13th Annual Canadian Blood Services International Symposium

Blood-Borne Pathogens: Defend, Detect, and Destroy

Canadian Blood Services
it's in you to give

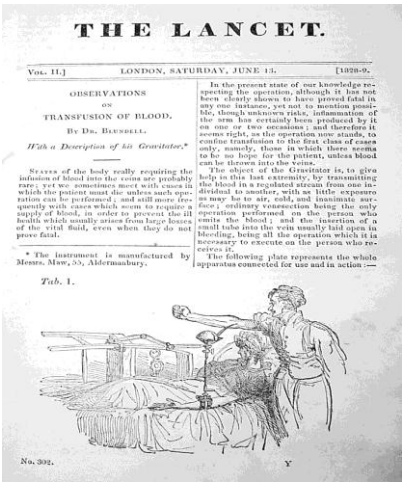
University of Bristol · University of Cambridge · The University of Edinburgh · University of Glasgow · Scottish National Blood Transfusion Service · NHS Blood and Transplant · Irish Blood Transfusion Service · Loughborough University · Heriot-Watt University · University of Dundee · Roslin Cells · Catapult Cell Therapy

Ex vivo production of red cells for clinical transfusion

wellcome trust · Scottish Funding Council · NOVOSANG

Blood Transfusion

- In 1816 John Henry Leacock from Barbados studying in Edinburgh carried out the first systematic experiments on intra-species transfusion.
- First human transfusions carried out in 1818 by James Blundell
- Mainstay of current clinical practice - @92m red cell transfusions *per annum* world-wide
- Limitations:
  - Sufficiency
  - Immunological compatibility
  - Transfusion Transmitted Infection
  - Iron loading



THE LANCET.

Vol. II.] LONDON, SATURDAY, JUNE 13. [1828-9.

OBSERVATIONS
ON
TRANSFUSION OF BLOOD.
By Dr. Blundell.

*With a Description of his Gravitator.**

Cases of the body really requiring the infusion of blood into the veins are probably rare; yet we sometimes meet with cases in which the patient must die unless such operation can be performed; and still more frequently with cases which seem to require a supply of blood, in order to prevent the ill health which usually arises from large losses of the vital fluid, even when they do not prove fatal.

* The instrument is manufactured by Messrs. Maw, 55, Aldermanbury.

In the present state of our knowledge respecting the operation, although it has not been clearly shown to have proved fatal in any one instance, yet not to mention possible, though unknown risks, inflammation of the arm has certainly been produced by it on one or two occasions; and therefore it seems right, as the operation now stands, to confine transfusion to the first class of cases only, namely, those in which there seems to be no hope for the patient, unless blood can be thrown into the veins.

The object of the Gravitator is, to give help in this last extremity, by transmitting the blood in a regulated stream from one individual to another, with as little exposure as may be to air, cold, and inanimate surface; ordinary venesection being the only operation performed on the person who emits the blood; and the insertion of a small tube into the vein usually laid open in bleeding, being all the operation which it is necessary to execute on the person who receives it.

The following plate represents the whole apparatus connected for use and in action:—

Tab. 1.

No. 302. Y

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Functional definition of stem cells

The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice
Author:McCulloch, Ernest A. ; Till, James E.
Issue Date:Jul-1960
Publisher:Radiation Research Society
Citation:Radiation Research 1960(Jul); 13(1): 115-125
Abstract (summary):SUMMARY: 1. A technique for measuring the number of viable cells in a suspension of bone marrow by quantitative transplantation into supralethally irradiated mice has been described. 2. The technique was used to measure the radiation sensitivity of normal mouse bone marrow cells and yielded a result of 105 ± 24 rads as the D37 for marrow cells.

- Capability for self renewal
- Multilineage differentiation
- Long term generation of tissue
- Variation in degree of potency

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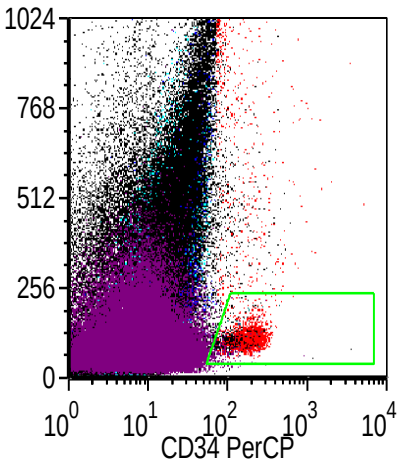
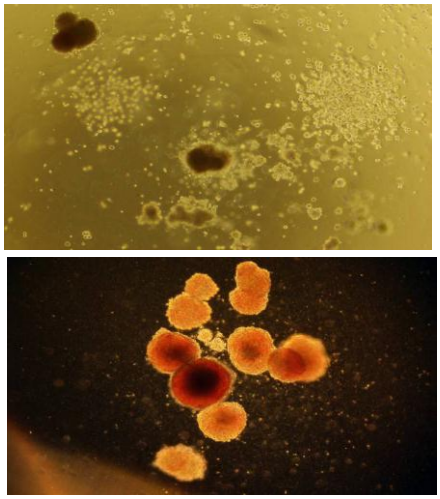
Haematopoietic stem and progenitor cells (HSPC)





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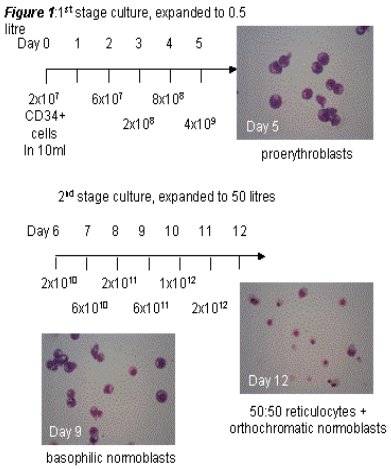
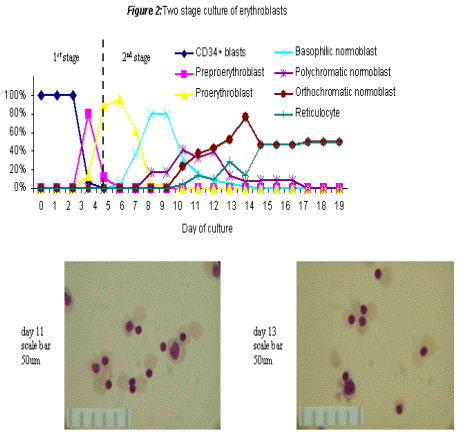
HSPC-derived erythroid cells





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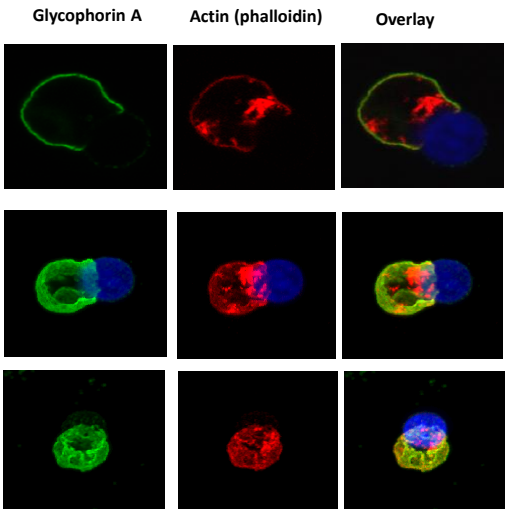
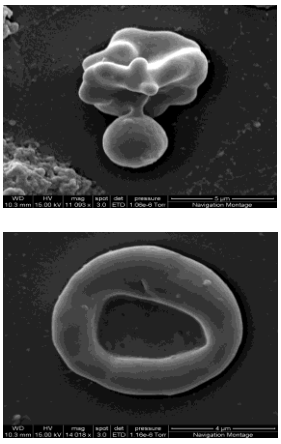
Enucleation





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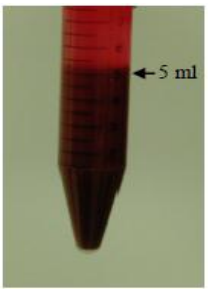
Manufacture of red cells from adult HPC

- Stirred culture vessels
- 5ml = 2x10E10 filtered reticulocytes
- Limited scalability



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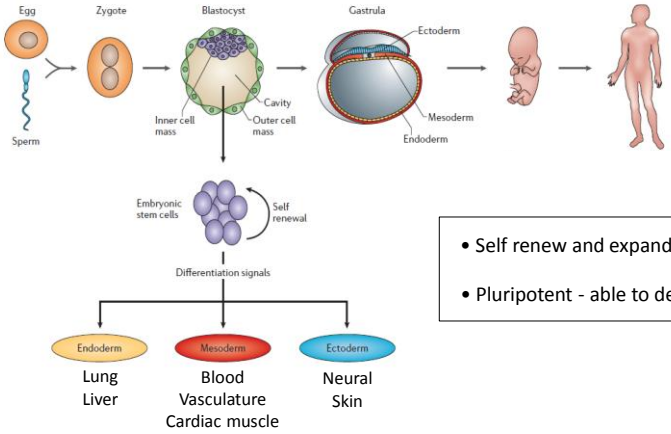
Human embryonic stem cells (hESC)



- Self renew and expand indefinitely in culture
- Pluripotent - able to derive all cells of the body

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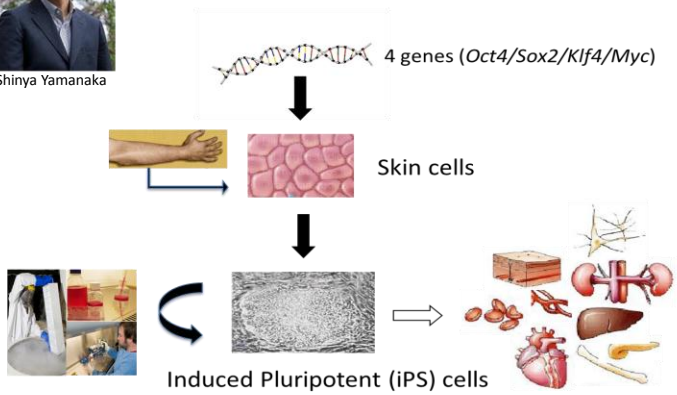
Induced pluripotent stem cells (iPSC)

(Takahashi & Yamanaka; 2006, 2007)

Shinya Yamanaka



Nobel Prize, 2012

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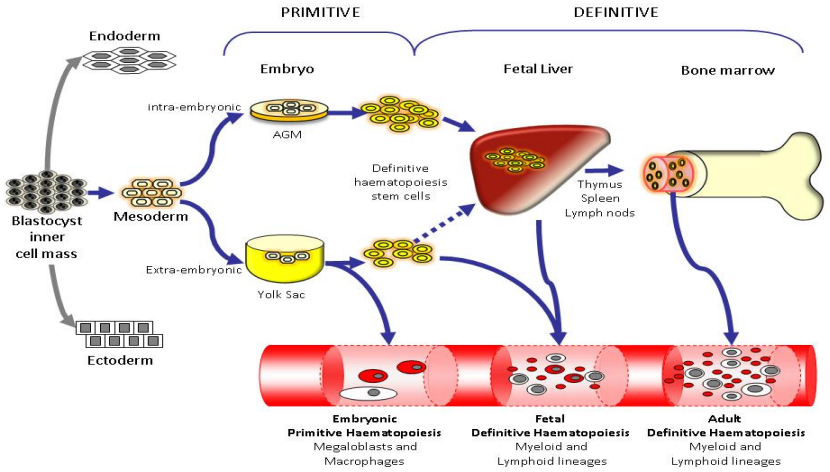
hPSC differentiation *in vivo*



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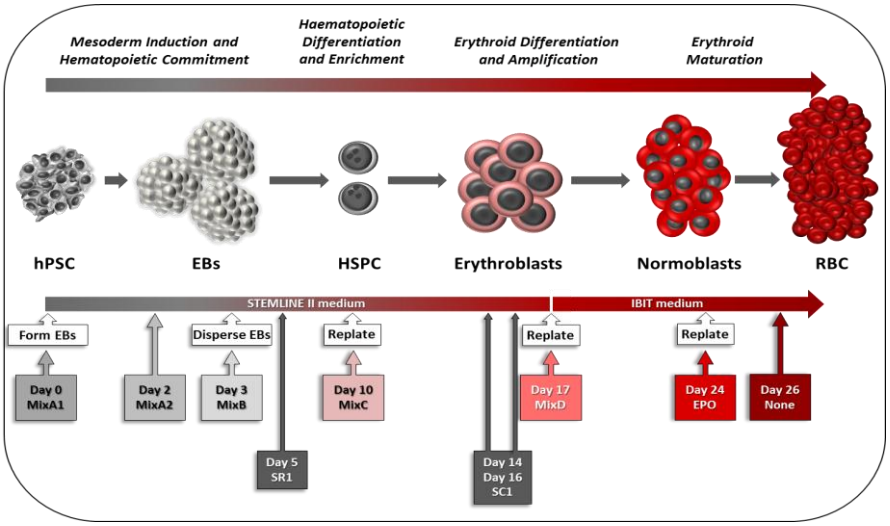
hPSC differentiation *in vitro* (feeder/serum free)



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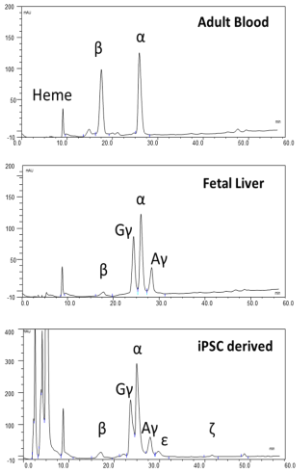
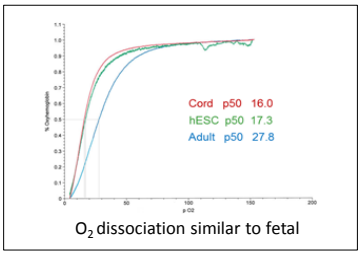
Cultures red cells express fetal globin



- Adult RBC express α/β globin chains
- Foetal RBC express α/γ globin chains
- Embryonic RBC express ζ/ε globin chains
- **hPSC RBC express α/γ globin chains**



O_2 dissociation similar to fetal

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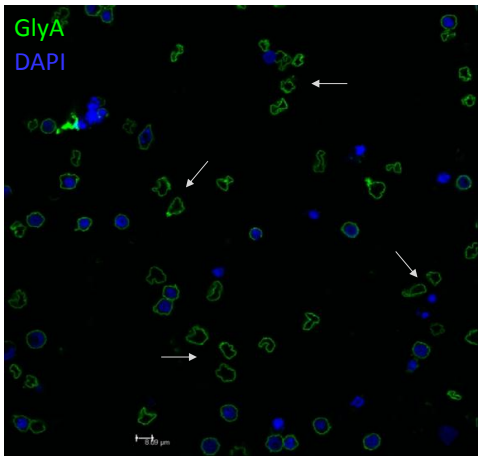
Enucleation



50% enucleated

- hPSC derived RBC can enucleate
- But they are fragile and difficult to maintain in culture

Data from Dave Anstee & Nicky Cogan, U of Bristol, NHSBT

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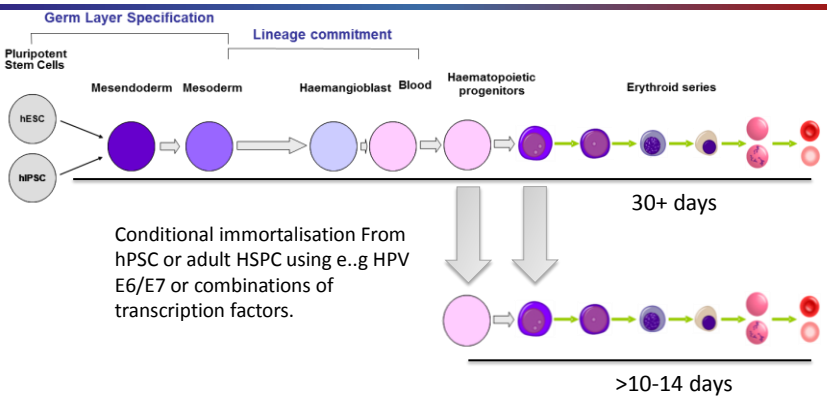
induced Multipotent Stem Cell lines



PROS:
1. Reduced differentiation time and complexity
2. Reduced cytokine/GF/media costs
3. Better enucleation
4. More adult phenotype

CONS:
1. Introduction of oncogenes
2. Cell line stability
3. Conversion to GMP/clinical grade

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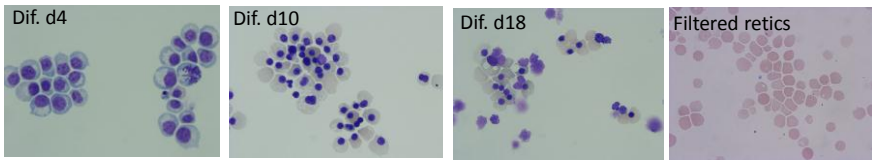
induced Multipotent Stem Cell lines

BEL-A2



Dr Jan Frayne and Prof David Anstee,
University of Bristol

PROS:
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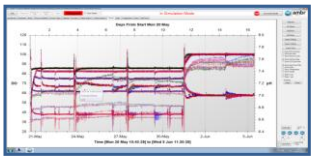
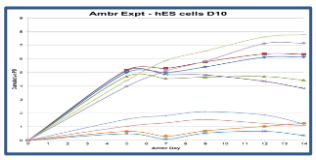
Scale-up, process control and intensification

- Maturity of technology: well established for production of therapeutics and regulatory compatible suppliers.
- Precise control of several physico-chemical parameters.
- Allows economic/rapid development screening in scaled-down version but staged scaling available (10ml, 250ml, multi-litre)





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Identifying productivity potential and limits

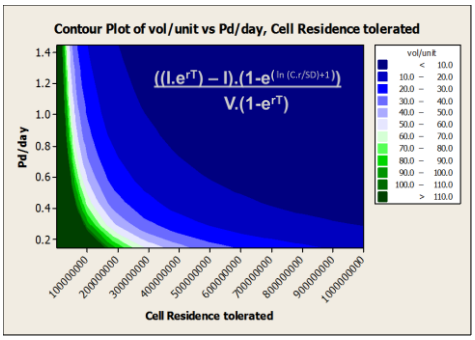
There are two gross limits to system efficiency:

-Absolute density limits of the system (mass transfer – Kla)

-Media volumetric productivity (litres of media/unit of blood)

-Density limit is calculated from ***specific oxygen uptake rate of the cells*** and the mass transfer coefficient of the system

-Volumetric Productivity is precisely determined by cell growth rate and ***specific support capacity of the media***



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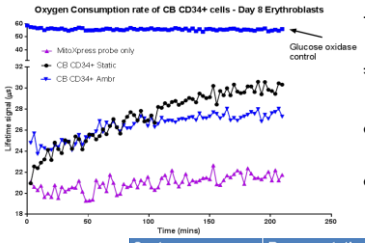
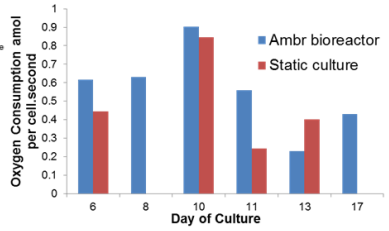
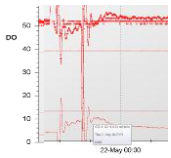
Absolute density limit (mass transfer)







System approximation	Representative Kla (hr^{-1})	Theoretical density supported (cells/ml)	System characteristics amenable to erythroid cell sensitivities
Pilot scale to large production sparged stirred tank (1-20k L)	60-380	1.6E9-9.8E9	No
10-50 L non-sparged stirred vessels	10-100 (depending on agitation and fill volume)	2.6E8-2.6E9	Yes
200L non-sparged (i.e. Cultibag Orb)	10-20	2.6E8-5.2E8	Potentially
5-10L Wave Bag	2-60 (dependent on rocking rate and head space gas exchange)	5.2E7-1.6E9	Potentially

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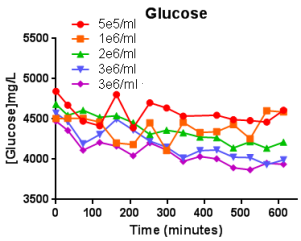
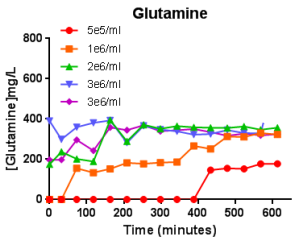
Vol. productivity limit (specific media capacity): Consumption and supplementation

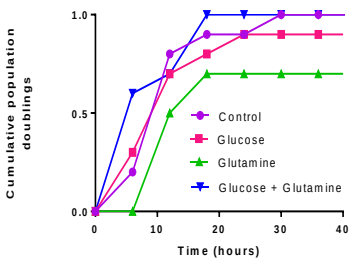


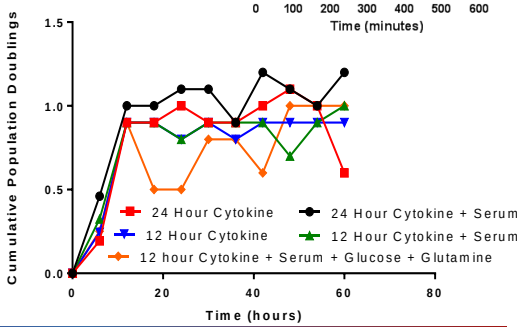
-Iron, glutamate, lit. on amino acids

-Specific rates unstable

-supplementation based on best estimate specific rates does not improve growth







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Challenges

- Control over genetic and epigenetic stability of cell lines
- Optimisation of differentiation pathway to allow stable enucleation
- Efficiency of the differentiation pathway
- Process control over multiple physical and biochemical factors
- Scale up and intensification to control the cost of goods
- Detailed characterisation of the product
- Demonstrating preclinical safety and efficacy
- Quality control and regulatory compliance
- Carefully design of clinical trials

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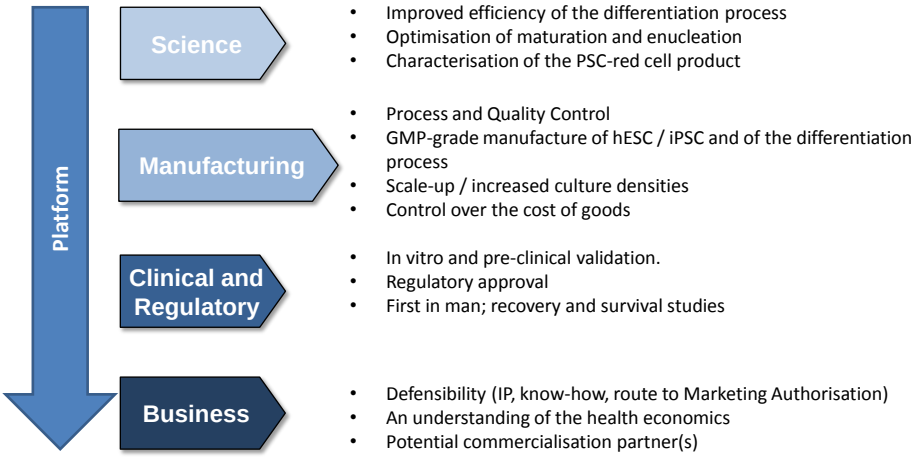
Multidisciplinarity

Start with the end in mind and create and deliver an integrated plan
Create a platform to leverage the investment required for manufacture and clinical trials



- Improved efficiency of the differentiation process
- Optimisation of maturation and enucleation
- Characterisation of the PSC-red cell product

- Process and Quality Control
- GMP-grade manufacture of hESC / iPSC and of the differentiation process
- Scale-up / increased culture densities
- Control over the cost of goods

- In vitro and pre-clinical validation.
- Regulatory approval
- First in man; recovery and survival studies

- Defensibility (IP, know-how, route to Marketing Authorisation)
- An understanding of the health economics
- Potential commercialisation partner(s)

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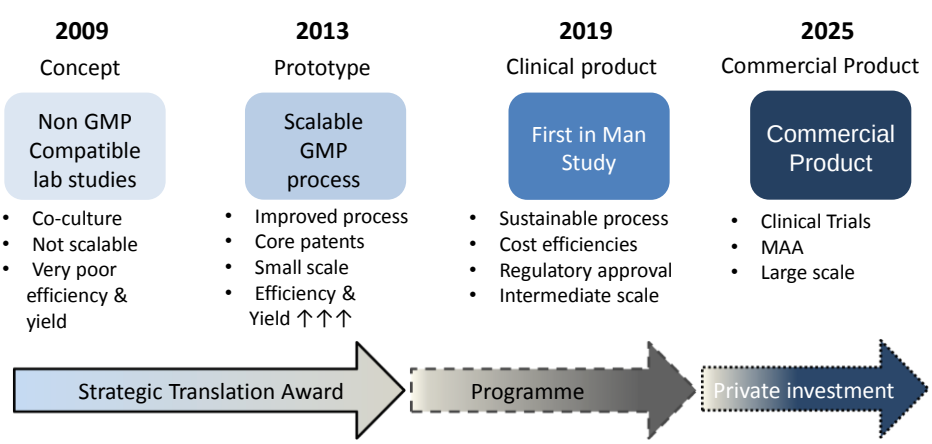
Path to commercial manufacture



Paradigm for the development of other cellular therapeutics

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