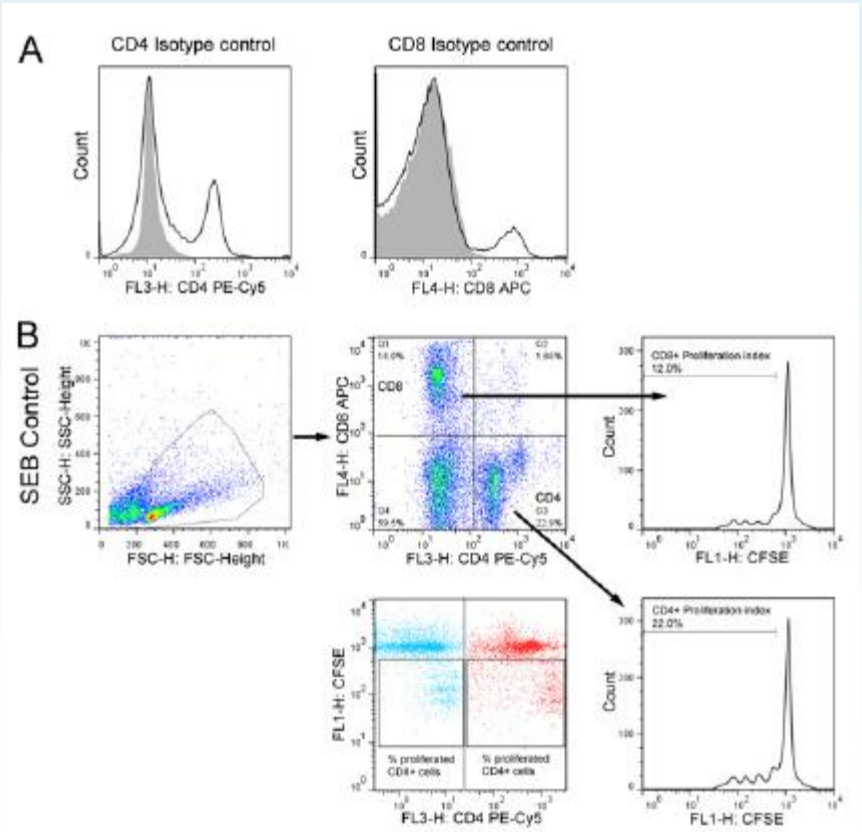
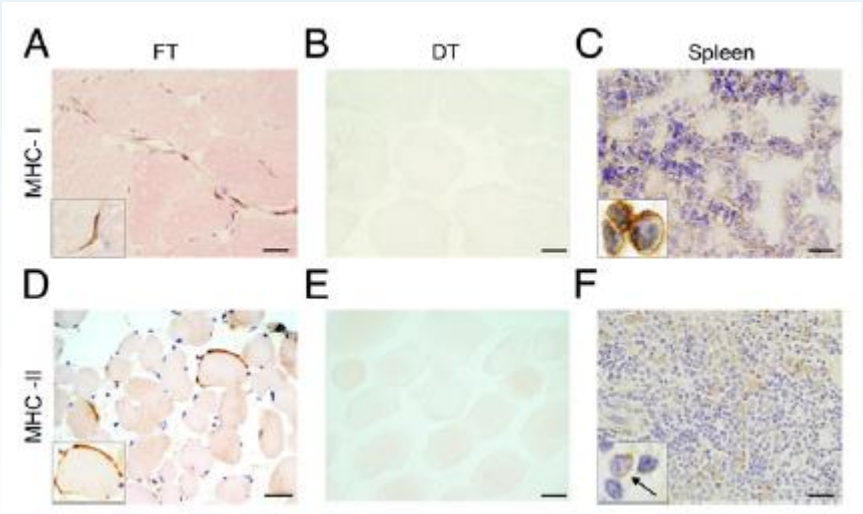
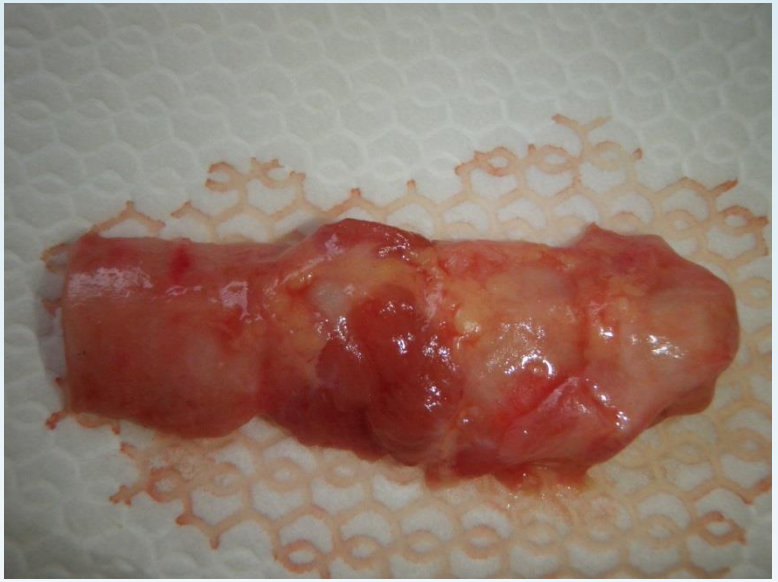
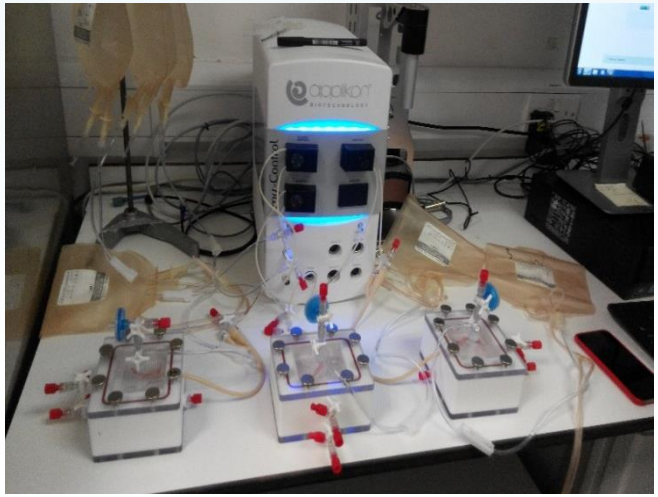
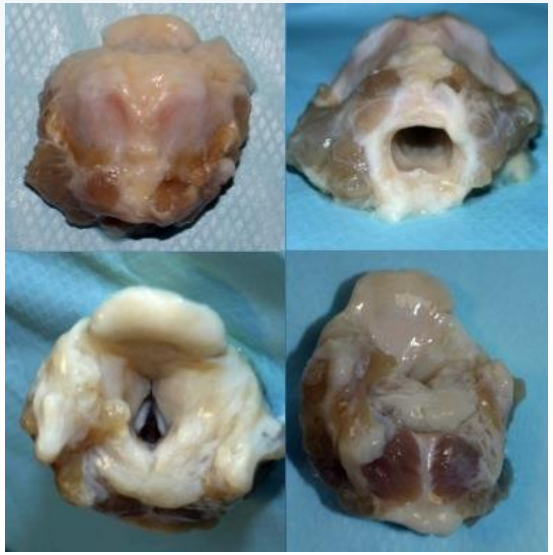




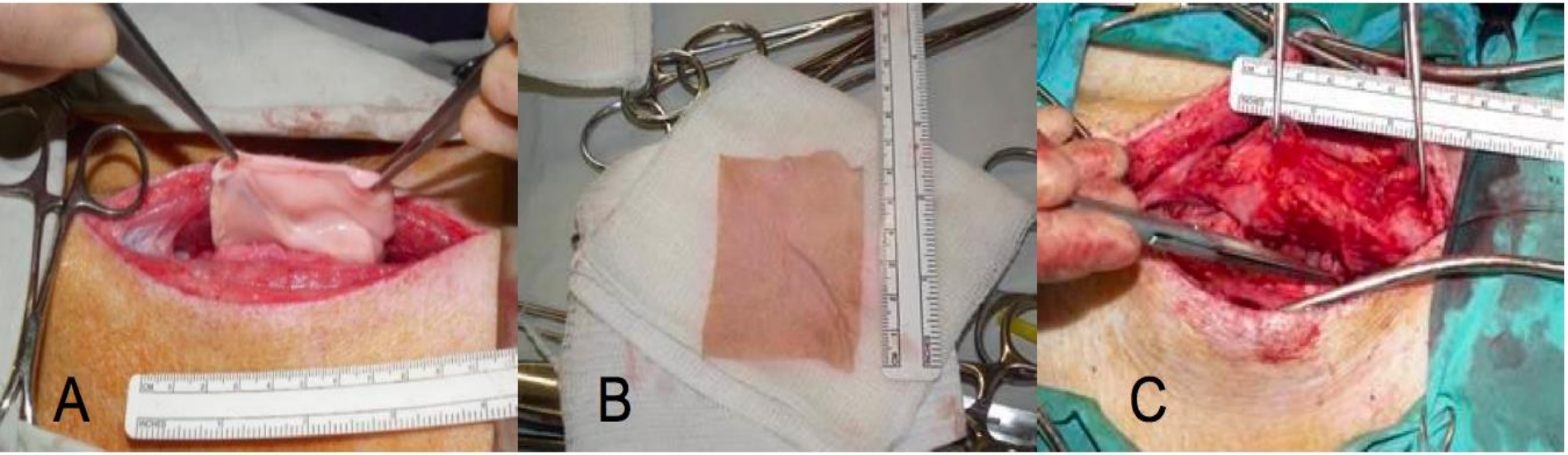
Larynx

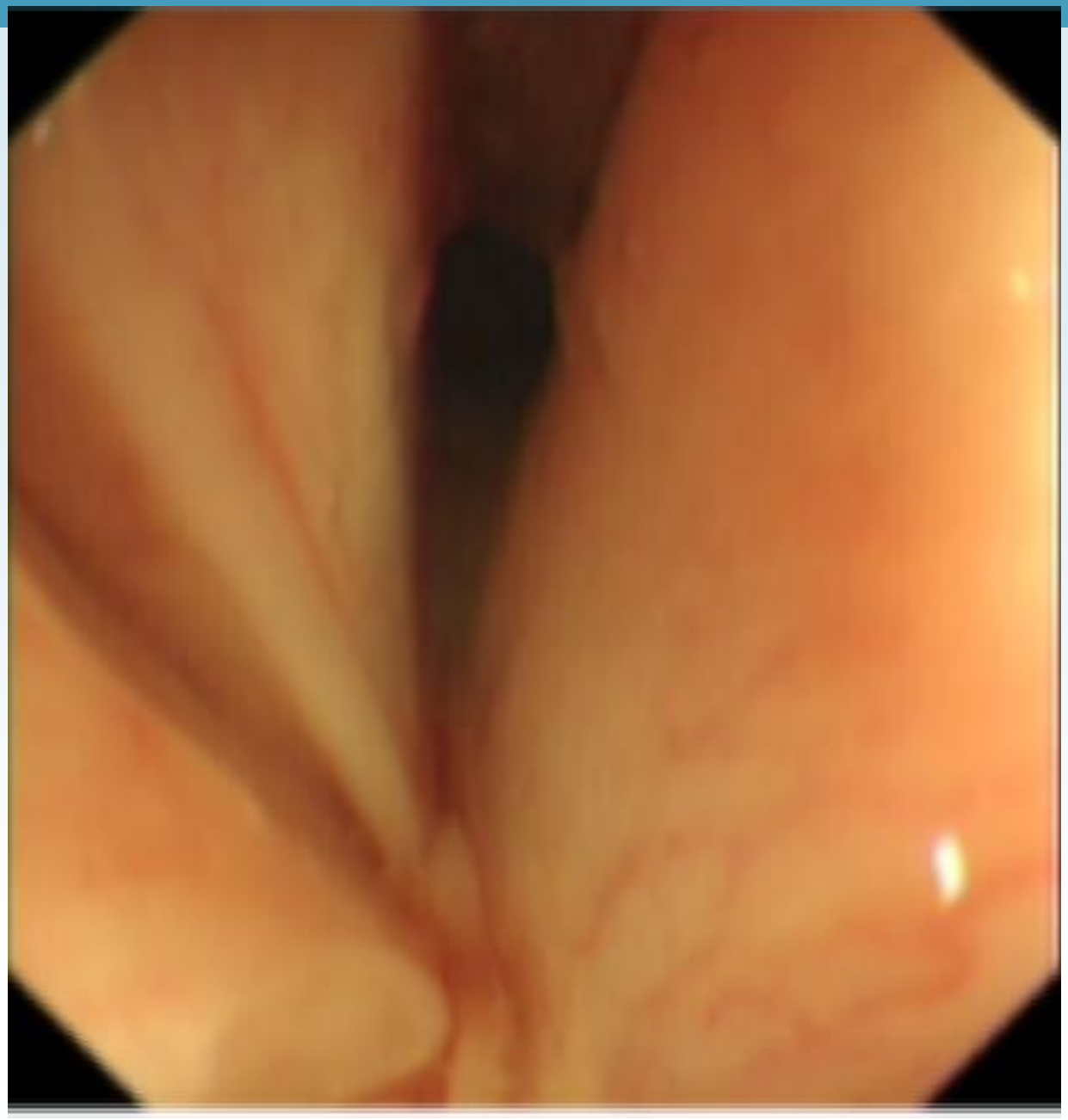




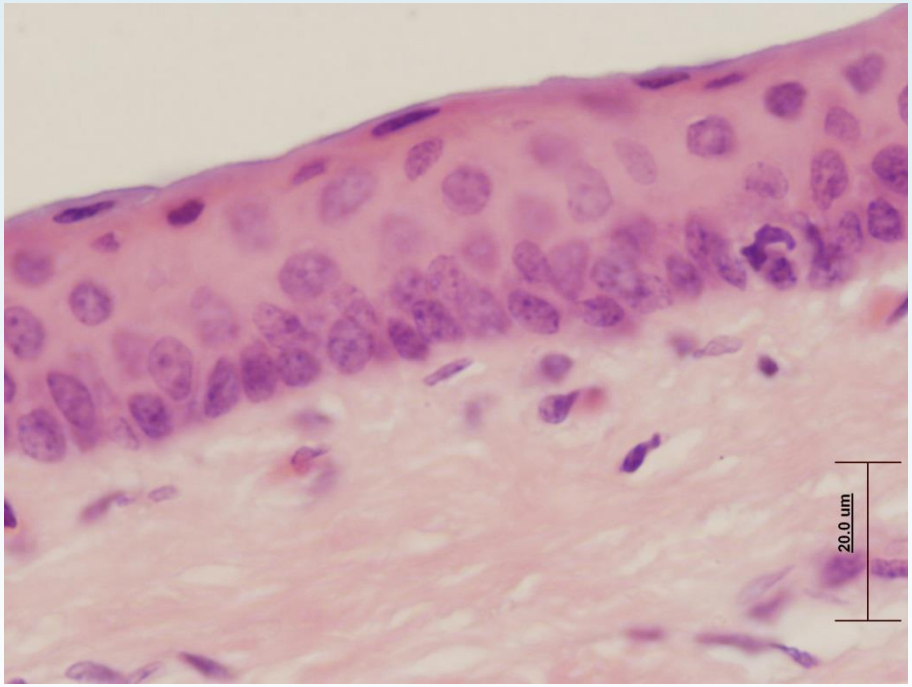


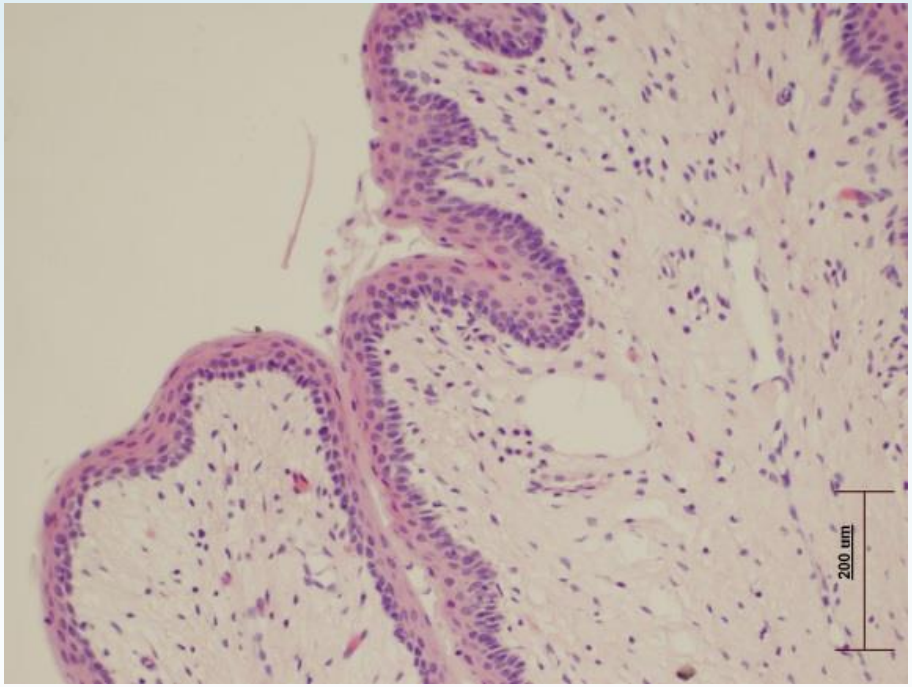
RegenVOX implant and NeoMucosa





5 months epithelialisation – porcine preclinical





organised epithelium over the regenerated vocal folds

Role of the microbiologist

Identifier	Date taken	Site description	Clinical details	Growth
G2214160000582	24.05.16 (10.00)	Blood	Extended culture 3 weeks, FAO Rebecca Gorton	Candida glabrata at 24 hrs
G221416000058	20.05.16 (15.45)	Blood	Extended culture 3 weeks, FAO Rebecca Gorton	No growth
G221416000058 20, overnight HBSS	13.05.16 (12.00)	Blood	Extended culture 3 weeks, FAO Rebecca Gorton	No growth
G221416000058 20, caspofungin + res	12.05.16 (18.15)	Bone Marrow random	Extended culture 3 weeks, FAO Rebecca Gorton	Candida albicans at 32 hours
G221416000058 20, caspofungin	12.05.16 (18.15)	Bone marrow random	Extended culture 3 weeks, FAO Rebecca Gorton	Candida albicans at 2 days
G22141600005, amphob + resep	11.05.16 (16.30)	Bone marrow	Extended culture 3 weeks, FAO Rebecca Gorton	No growth
G221416000058 20, amphb + caspofungin	11.05.16 (16.30)	Bone marrow	Extended culture 3 weeks, FAO Rebecca Gorton	No growth
G221416000058 20	10.05.16 (14.00)	Blood	Ampho b treated only without resep	Candid glabrata at 9 days
G221416000058 20	10.05.16 (14.00)	Blood	Ampho b treated only without resep	Candida glabrata at 8 days
G221416000058 20 (Incorrectly booked as A22141600005)	14.04.16	Blood	End of process	C albicans and C glabrata positive
G221416000058 20	08.04.16 (18.08)	Bone marrow random	G22141600005281 overnight wash	No growth
G2214160005820	07.04.16 (12.00)	Bone marrow pre	Pre Decell 1d	No growth
G221416000058 20	06.04.16 (20.45)	Bone marrow pre	Pre-decell-1d	Candida glabrata and Candida albicans at 5 days

Royal Free London NHS Foundation Trust
Microbiology Department
London NW3 2QG Tel: 020 7794 0500
Accredited Medical Laboratory Reference No. 1061
Results produced by HSL

CPA

Bone Marrow Processing

PAUL O'GOORMAN LAB.
LOWER GROUND FLOOR
ROYAL FREE HOSPITAL

Name: G22141600005,POST SONICATION
Hosp No:
DOB
Dept: Bone Marrow Processing

Laboratory Number: 16M206366
Date sample booked in: 01/07/16 at 17:56
Sample collected: 01/07/16

Specimen: Blood Culture

Site: Bone Marrow Post

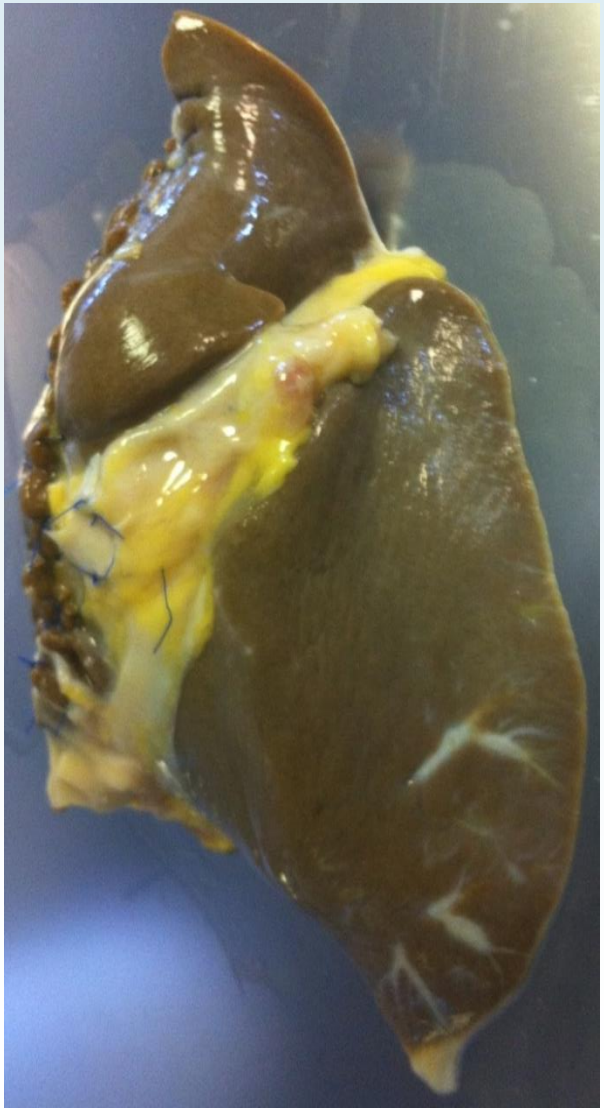
Test: BLOOD CULTURE

Clinical Details: non clinical sample-do not report to consultant

Interim Report	No bacterial growth after 36 hours incubation
Aerobic bottle culture	No growth after 21 days of incubation
Anaerobic bottle culture	No growth after 21 days of incubation

So where will we be in 3 years?

Human liver for allogeneic Tx transplant



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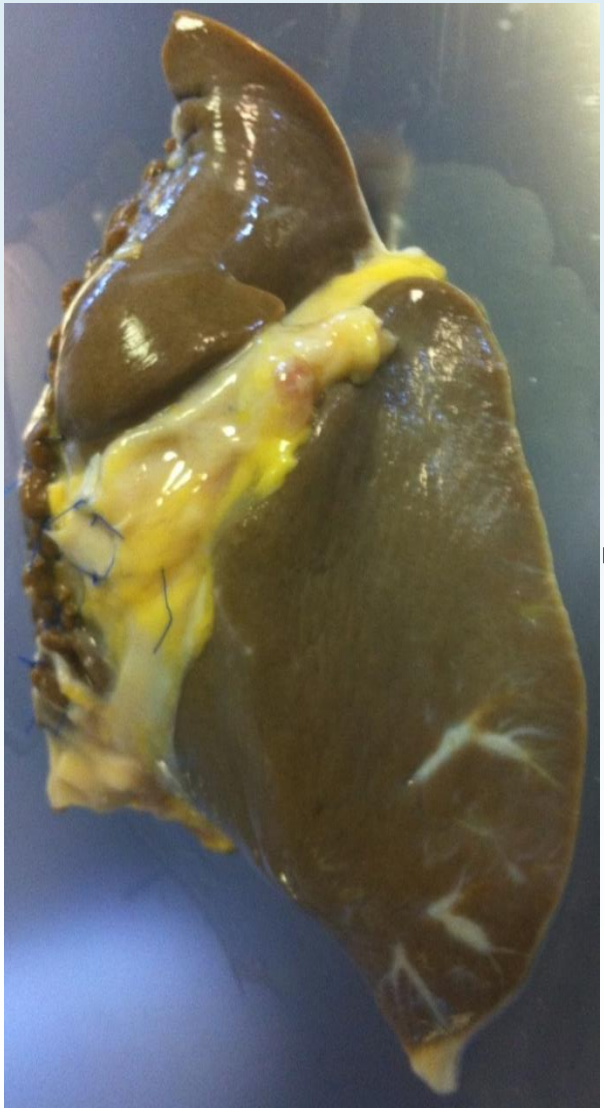
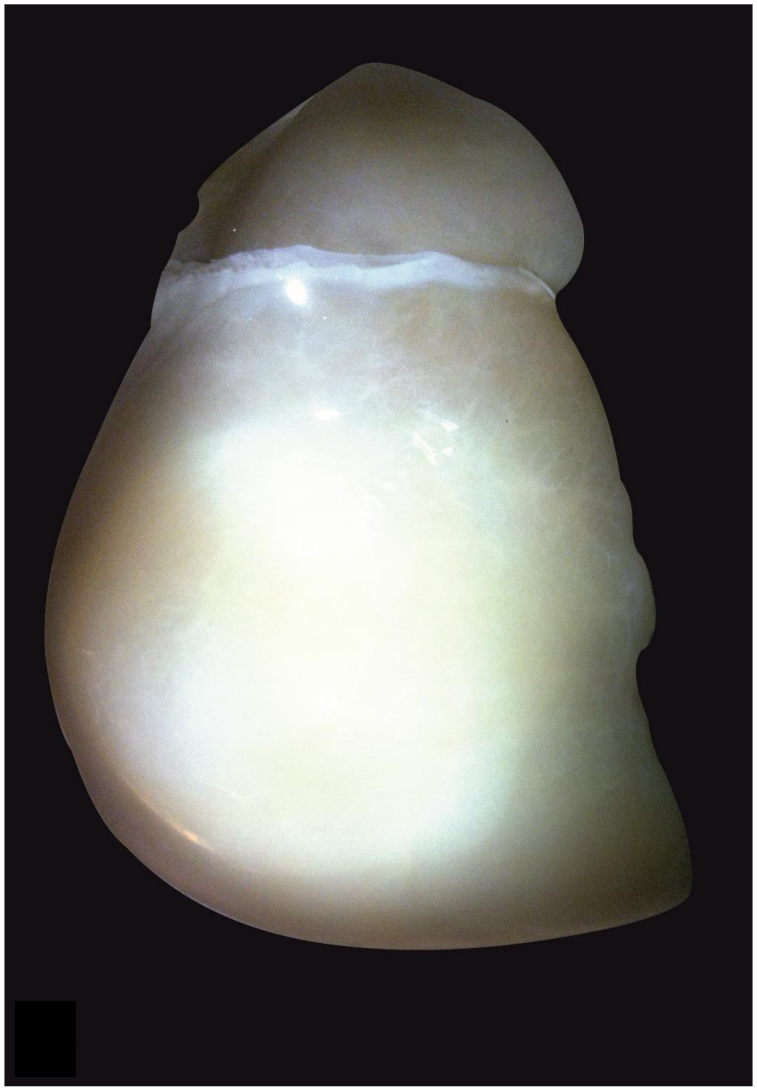


Decellularised human liver for TE autologous transplant



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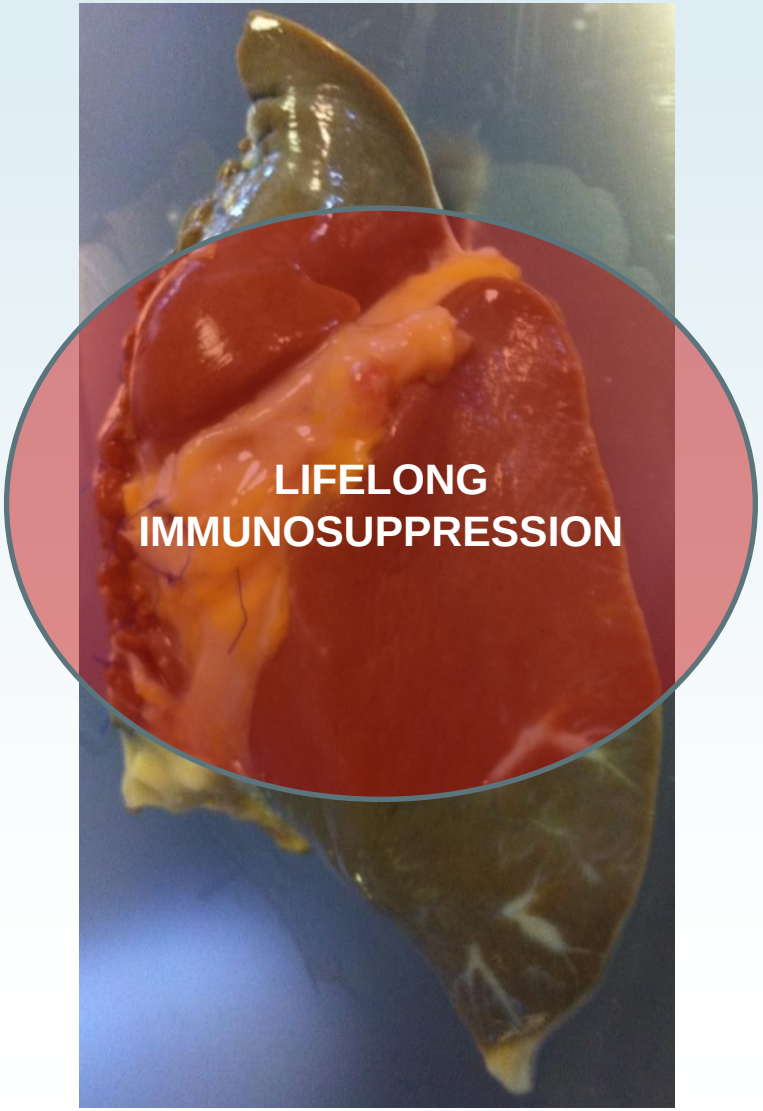

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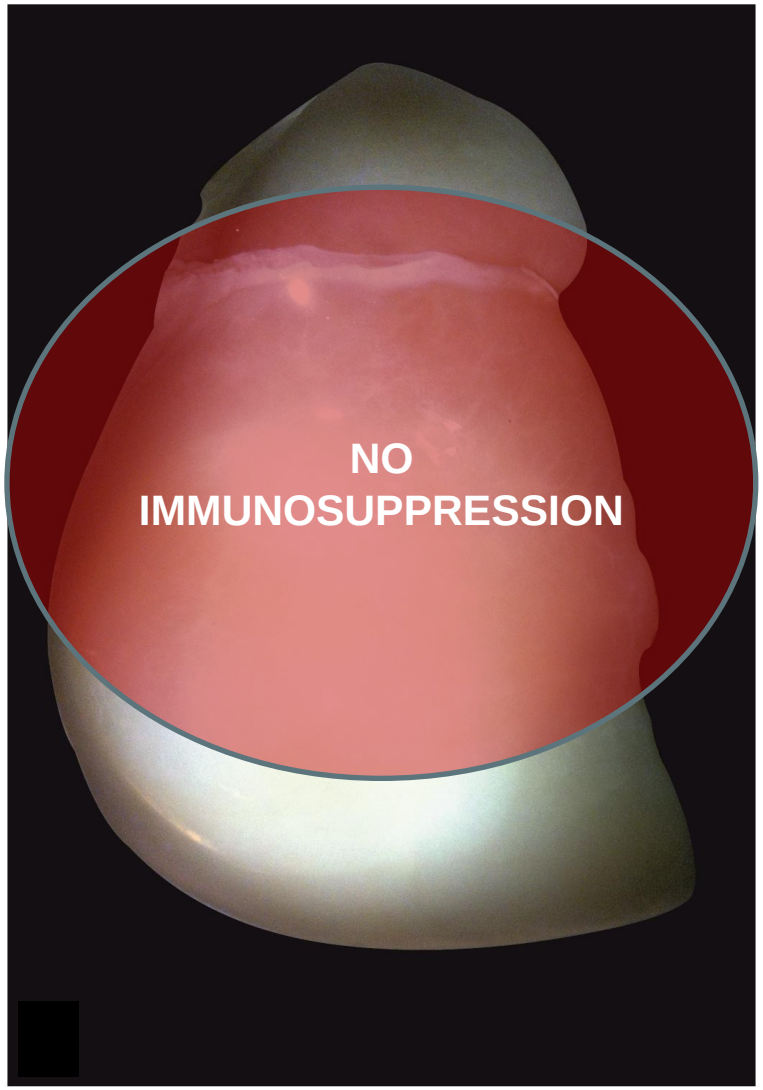

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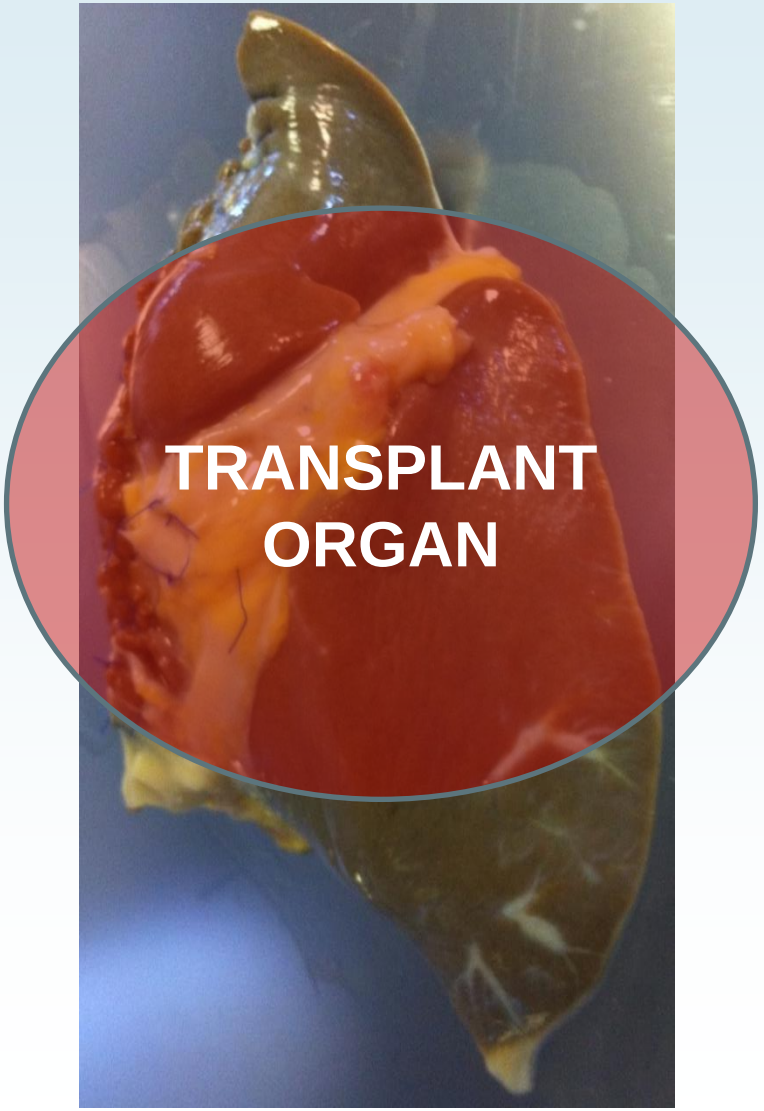

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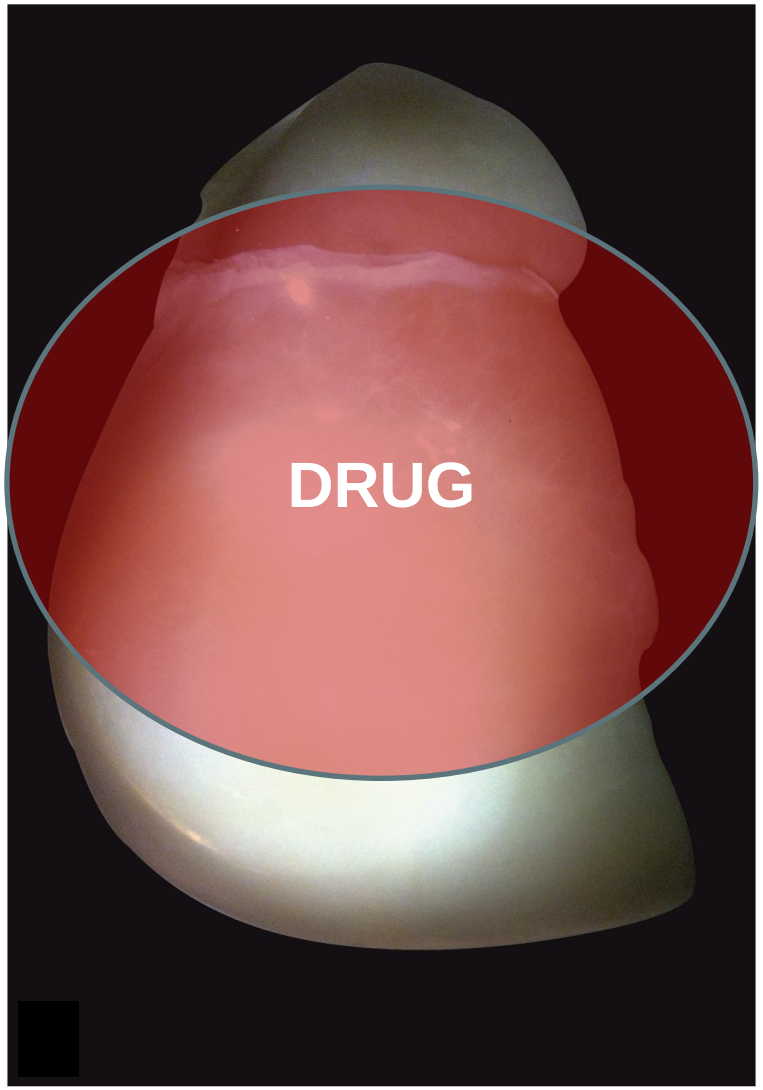

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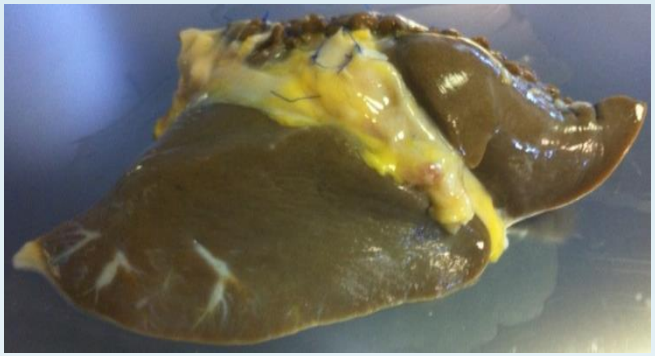
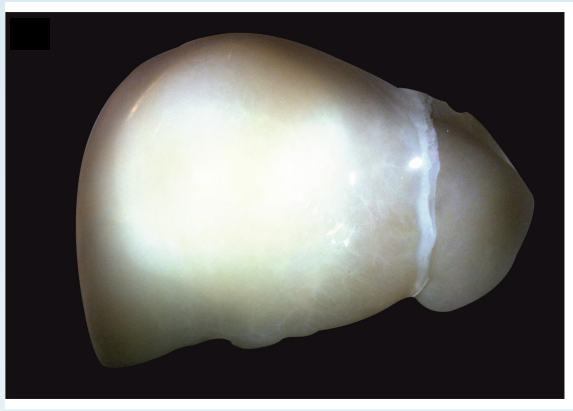


- Transplant organ
  - Philanthropic donation
  - Public sector supply
  - Very limited regulation
  - No proof of concept
  - No proof of efficacy
  - Lifelong immunosuppression



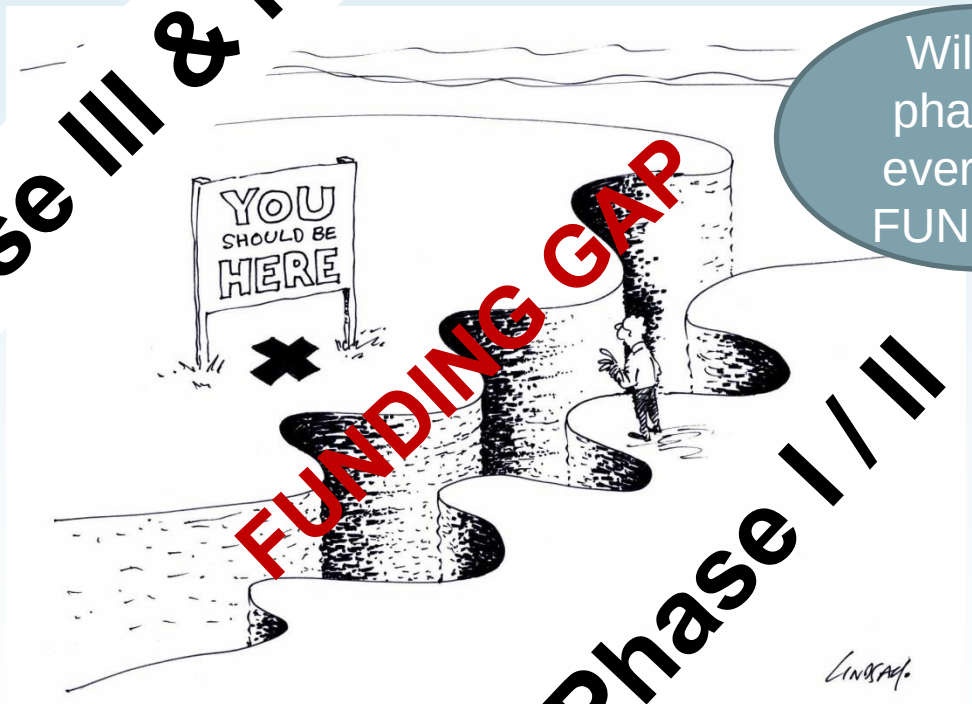
- Recellularised liver scaffold
  - Regulated as a medicine
  - Complex & expensive manufacture **with QC**
  - Formal clinical trials
  - No need for immunosuppression
  - Private sector supply
  - Philanthropic donation?

CHALLENGE – Public education essential

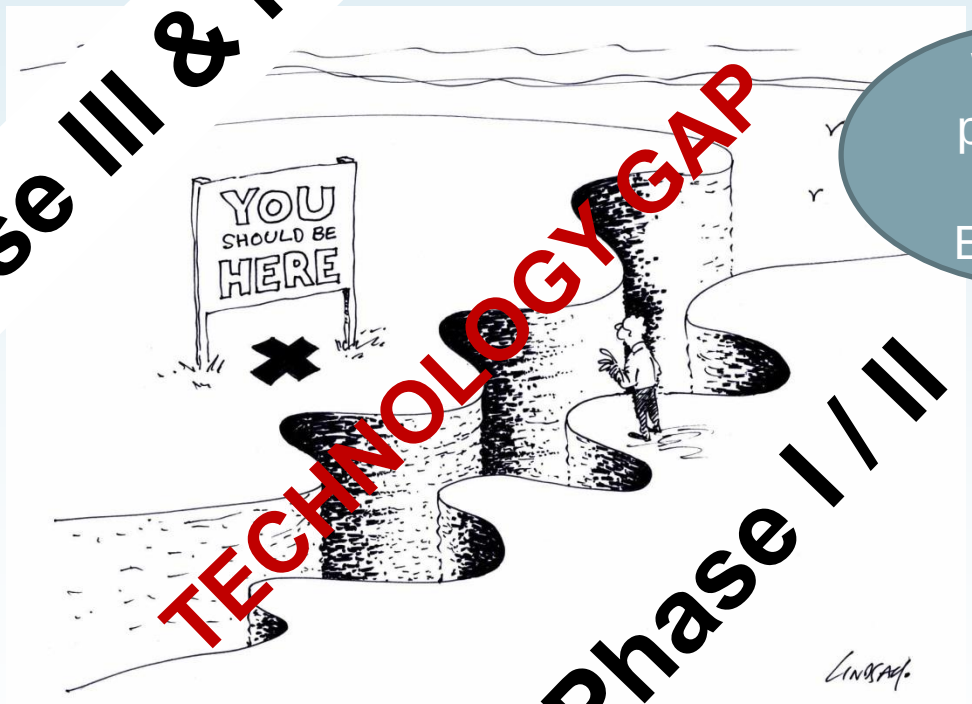
GUIDING PRINCIPLES ON HUMAN CELL, TISSUE AND ORGAN TRANSPLANTATION

"The need to cover legitimate costs ……. accepted as long as the human body and its parts as such are **not a source of financial gain**".

“The problem with cell therapy” - 2014



“The problem with cell therapy” - 2016



How much can I change my process to move from academic phase I/II to MA?

Phase I / II
10 patients

"Who knows how far up we are?
Raise your hands."

Phase III & MA
10k patients

Some of the challenges

- Closing the process
  - Equipment
  - Compatible plastics
  - Comparability vs “open” process
- Robust QC assays
  - Reagents
  - Equipment
  - Controls
  - QA
- Informative potency assays
  - FIO data from early phase trials
- REDUNDANCY
  - More than one of each critical supplier!!!
- Cold supply Chain
  - Do you need -155oC?

British Journal of Haematology, 2002, 117, 821–827

Evidence that continued remission in patients treated for acute leukaemia is dependent upon autologous natural killer cells

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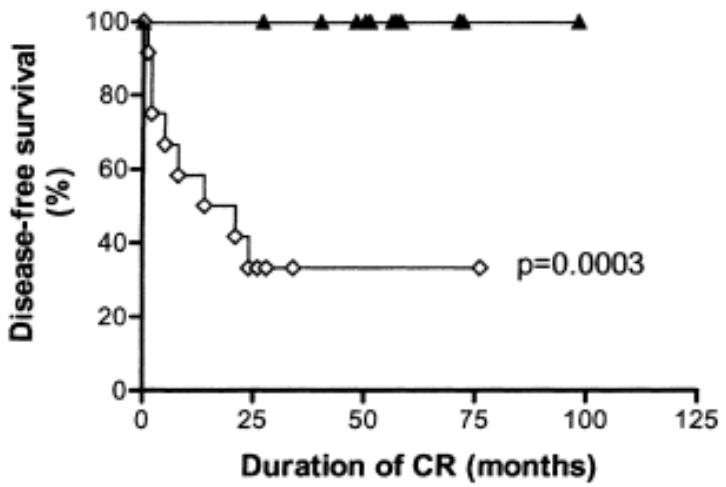
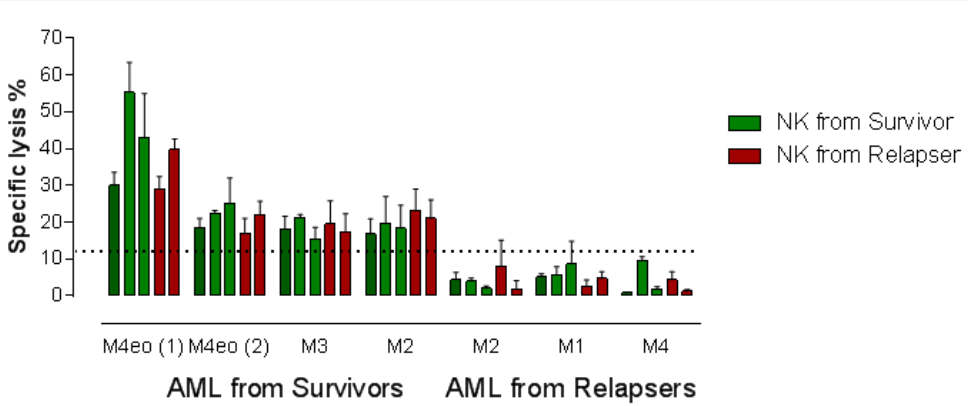


Fig 2. Prediction of disease-free survival by virtue of LCA-max activity. <12·2% LCA, ◇; >12·2% LCA, ▲.

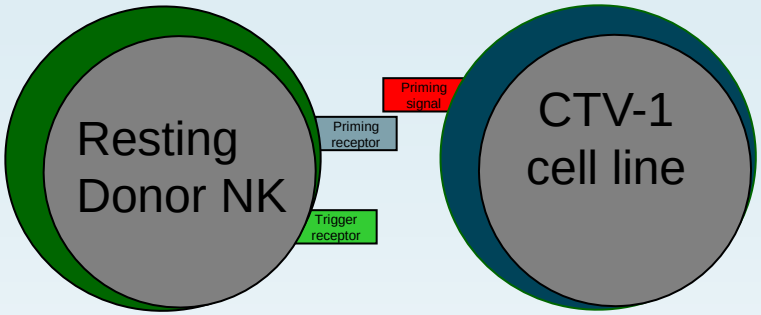


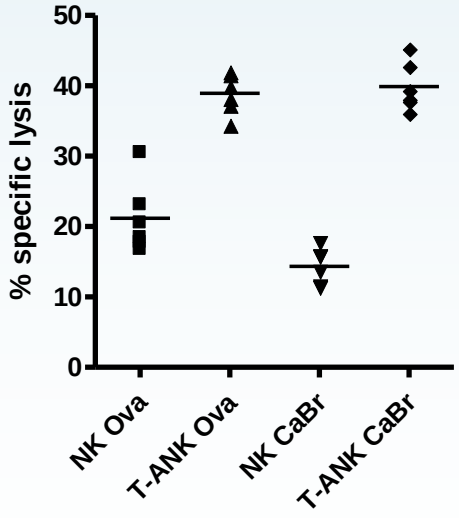
Hypothesis

- NK-mediated tumour cell lysis requires two signals
  - S1 = “priming”
  - S2 = “triggering”

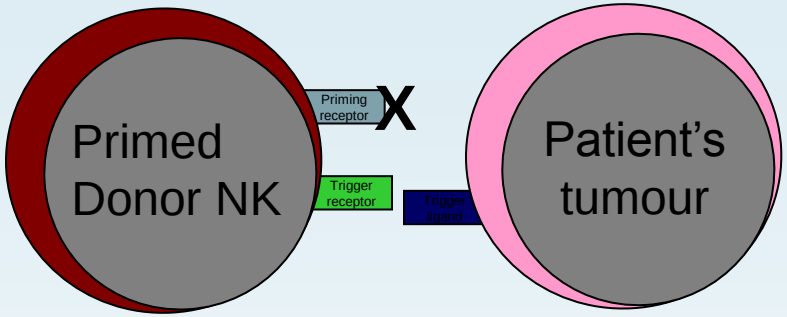
- NK-resistance through
  - Missing S1 – failure to “prime”
  - Missing S2 – failure to “trigger”

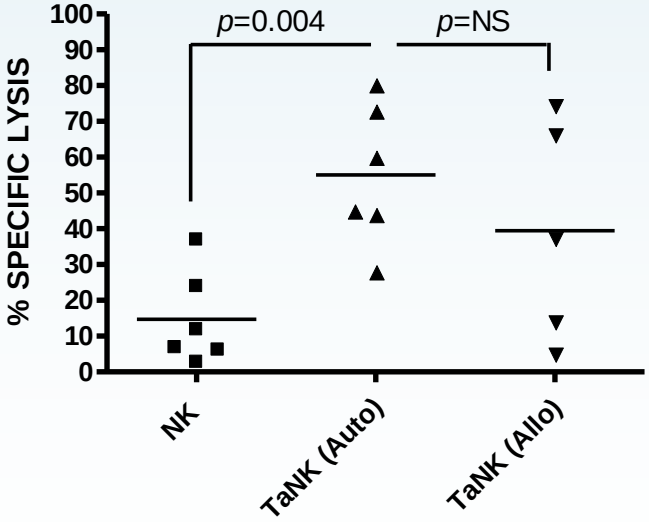
Only Signal 1 so NK cell doesn't kill but is "ready to kill"





Patient's tumour has Signal 2 and can thus be killed by primed NK cell.



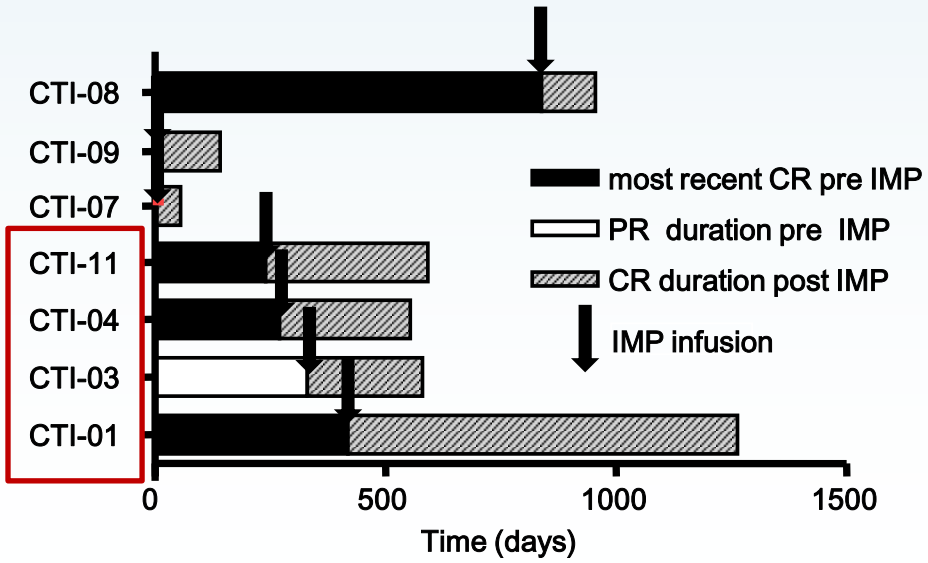


Signal 2 "triggering"

LYSIS

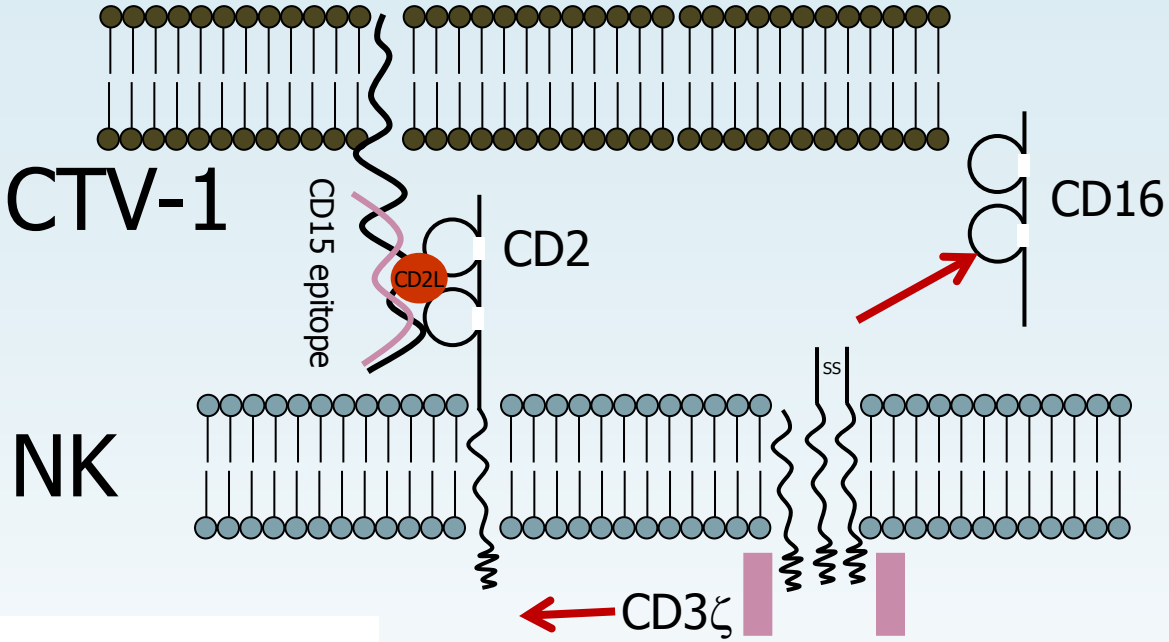
The EU GMP PD/PV pathway for TpNK

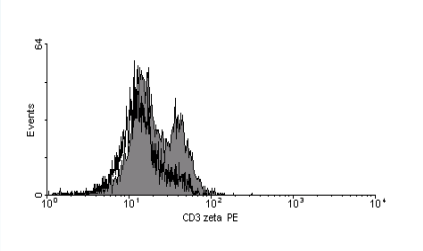
- Development of PhI GMP
  - CTV-1 sourcing / testing
  - CTV-1 expansion
  - **Lysate manufacture / testing**
  - **Clinical-grade NK selection**
  - **? Adequate T cell depletion**
  - Effect on trial design (max IMP dose)
- Engineering PhI GMP
  - CTV-1 scale-up and closure
  - **Assay design/testing**
- Validation PhI GMP
  - Reproducibility of lysate batches
    - Pool
  - **NK yield / purity / TCD**
  - Dose success rate

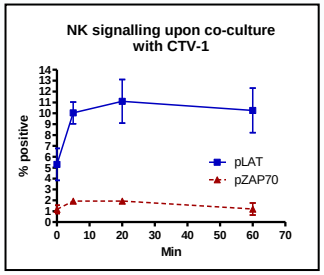


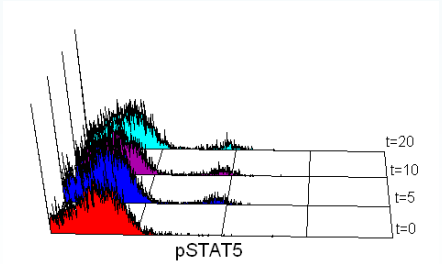
The tech transfer pathway to the US

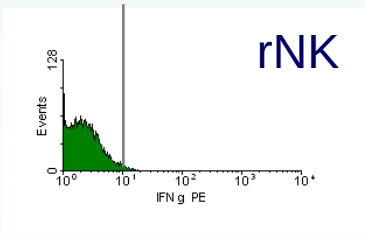
- Technology transfer for TpNK
  - New SOPs
  - New BMR
  - New PD
  - New PE
  - New PV
  - Apheresate sourcing
  - Shipping validation
  - Assay transfer and qualification
- Transfer TpNK process to **CMO 1** with UCL lysate
- CTV-1 MCB and WCB – **CMO 1 *I* 2**
- Lysate manufacture technology transfer – **CMO 1**
- US phase I and II clinical trial protocols
- IND submission
- Scale-up lysate manufacture x10 – **CMO 1**
- Technology transfer of lysate QC
- **COMPARABILITY ASSAY DEVELOPMENT**
- STABILITY TESTING
- ADDITIONAL RELEASE CRITERIA
- COST REDUCTION

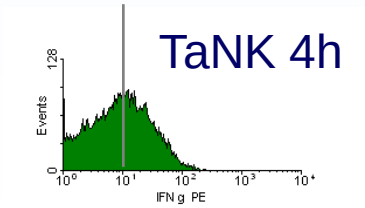

CTV-1
CD15 epitope
CD2
CD2L
CD16
SS
NK
CD3ζ


Events
CD3 zeta PE


NK signalling upon co-culture with CTV-1
% positive
pLAT
pZAP70
Min


t=20
t=10
t=5
t=0
pSTAT5


rNK
Events
IFN g PE

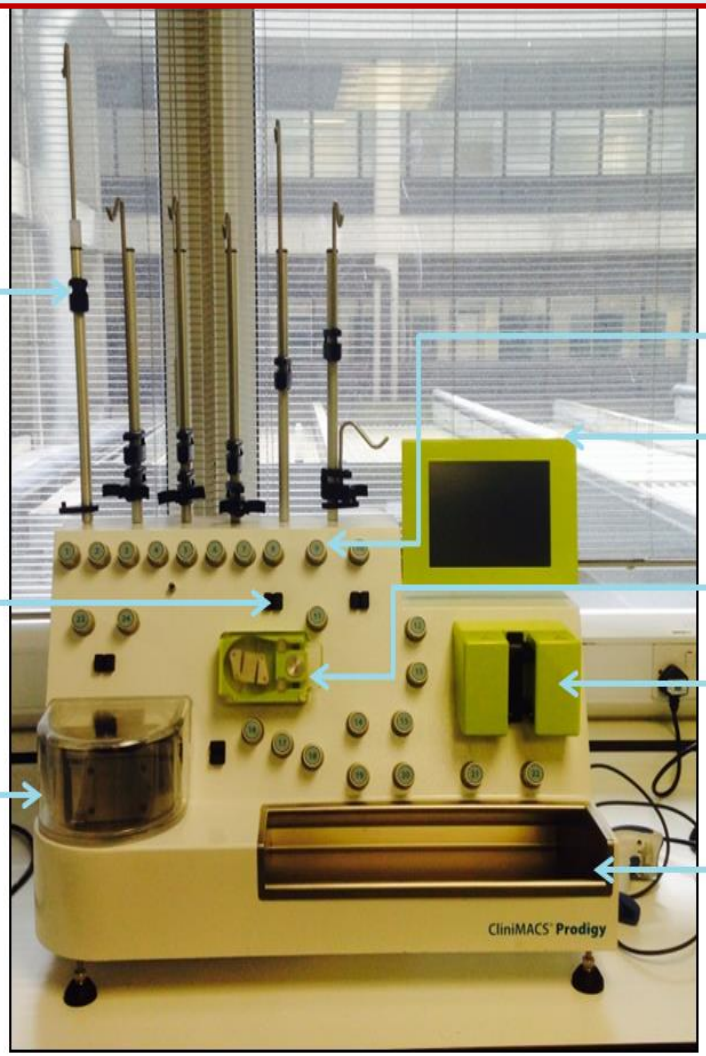

TaNK 4h
Events
IFN g PE

The tech transfer pathway to scale-out

Bag hangers for:
- Sample material
- Solution bags
- CliniMACS reagents

Liquid sensor

CentriCult unit for:
- Cell washing
- Labelling
- Ficol



Pinch valves (24)

Touchscreen monitor

Peristaltic pump

Magnetic cell separation column

Bag compartment for:
- Target cell fraction
- Non-target cell fraction
- Waste

BJHaem - *in press*

The expanding role of the haematologist in the new world of advanced therapy medicinal products (ATMPs).

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Director of the Centre for Cell, Gene & Tissue Therapeutics
Royal Free London NHS Foundation Trust & University College London

Dr Amy Thomas
Regulation Manager
Human Tissue Authority
Buckingham Palace Road
London

- Procurement (HTA licence and DI)
- Storage
- Dispensing
- Infusion

Product	ATMP type	Starting material	Regulatory status
Holoclar – limbal epithelial cell construct	Combination ATMP: Tissue engineered product plus engineered scaffold	Limbal biopsy	Licensed medicine
ChondroCelect	Tissue engineered product	Autologous chondrocytes	Licensed medicine
Mesenchymal stromal cells for immunomodulation	Somatic cell therapy	Bone marrow aspirate	Investigational or unlicensed medicine
Mesenchymal stromal cells for tissue regeneration	Tissue engineered product	Bone marrow aspirate	Investigational or unlicensed medicine
Dendritic cell vaccines	Somatic cell therapy	Autologous peripheral blood monocytes from apheresate	Investigational or unlicensed medicine
TCR-modified T cells	Gene therapy product (GM autologous somatic cells)	Autologous apheresate	Investigational medicine
CAR-T cell	Gene therapy product (GM autologous somatic cells)	Autologous apheresate	Investigational medicine
Activated NK cells	Somatic cell therapy	Autologous apheresate	Investigational medicine
Regulatory T cells	Somatic cell therapy	Autologous apheresate	Investigational medicine
Tracheal construct	Combination ATMP: Tissue engineered product plus allogeneic human scaffold	Autologous bone marrow aspirate and Tracheal biopsy	Investigational medicine
iPS cells	Gene therapy product (GM autologous somatic cells)	Skin biopsy	Investigational medicine

Summary

- ATMPs for regenerative medicine and immunotherapy are the most rapidly advancing field of drug development
- Uniquely these need hospital participation in their manufacture and delivery
- Skills of pathologists are essential from pre-clinical to licensed product manufacture – pharmaceutical pathology?
- Standards for most assays and QA do not exist – BSI is trying to set standards (!)
- Time for our colleagues and our College to get involved and to inspire the pathologists of the future

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  - Ed Samuel
  - Colin Butler
  - Leanne Partington

Patients and their families

Any questions?