the london project

to cure blindness

RPE Cell Therapy for Age Related Macular Degeneration (AMD): the road towards the clinic & some lessons learned

A partnership between

UCL Institute of Ophthalmology (Pete Coffey)

Moorfields Eye Hospital (Lyndon da Cruz)

Pfizer Neusentis

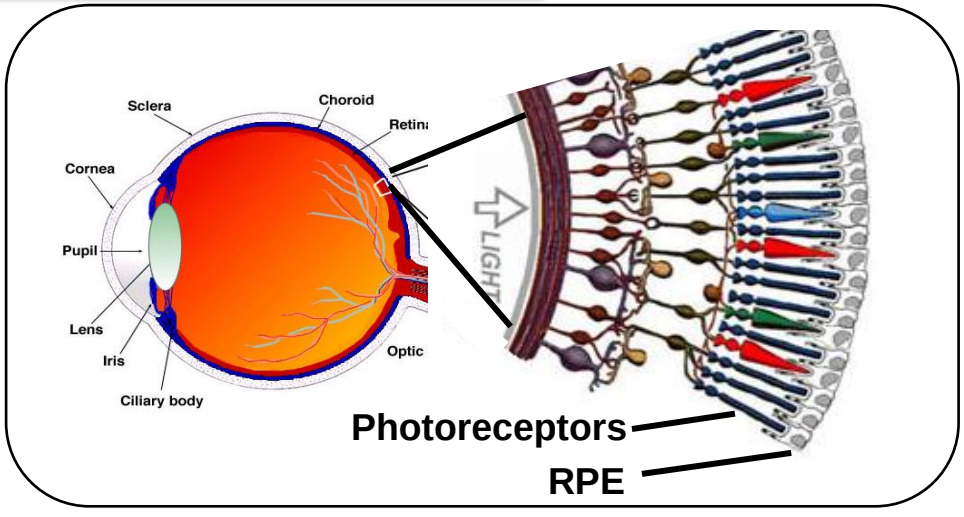
UCL

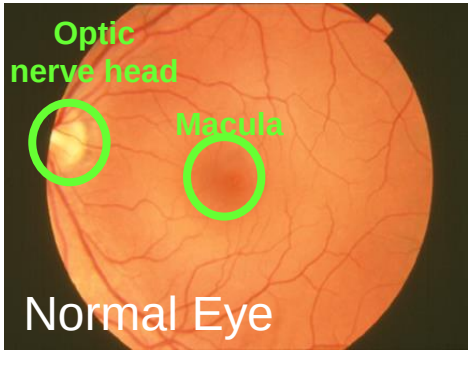
Moorfields Eye Hospital NHS
NHS Foundation Trust

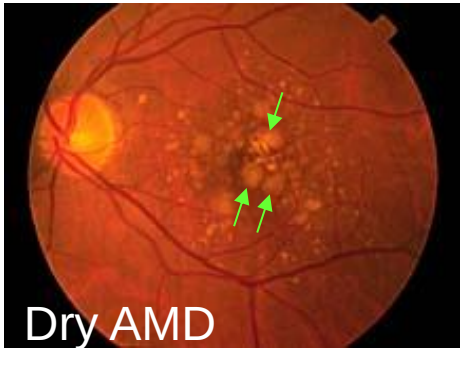
AMD is the major cause of vision loss in adults over 65

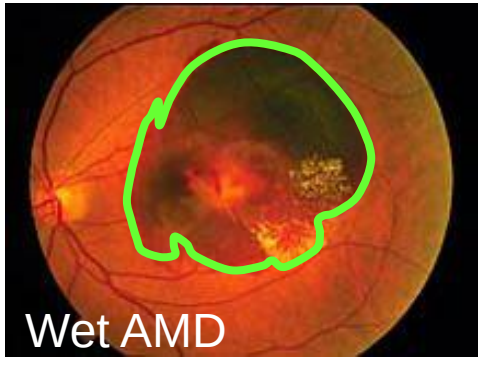
- 2010 US prevalence of AMD 1:6
- Predicted to almost double by 2030

Neusentis
A Pfizer research unit









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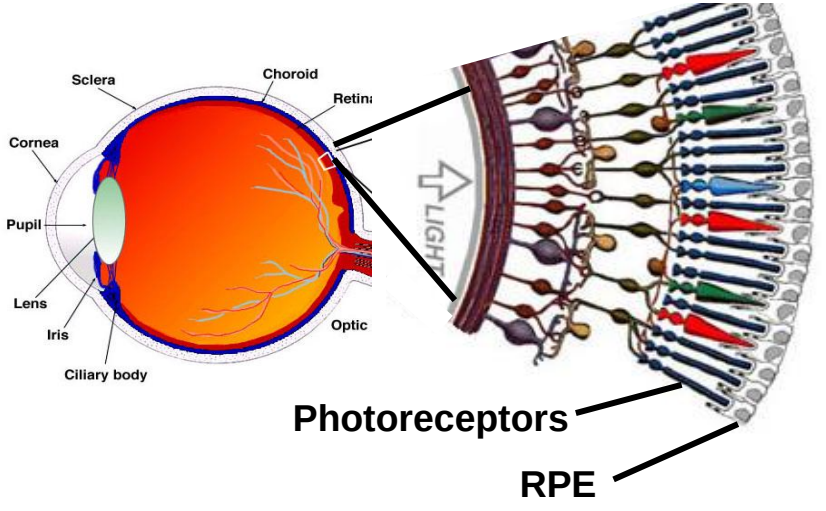
Rationale for RPE transplantation for AMD

- **RPE cells are critical for retinal function**
  - Trophic factor support for photoreceptors & choriocapillaris
  - Phagocytosis of shed photoreceptor outer segments
  - Nutrient supply to & from subretinal space & blood
  - Re-isomerisation of all-trans retinal (visual cycle)
  - Maintenance of immune privilege of posterior chamber

- **Transplantation of RPE cells show efficacy in preclinical models of retinal degeneration**
  - Sub-retinal delivery maintains visual function (optokinesis) in RCS dystrophic rat

- **Transplantation of RPE layer, or macular translocation shows clinical efficacy**
  - Macular translocation: 10 / 40 patients maintain 3 line gain in acuity after 3 years



"viable RPE → functional photoreceptors → maintenance of vision"

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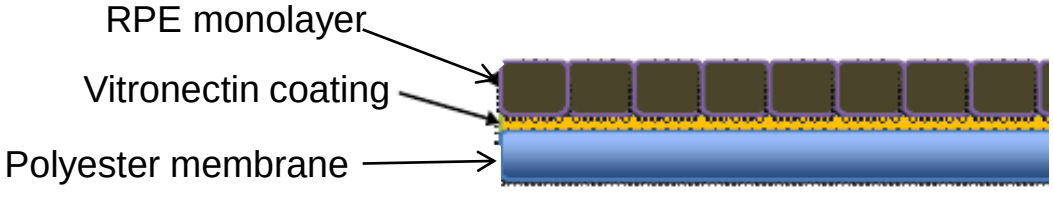
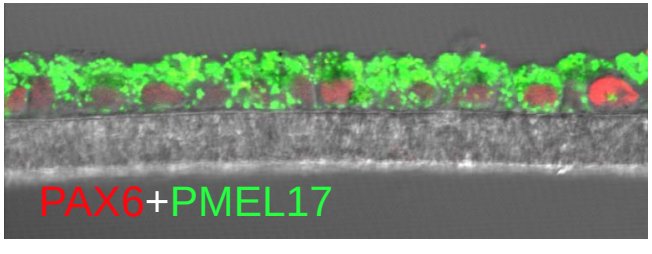
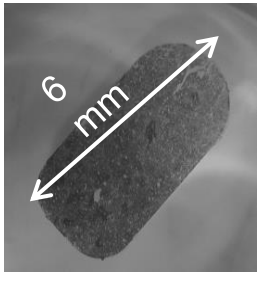
The cell therapy

- Human embryonic stem cell derived RPE cells phenotypically & functionally equivalent to native RPE
- RPE cells seeded as a monolayer on vitronectin coated polyester membrane
- Monolayer of RPE cells mimics normal morphology enabling optimal function in-situ



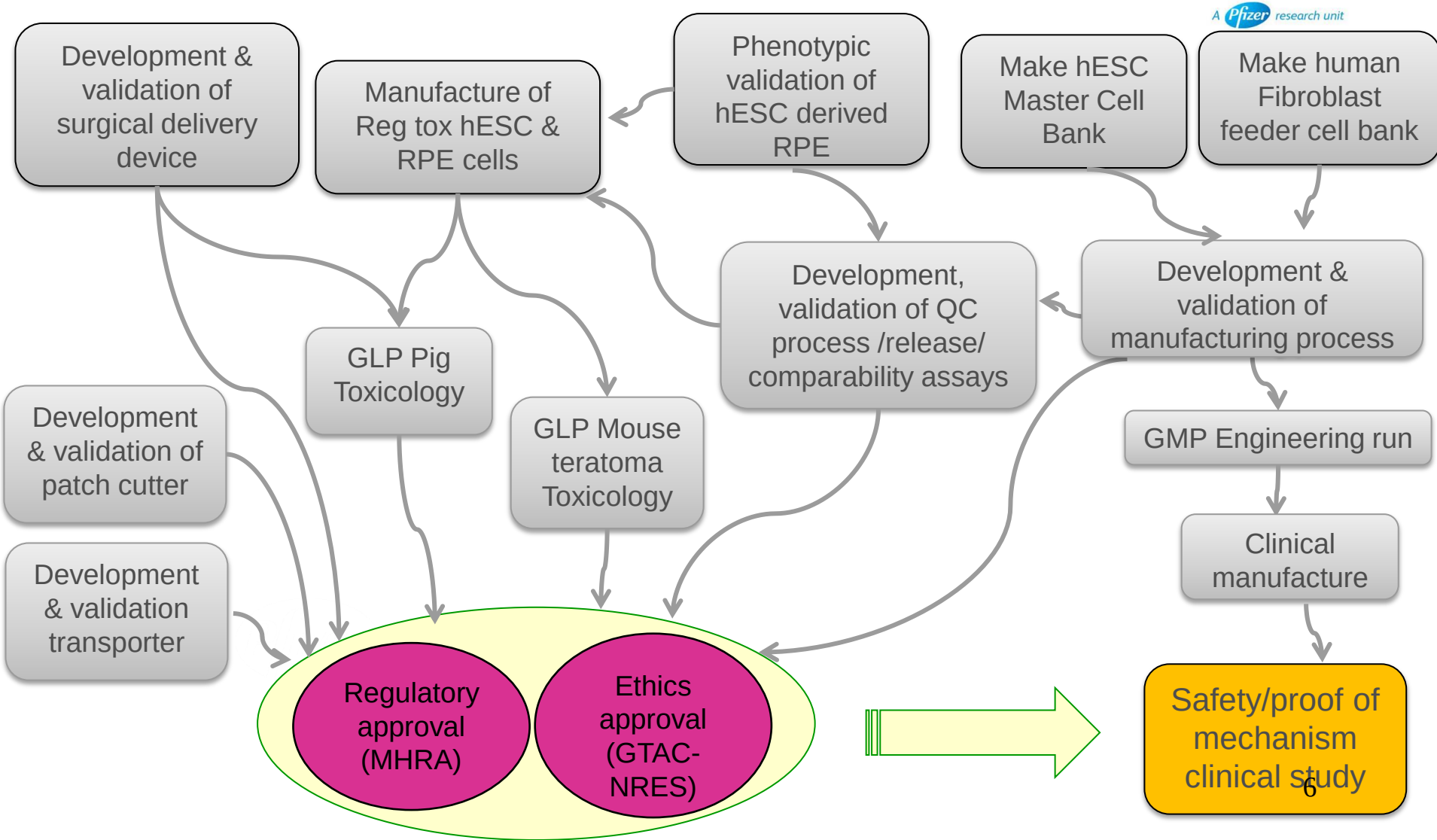




En face view Polyester membrane seeded with 100,000 RPE cells

- Bespoke surgical delivery device enables sub-retinal delivery product
- Developed & validated in procedures performed on >40 pigs
- ~ 50 min procedure

What needed to be achieved



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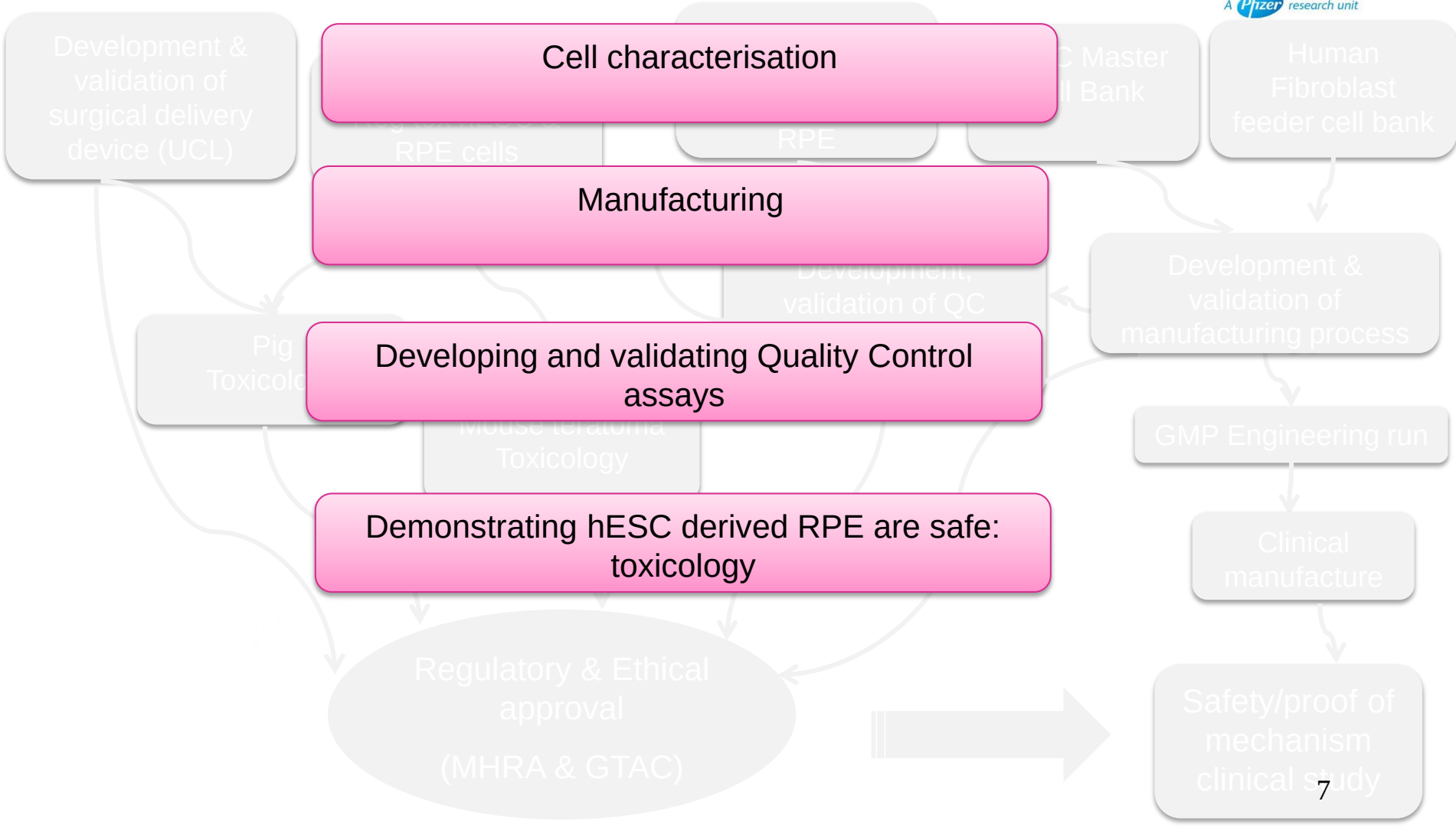
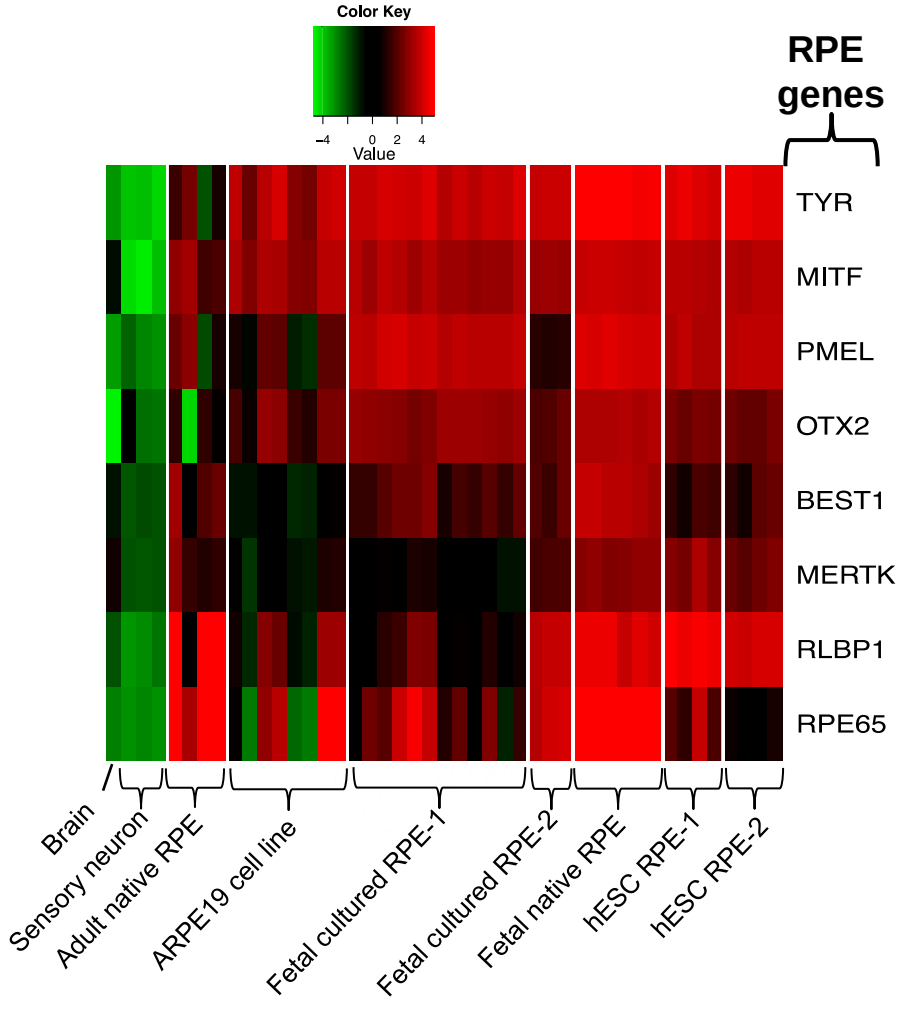
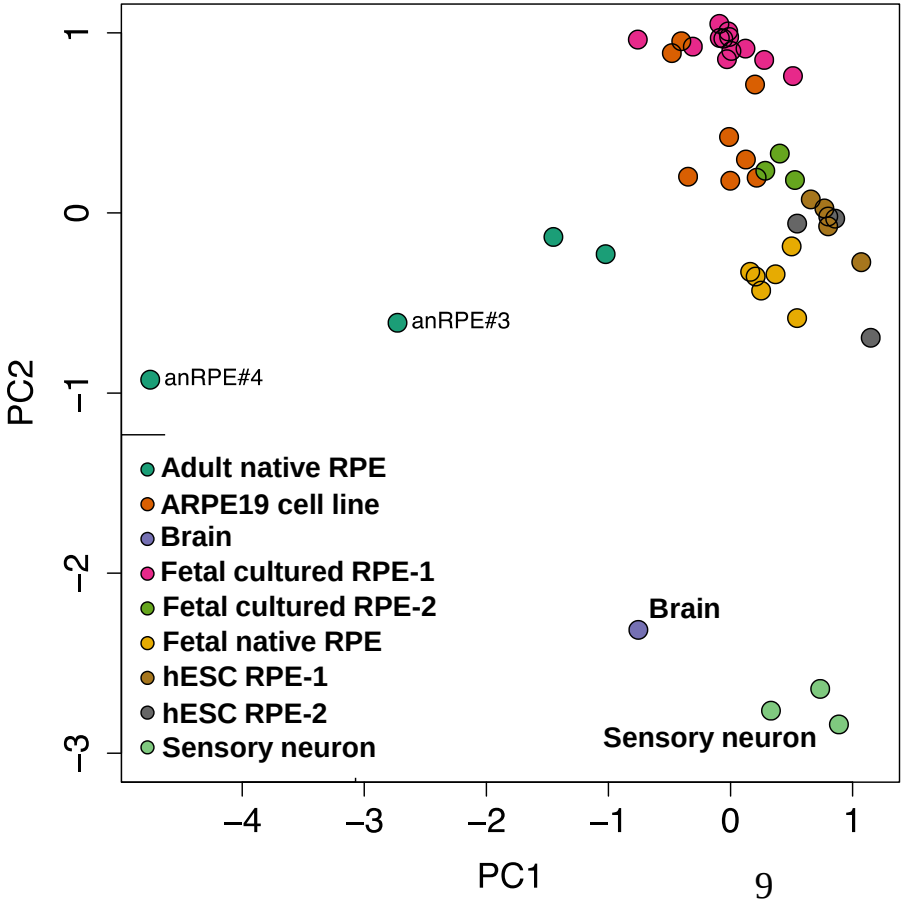


Phenotypic/functional properties of hESC derived RPE are indistinguishable from native RPE

	Property	hESC derived RPE	Native fetal / adult human RPE
Phenotype	"Cobblestone" appearance	✓	✓
	Pigmentation	✓	✓
	Expression of RPE phenotypic markers (whole genome transcript, and protein)	✓	✓
Ultra-structure	Expression of and formation of tight junction proteins	✓	✓
	Apical and basolateral polarisation	✓	✓
Immune- activity	Secrete immunomodulatory cytokines	✓	✓
	Inhibit T cell activation	✓	✓
In-vitro function	Vectoral secretion of trophic factors such as VEGF and PEDF	✓	✓
	Phagocytosis of rod outer segments	✓	✓
In-vivo function	Maintain vision in the RCS rat	✓	✓

Whole genome transcript analysis: hESC derived RPE indistinguishable from native RPE





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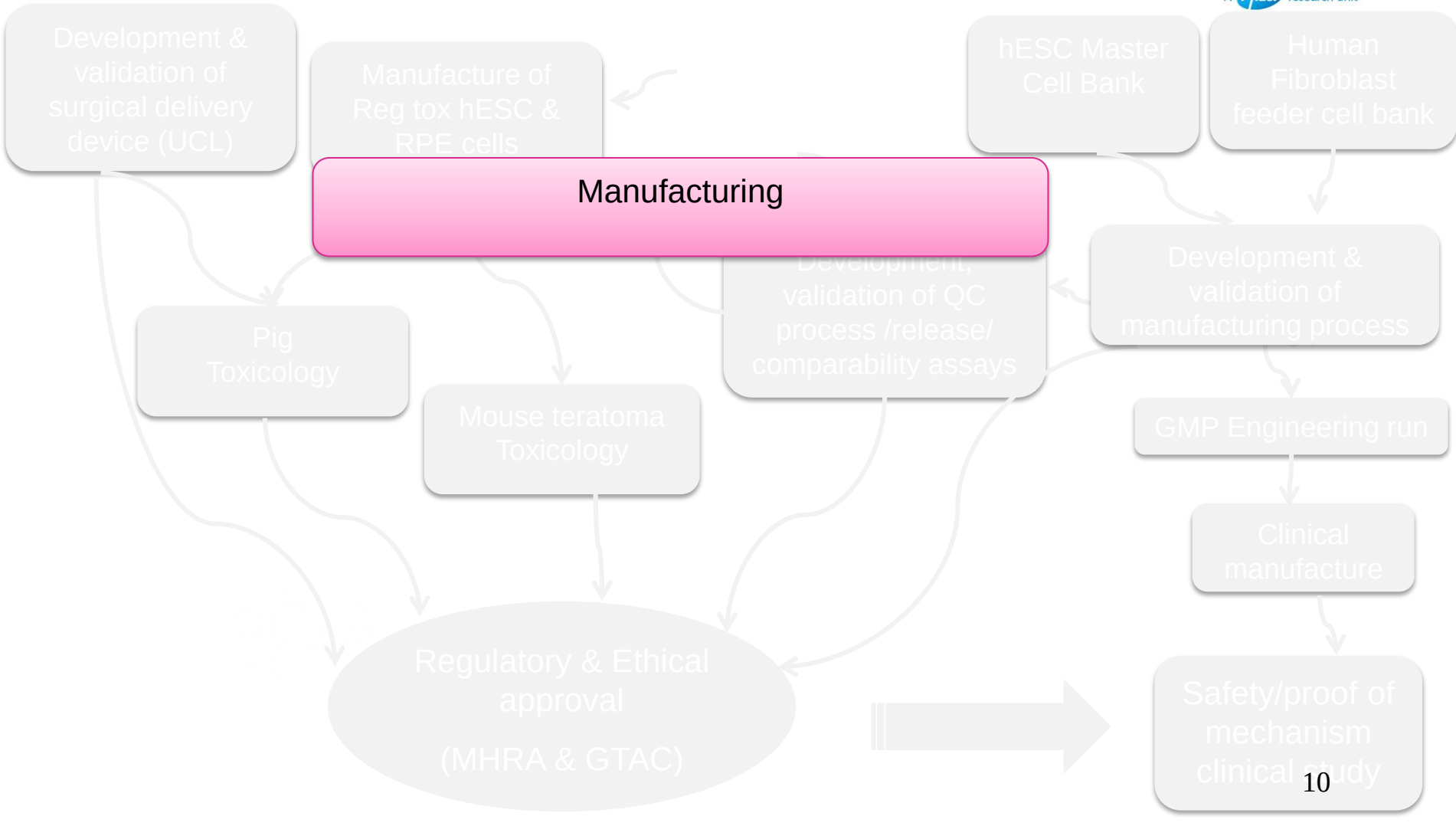
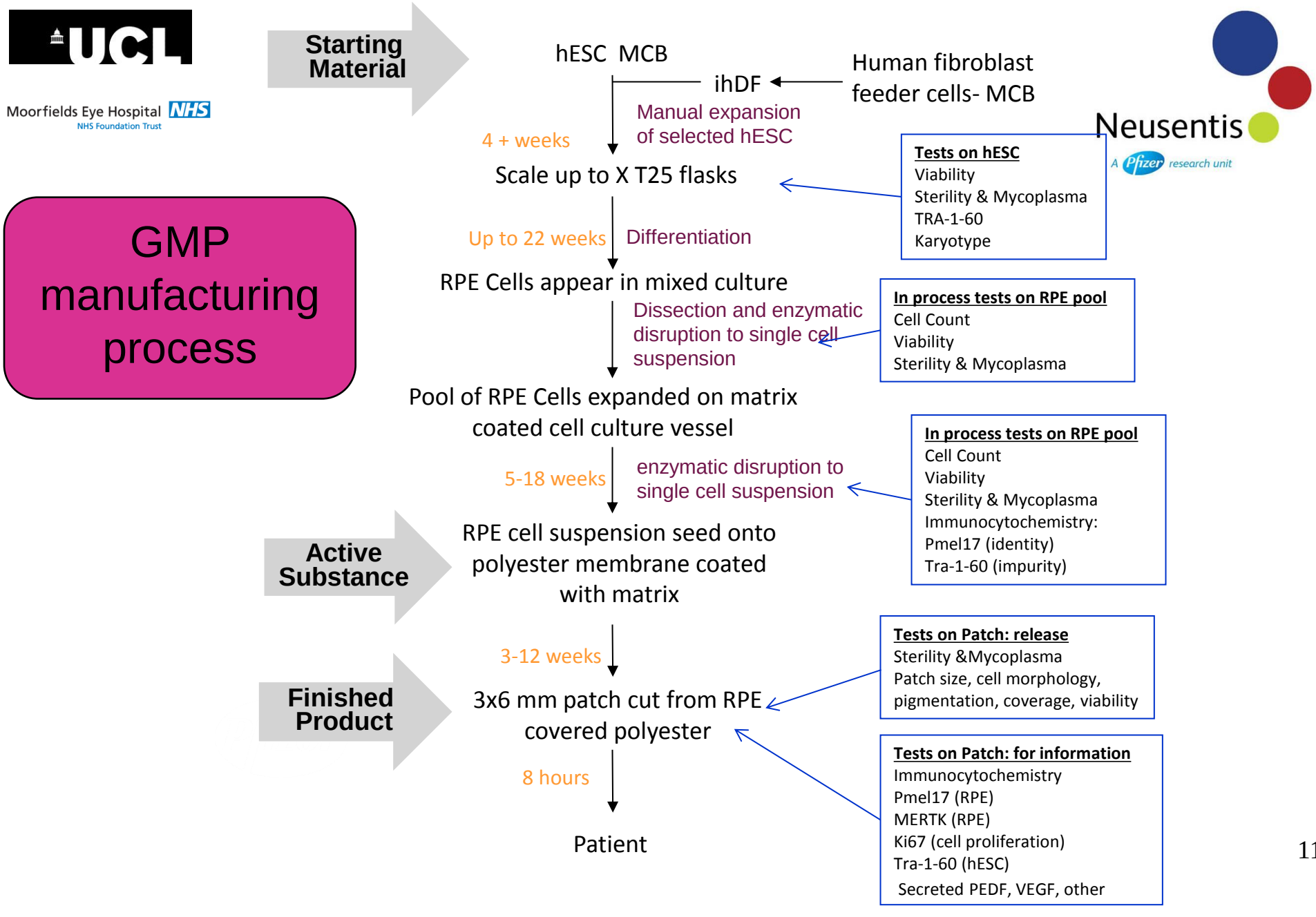
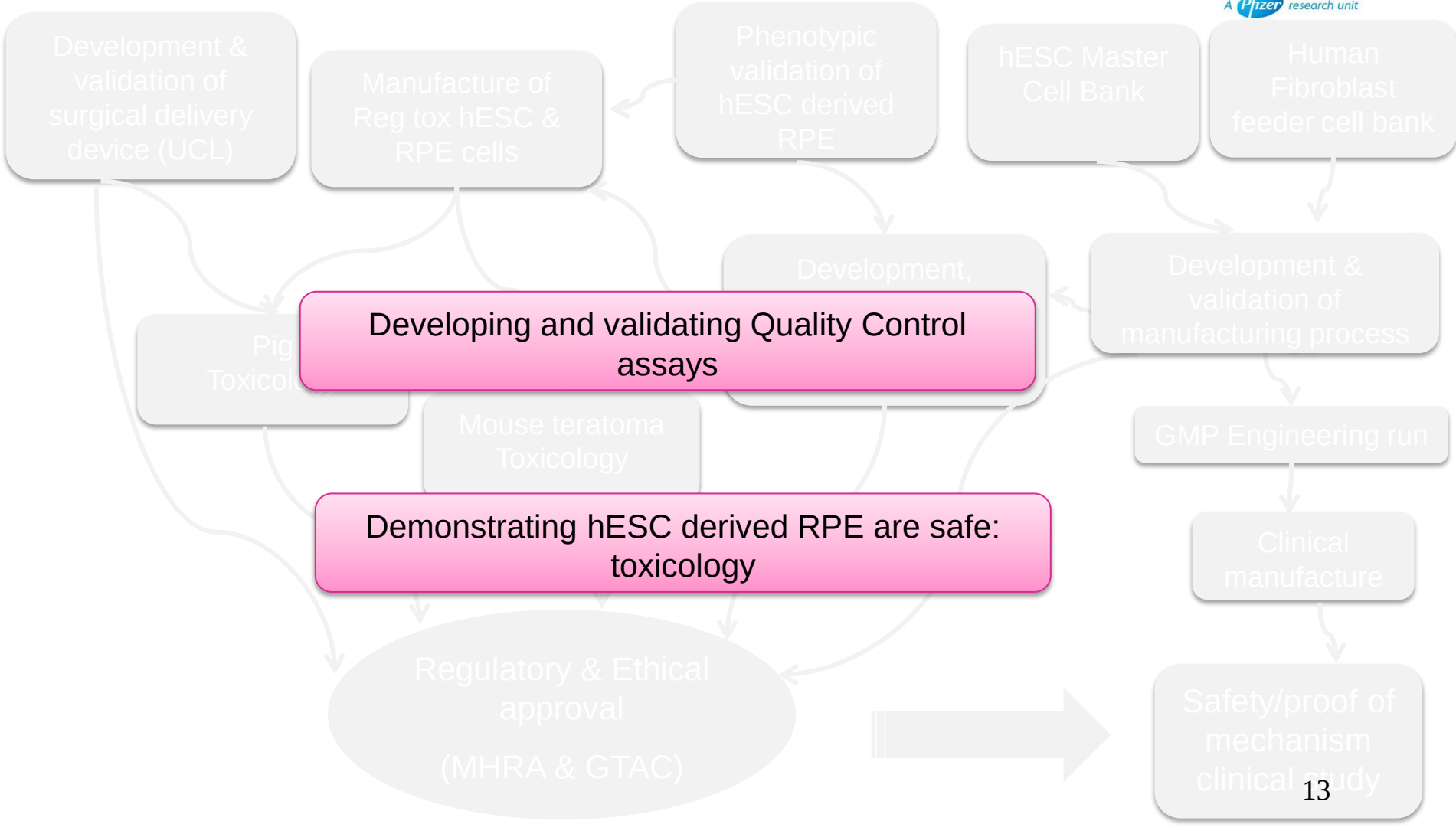



Starting Material
hESC MCB
ihDF
Human fibroblast feeder cells- MCB
Manual expansion of selected hESC
4 + weeks
Scale up to X T25 flasks
Tests on hESC
Viability
Sterility & Mycoplasma
TRA-1-60
Karyotype
Up to 22 weeks
Differentiation
RPE Cells appear in mixed culture
Dissection and enzymatic disruption to single cell suspension
In process tests on RPE pool
Cell Count
Viability
Sterility & Mycoplasma
Pool of RPE Cells expanded on matrix coated cell culture vessel
5-18 weeks
enzymatic disruption to single cell suspension
In process tests on RPE pool
Cell Count
Viability
Sterility & Mycoplasma
Immunocytochemistry:
Pmel17 (identity)
Tra-1-60 (impurity)
Active Substance
RPE cell suspension seed onto polyester membrane coated with matrix
3-12 weeks
Finished Product
3x6 mm patch cut from RPE covered polyester
Tests on Patch: release
Sterility &Mycoplasma
Patch size, cell morphology, pigmentation, coverage, viability
Tests on Patch: for information
Immunocytochemistry
Pmel17 (RPE)
MERTK (RPE)
Ki67 (cell proliferation)
Tra-1-60 (hESC)
Secreted PEDF, VEGF, other
8 hours
Patient
GMP manufacturing process
Moorfields Eye Hospital NHS
NHS Foundation Trust
UCL
Neusentis
A Pfizer research unit

Manufacturing: Lessons learned

- Turning a “research” process into a cGMP process is not trivial
- A long, linear manufacturing process is not ideal
  - Introduce a cryopreservation point
- Once you have your manufacturing process in place & approved you are locked in
  - “Fit for purpose” may require more than you think
  - Major changes will be challenging and costly in terms of time and money
  - More time spent early on will be recouped

What needed to be achieved



A key concern for Regulators: are there contaminating hESC in the product that could lead to teratoma formation?

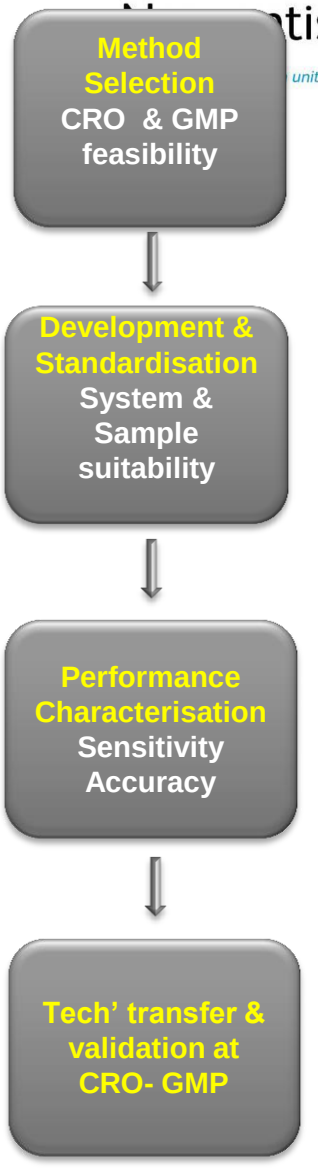
1
Do hESC survive the production process?

2
Can we detect hESC in the RPE product?

3
Does the RPE product lead to teratoma formation in immunodeficient mice?

Quality control assays

- "In-process" & "Release" assays
- *Pharmacopeia assays for sterility & mycoplasma*
- "Viability", "Identity", "Purity", "Impurity", ("Potency")
  - Population analysis vs single cell resolution
- Sensitivity, accuracy, robustness
- "GMP-ability"
  - Research lab development → GMP qualification & validation
- Fit for purpose (ie sufficient to support the stage of clinical development)



Quality Control Assay: testing for impurity

Moorfields Eye Hospital NHS
NHS Foundation Trust

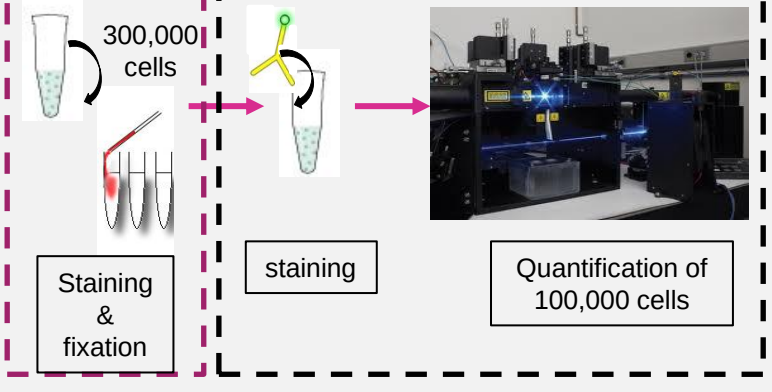
Neusentis

Choices of analytical test methods

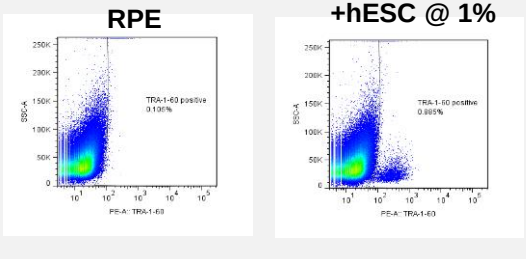
Flow Cytometry

Manufacturing Facility

GMP CRO



Selected population (double gated)



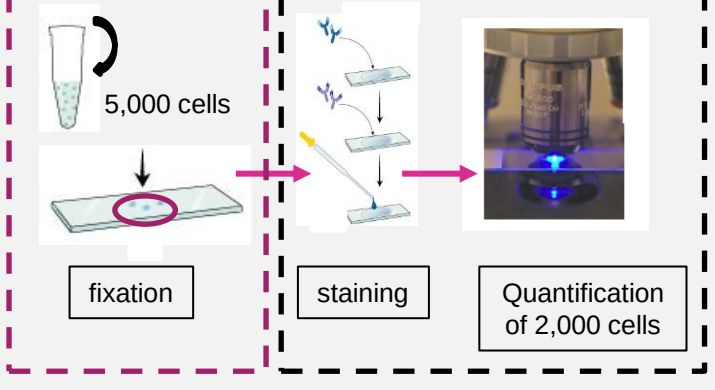
Sample use: 50 fold more cells required

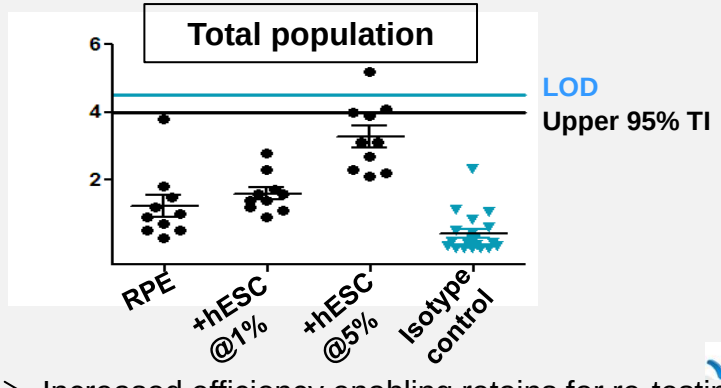
Single test opportunity

Image Cytometry

Manufacturing Facility

GMP CRO

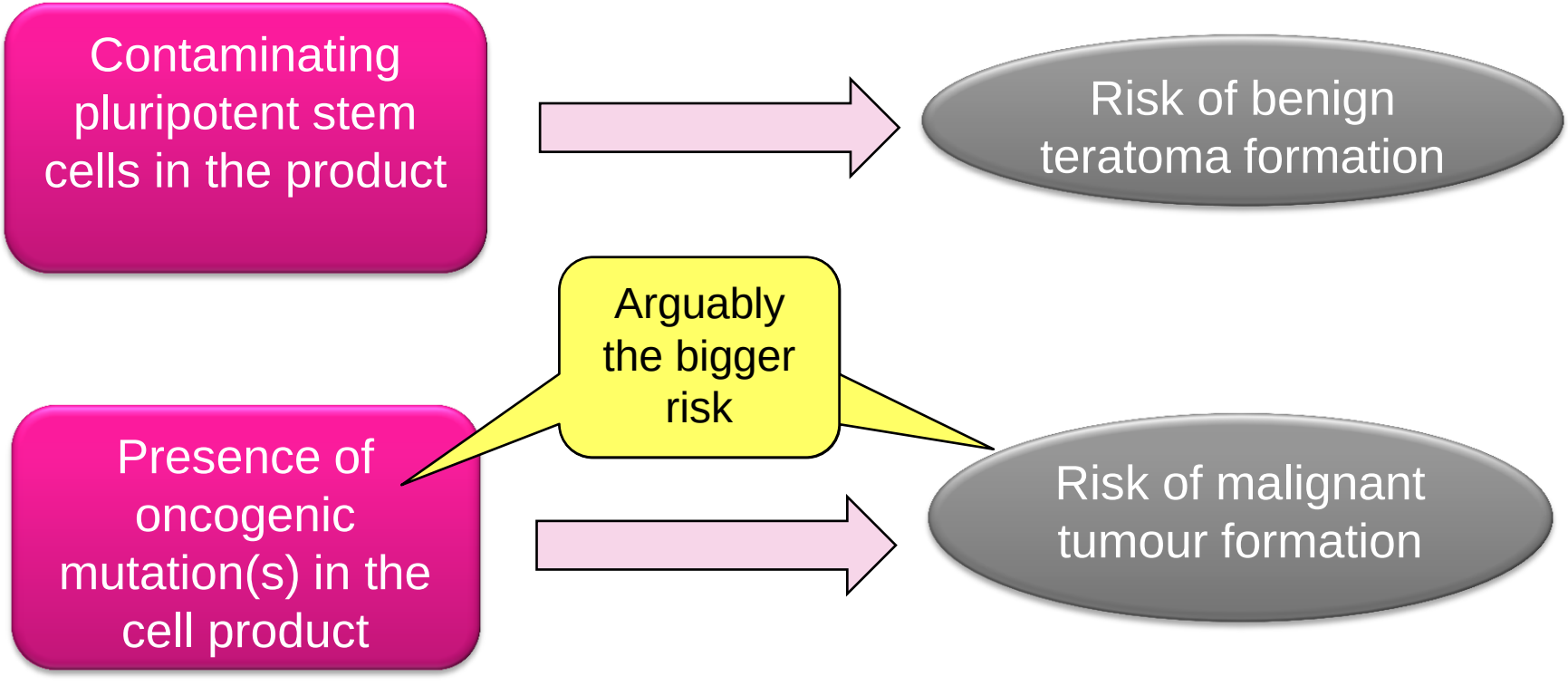




Increased efficiency enabling retains for re-testing

Images of individual cells preserved

Teratoma vs tumourgenicity
Clarity over what we mean



hESC do not survive the production process

Moorfields Eye Hospital NHS
NHS Foundation Trust

RPE cells spiked with hESC produce RPE cultures free of hESC

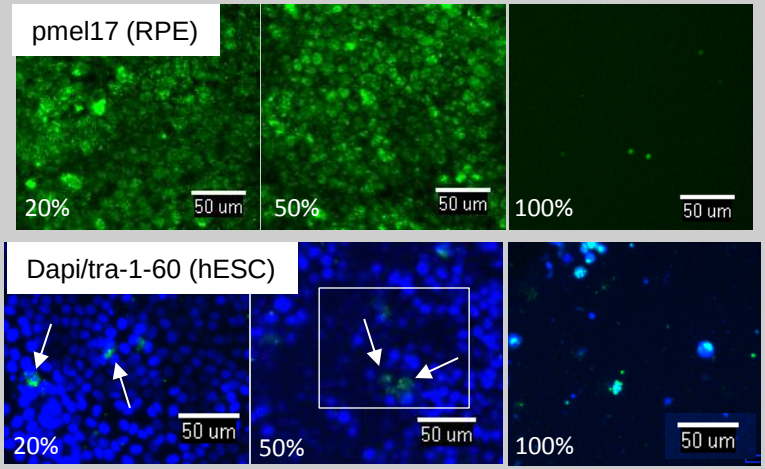
Dissociated RPE cells spiked with
0 →100% hESC
Culture for 6 weeks

↓

Quantify hESC & RPE by image analysis & flow cytometry

↓

No hESC (TRA-1- 60 events) detected



RPE culture conditions do not support hESC survival

hESC dissociated using RPE conditions

↓

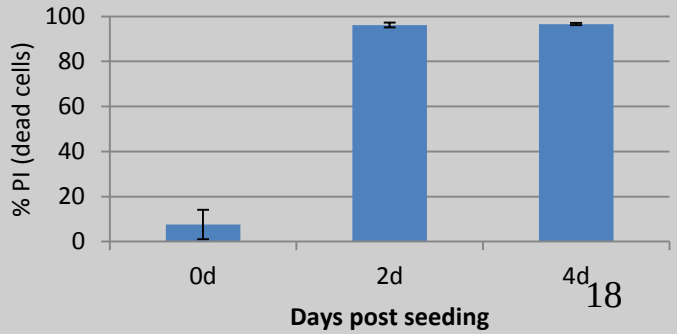
hESC maintained under RPE culture conditions for 0, 2 & 4 days

↓

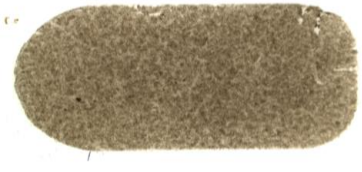
Quantify hESC by image analysis & flow cytometry

↓

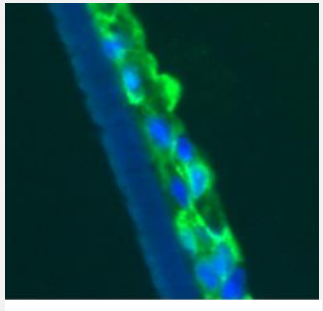
96% of cells dead by day 4
Remaining live cells were TRA-1-60 negative



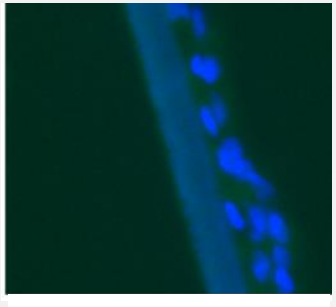
No hESC detected in the final product



- Sections cut from patch
- Quantitative immuno-cytochemistry for markers of RPE and hESC



CRALBP
(+ive control RPE)



TRA160
(+ive control hESC)

- 4 manufacturing runs
- 23, 221 total cells counted
- 0 Tra-1-60 events (hESC) detected

- This is a destructive assay which takes days to process & analyse
- For information, NOT a Release test

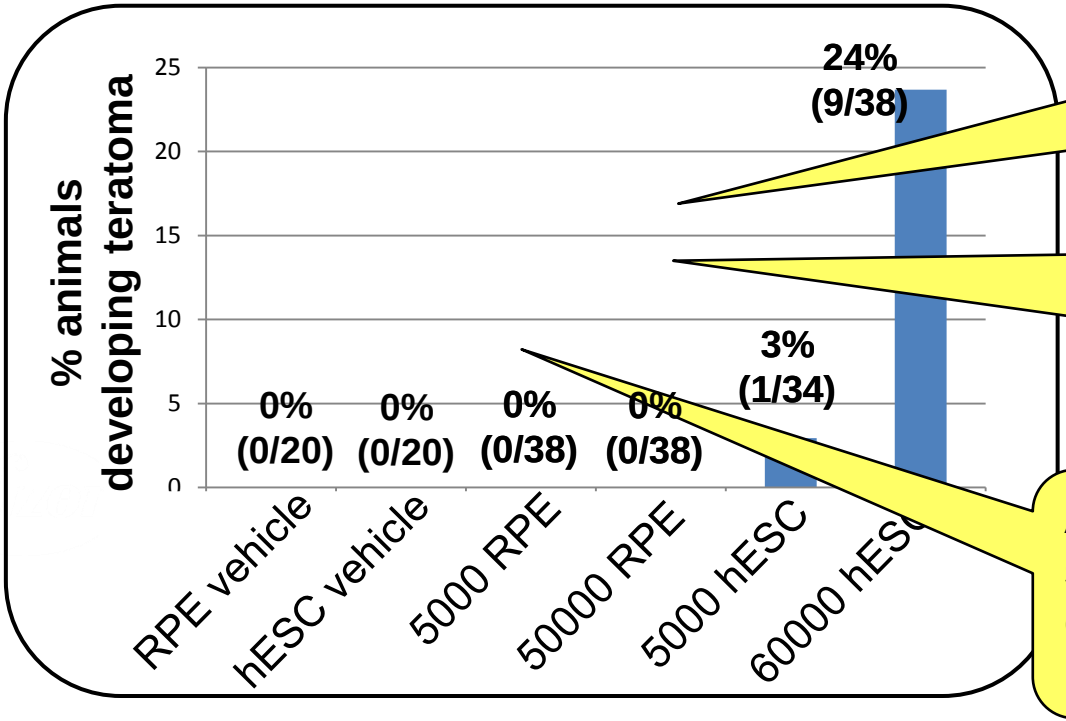
hESC derived RPE does not form teratomas in immunodeficient mice

Moorfields Eye Hospital NHS
NHS Foundation Trust

Neusentis
A Pfizer research unit

Intra-ocular administration
- RPE cells
- hESC (+ive control)

6 months → Pathology

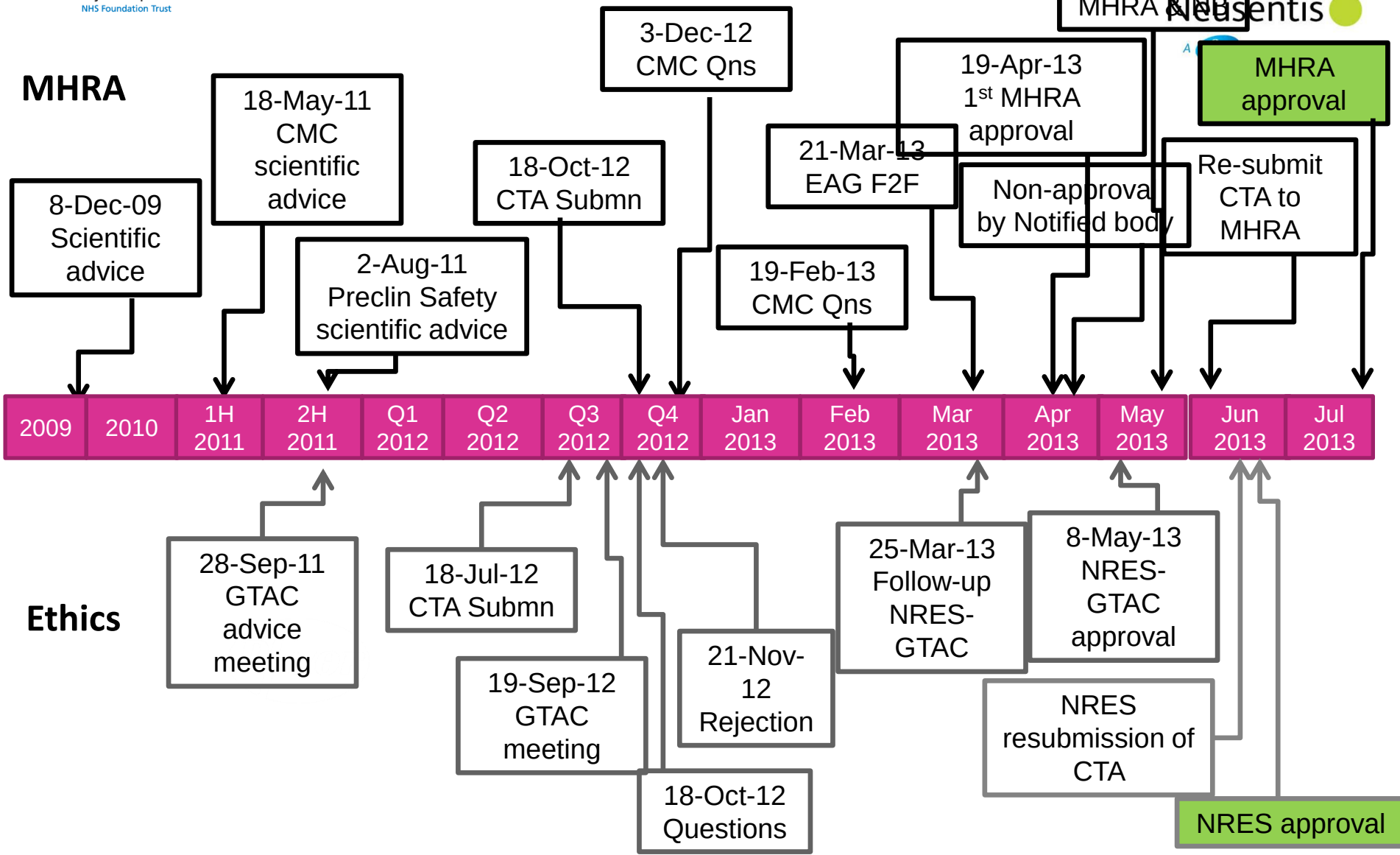


How many cells should be dosed to mitigate risk?

Ideally cells need to be given to the clinically relevant site

A GLP teratoma study costs hundreds of thousands of pounds

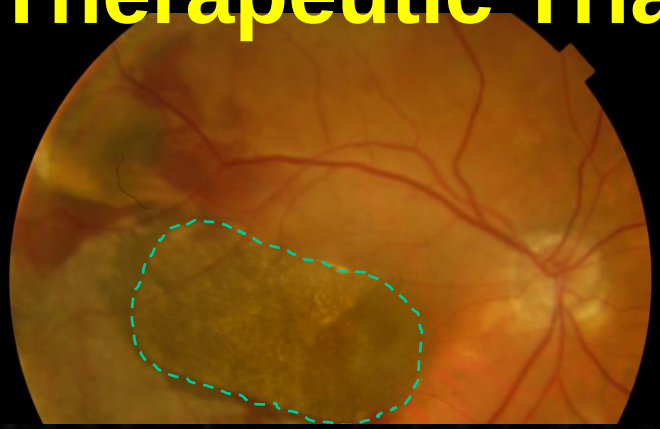
Regulatory Interactions

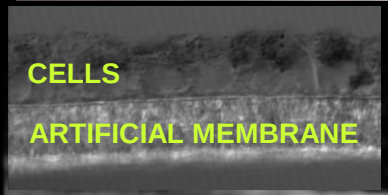


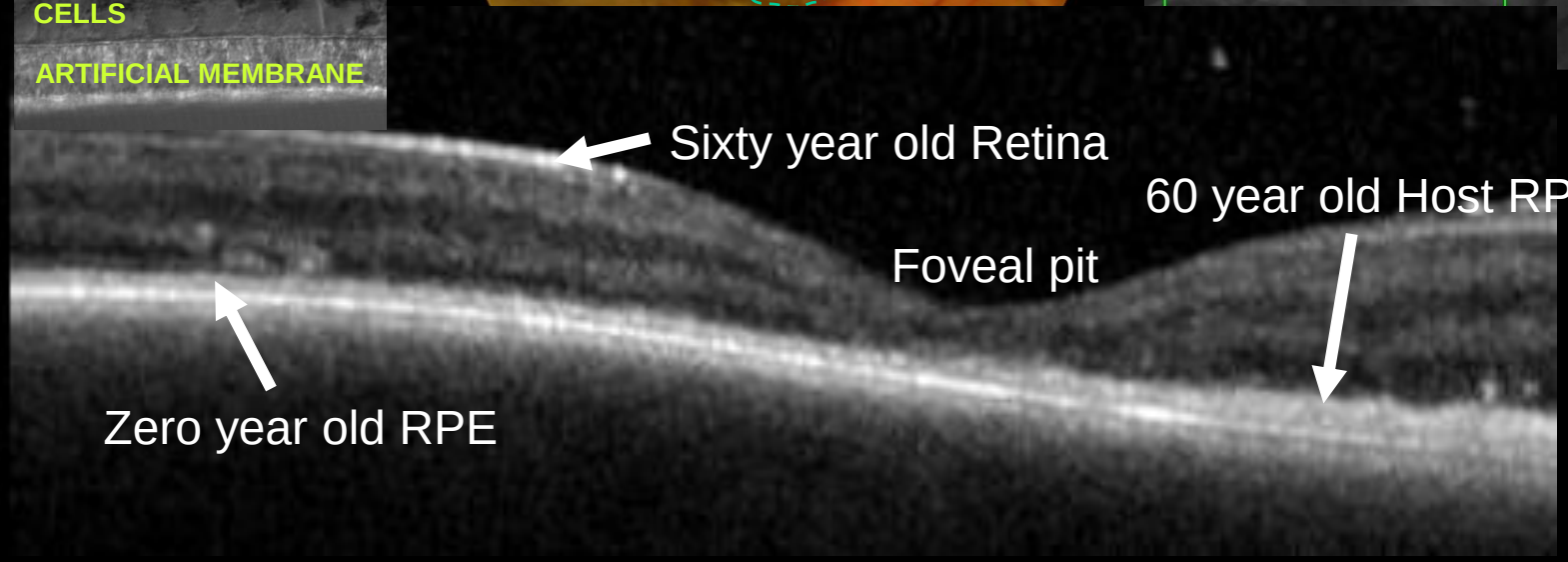
Cost!!!


Moorfields Eye Hospital NHS
NHS Foundation Trust
Meeting with MHRA & NB
MHRA approval
MHRA
the london project
to cure blindness
Re-submit CTA to MHRA
8-Dec-09 Scientific advice
2-Aug-11 Preclin Safety scientific advice
£10m
CMC Qns
2009
2010
1H 2011
2H 2011
Q1 2012
Q2 2012
Q3 2012
Q4 2012
Jan 2013
Feb 2013
Mar 2013
Apr 2013
May 2013
Jun 2013
Jul 2013
$20m (2010-2015)
Ethics
CTA Submi
Follow-up NRES-
GTAC
the california project
to cure blindness
Questions
NRES approval

Regulatory Approved – Phase I/II Therapeutic Trial







Cost/Benefit

- 40 patients at a cost of £4k/patient ~ total £160K
- It costs £15k/year to keep a person blind
- 16 patients recovered vision up to 8years
- Cost of procedures to NHS **£160K**
- **Saving to NHS ~ £2m**

Smart Cell Processing System

Based on the Open Innovation Concept

TOKYO ELECTRON

At Stevenage

- **80,000 Patches produced to GMP**

Summary

- Dont under-estimate the challenges or the costs!
- Ongoing structured dialogue with the Regulators
- Safety is the key concern
- Pick the right patient population
- Fit for purpose process appropriate to the stage of development