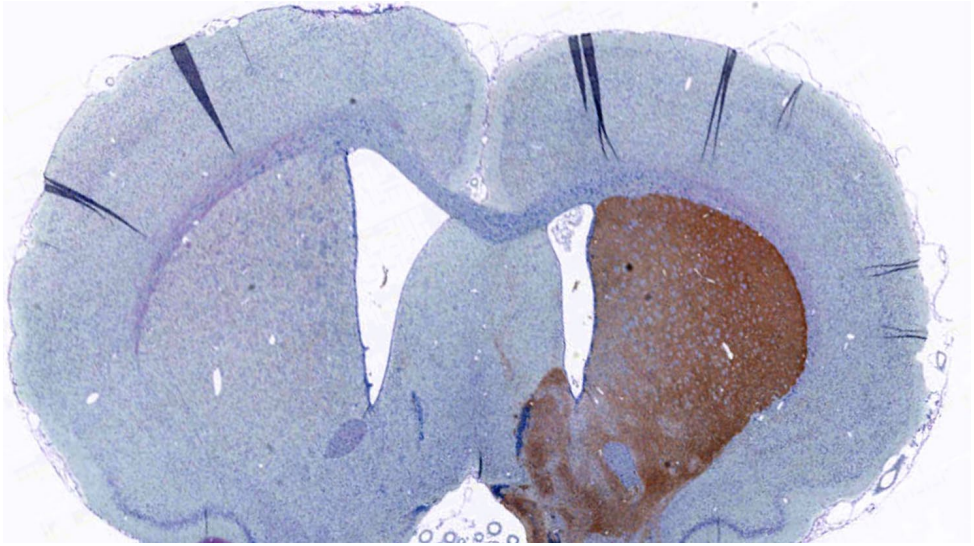
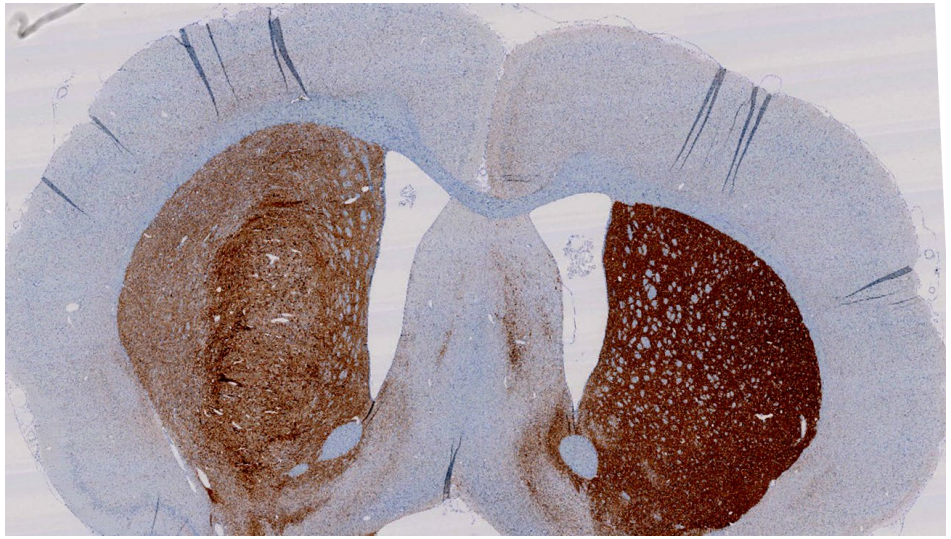
Cell Therapies

Lorenz P. Studer
Director, Center for Stem Cell Biology,
Memorial Sloan Kettering Cancer Center, New York, NY

studerl@mskcc.org www.ski.edu/studer @studerl





Disclosure Statement

- I am a scientific co-founder of BlueRock Therapeutics, a now wholly owned subsidiary of Bayer
- BlueRock has licensed mDA neuron differentiation technology for cell therapy from MSKCC and sponsors Phase I trial

- I am scientific co-founder of DaCapo Brainscience, an early start up using AI & stem cell technology for drug discovery

Outline – Cell Therapies

Cell sources
- Definition of stem cells and potency
- Pluripotent stem cells, directed differentiation, cell maturation and aging
- Other types of stem cells, tissue-specific stem cells, engineered cells (synthetic biology?)

Currently approved and investigational Cell therapies
- Approved cell therapies
- Investigational cell therapies
- Unproven cell therapies and stem cell tourism

Preclinical research
- Choice of disease and choice of candidate cell type
- In vivo model systems for preclinical research

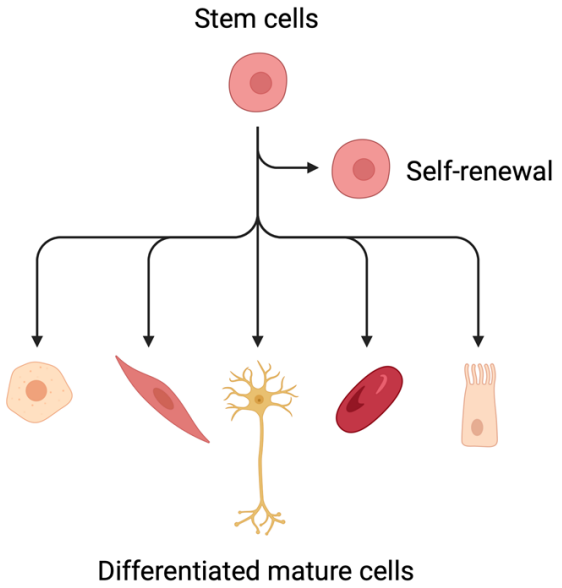
Product development and clinical grade manufacturing
- Clinical grade manufacturing, GMP compliance, Critical quality attributes
- IND enabling studies, Device and cell delivery

  ✓ Case study**:** Manufacturing a **dopamine neuron cell product for Parkinson's disease**

  ✓ Case study: Manufacturing an **enteric neural precursor cell product for Hirschsprung's disease**

❑ **Design you own study (DIY):**

Cell Sources – Definition of Stem Cells, Nomenclature & Potency



Names	Criteria	Potency
• *Stem Cell*	• ***Self-Renewal***	• *Totipotent*
• *Progenitor Cell*	• ***Ability to generate specialized cells***	• *Pluripotent*
• *Precursor Cell*	*(Multi)-lineage Differentiation*	• *Multipotent*
• *(Commitment)*	• *(Regeneration of Organ System)*	• *Unipotent*
• *(Stem Cell Niche)*	• *(Regulation of size of stem cell pool (niche)*	

Cell Sources – Stem cells & Potency throughout life span

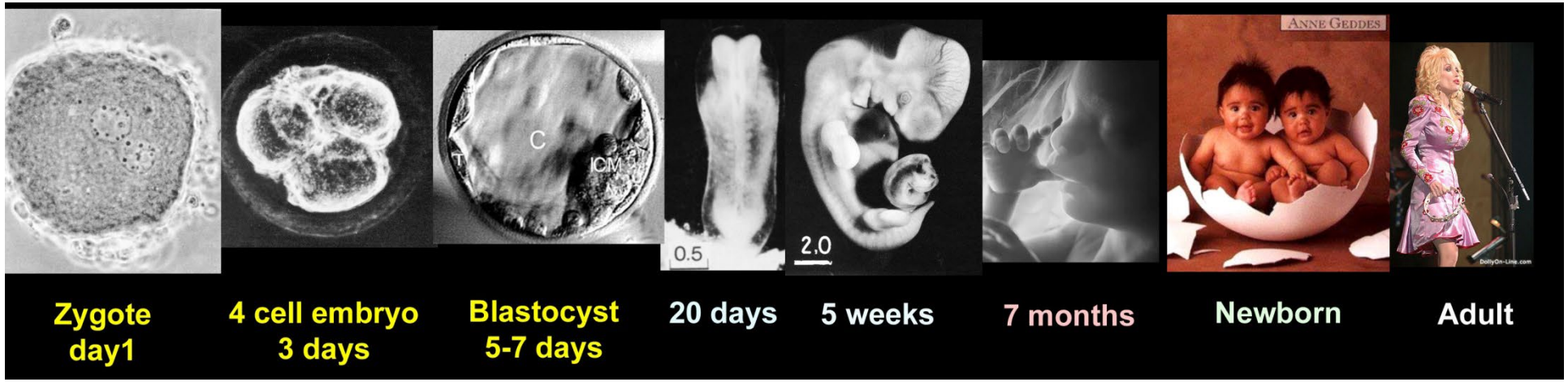
Uni-/ Multipotent Stem Cells:

Tissue Specific Cells

Embryonic | Fetal | Neonatal (Umbilical Cord) | Adult

Pluripotent Stem Cells:

ES Cells | EG Cells | EC Cells | SSC (?)



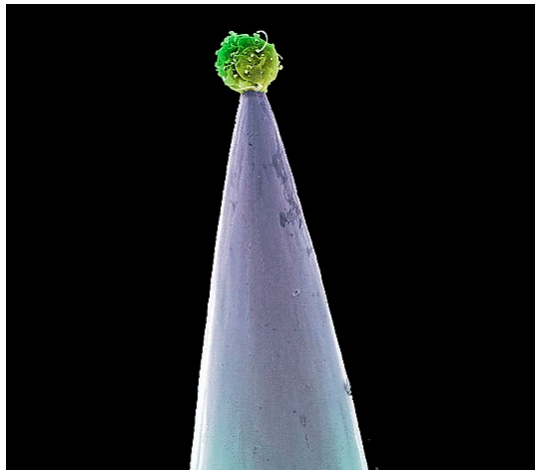
EMBRYO → FETUS ⇢ ADULT

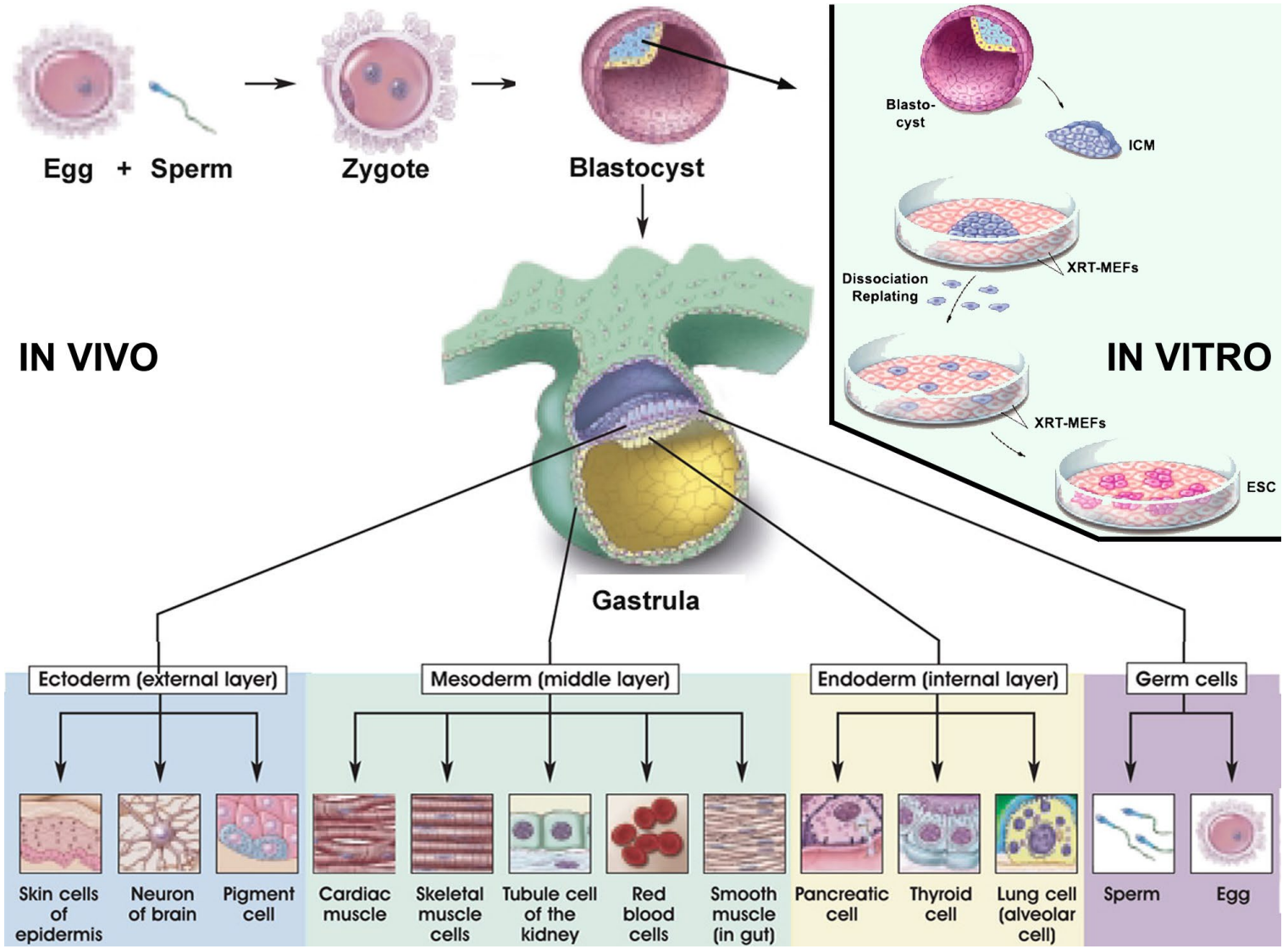
fertilization | 14 days | 3 mts | 9 mts

Reprogramming ←

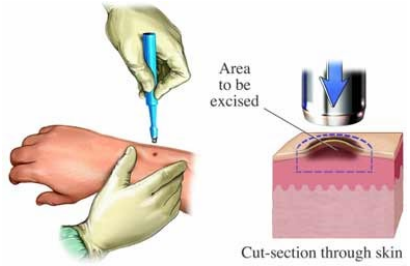
Cell Sources – human embryonic stem cells

Jamie Thomson

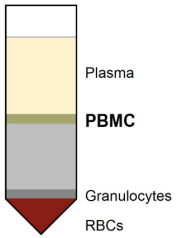


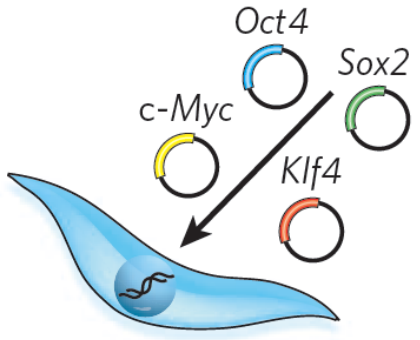


Cell Sources – human induced pluripotent stem cells

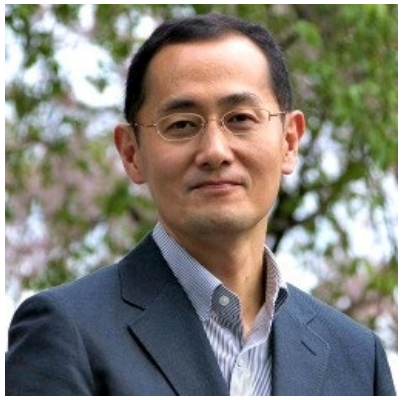


Blood, skin, urine

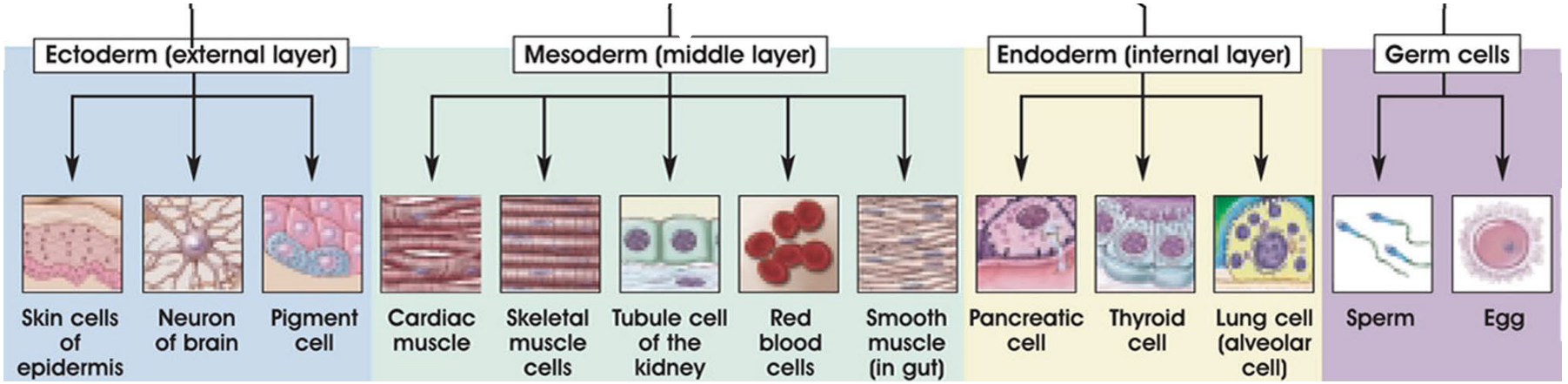




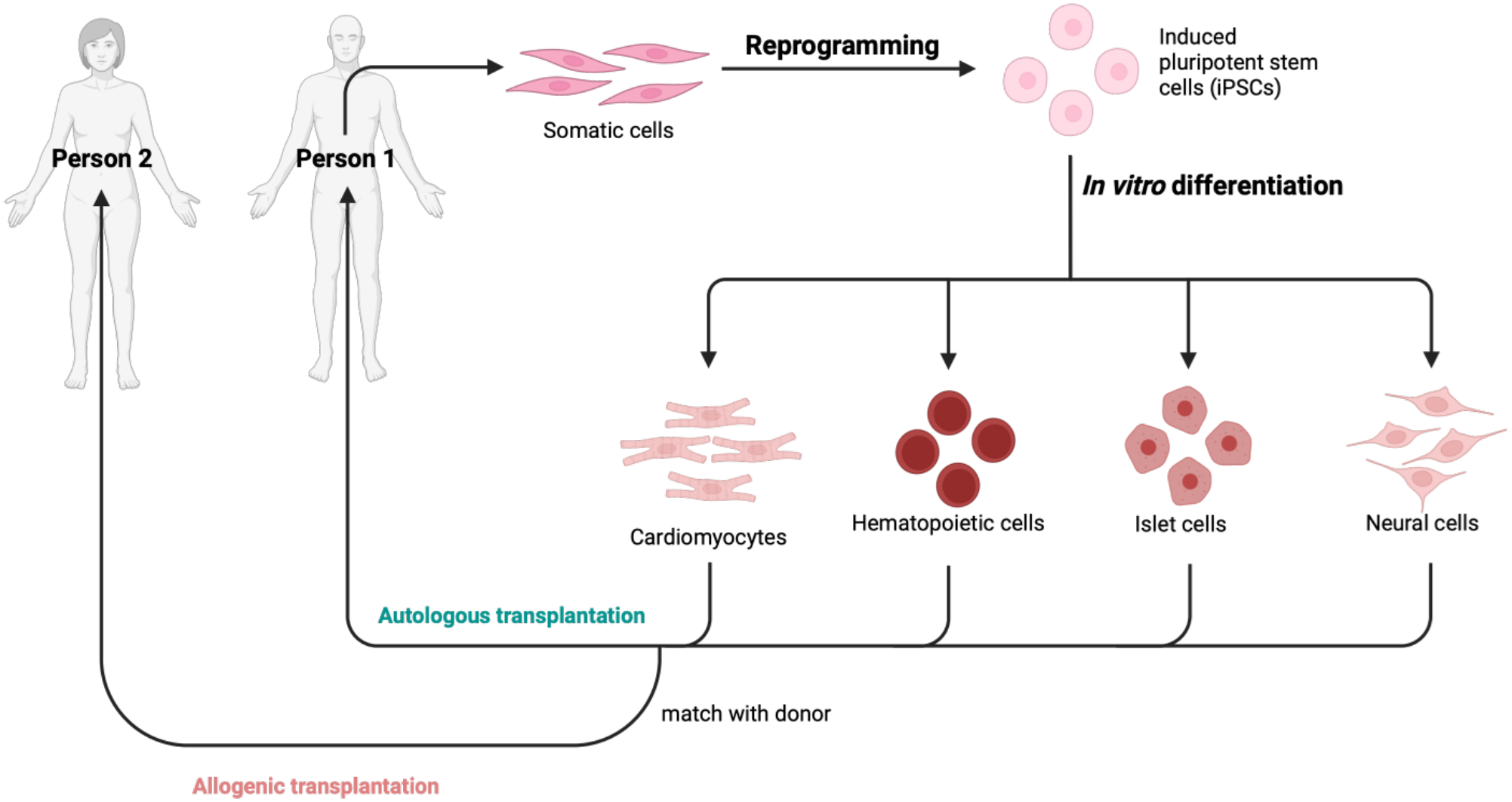
iPS cell



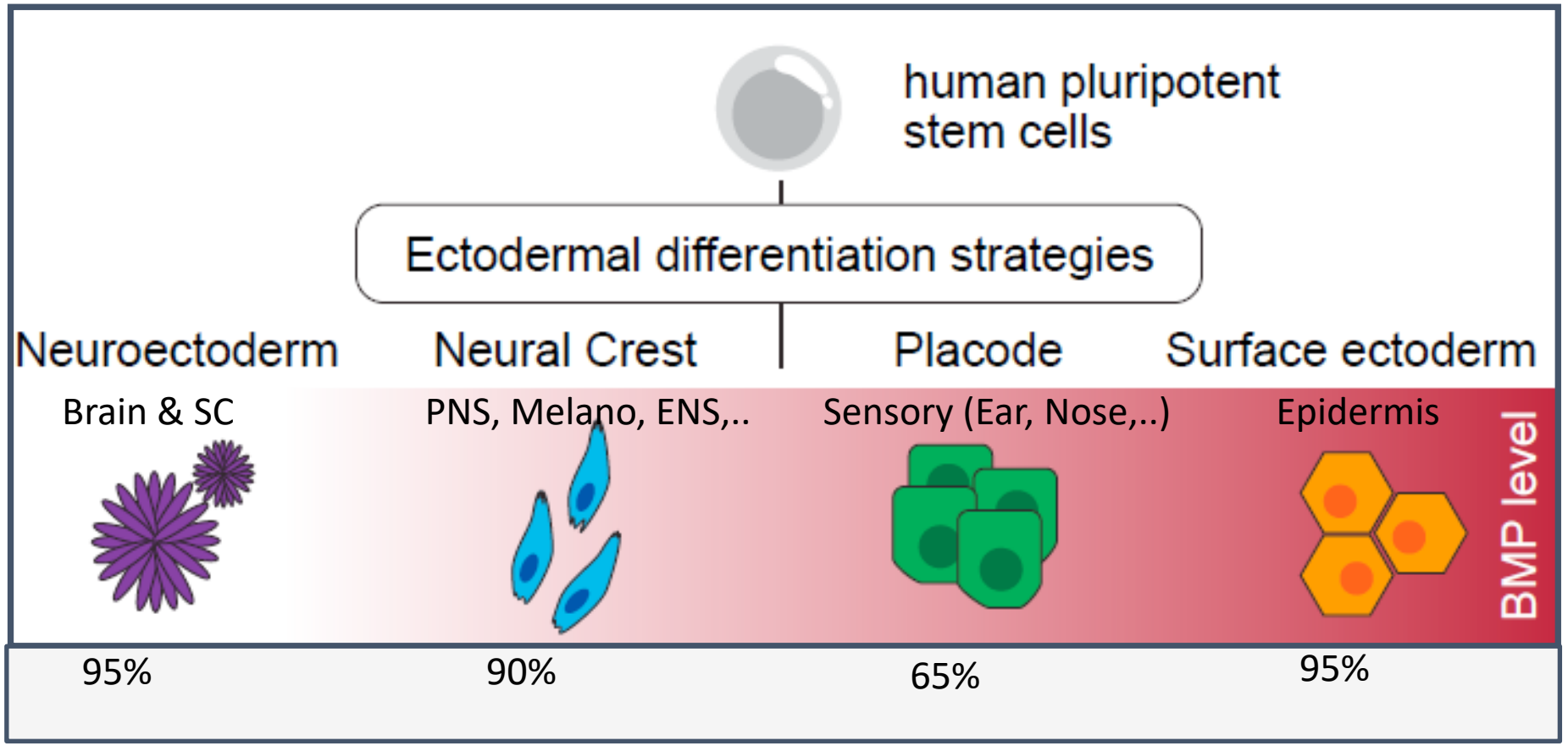
Shinya Yamanaka
(Nobel Prize 2012)



Cell Sources – human induced pluripotent stem cells



Cell Sources – Directing human PSC fate (from ESC or iPSC)

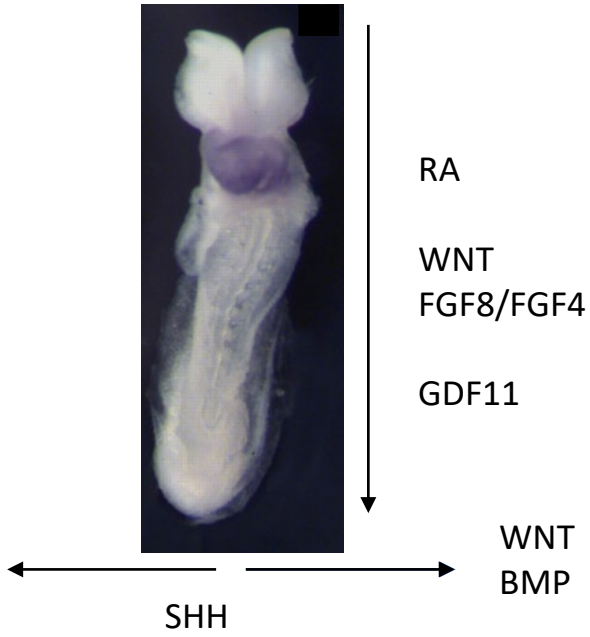


-TGFβ/Activin/Nodal	-TGFβ/Activin/Nodal	-TGFβ/Activin/Nodal	-TGFβ/Activin/Nodal
	+ WNT	+ FGF	FGF-i
(inhibition of BMP)	(lowest BMP)	(low- medium BMP)	(medium-high BMP)

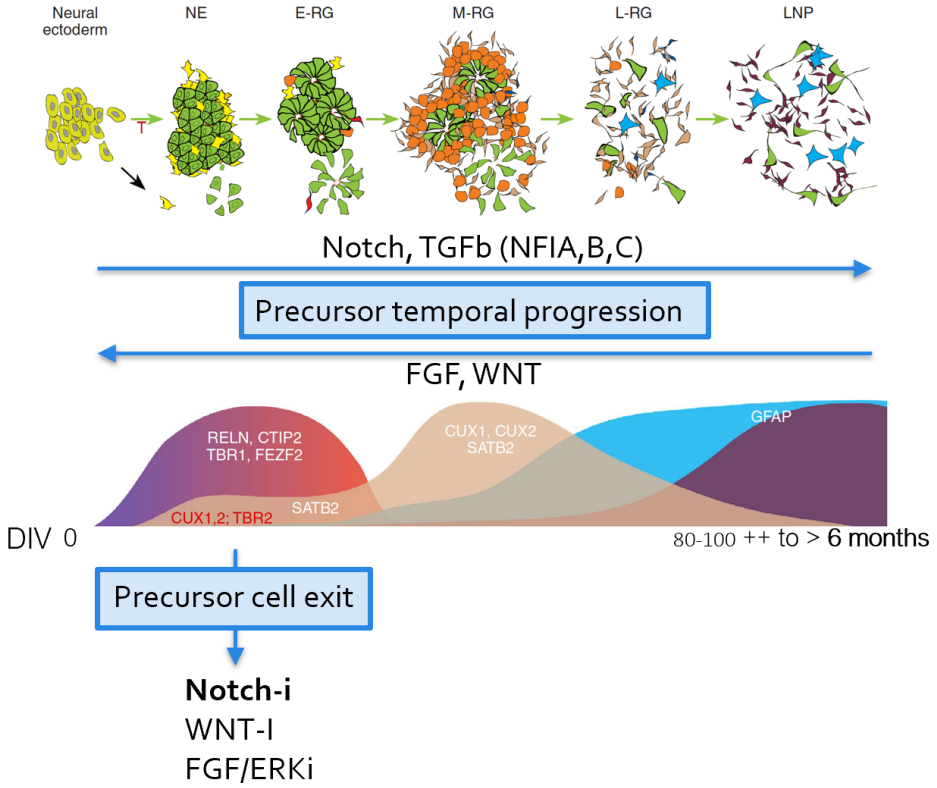
Tchieu et al., Cell Stem Cell 2017

Cell Sources – Directing human PSC fate

Directing **position** (AP / DV)



Directing progenitor **timing**



→ Building an "atlas" of the human nervous system (2D and 3D organoid approaches)

Repertoire of hPSC-derived cells (>60 cell types)

ALS / **Lou Gehrig's** disease (spinal & oculomotor neurons)

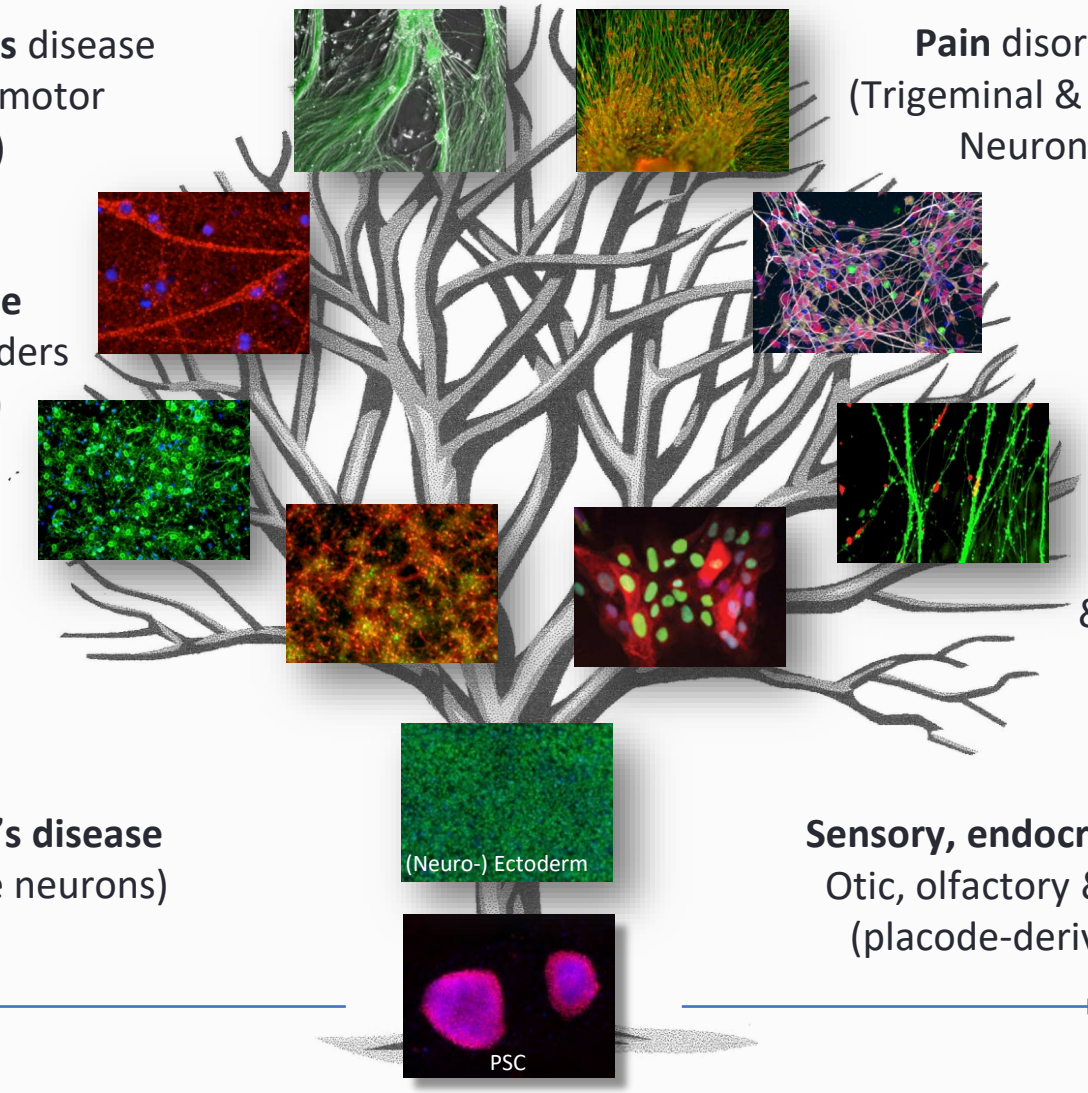
Pain disorders (Trigeminal & Sensory Neurons)

Alzheimer's disease
Autism, Seizure disorders (Cortical neurons)

Hirschsprung's disease (ENS precursors)

Radiation damage repair (oligodendrocytes)

Peripheral nerve & **Spinal cord** damage (Schwann cells)

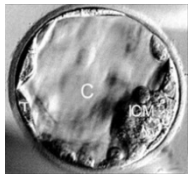


Parkinson's disease (Dopamine neurons)

Sensory, endocrine disease Otic, olfactory & pituitary (placode-derived cells)

CNS lineages ← → PNS lineages

Challenge of directing / accelerating developmental timing

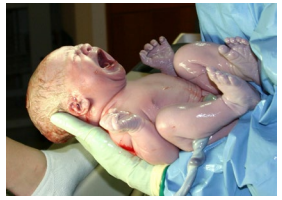


Pluripotency

weeks-months →

Cell Fate

months-years →



Maturation

years – decades →

Age

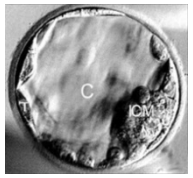
ms v

an

Species	Mouse	Domestic cat	Human	African elephant
Gestation	20 days	2 months	9 months	12 months
In vitro neuron maturation	Days to weeks	Weeks to months	Several months	Unknown
	2 cm^3	60 cm^3	1200 cm^3	4000 cm^3
Brain volume				

Emiliano Hergenreder

Challenge of directing / accelerating developmental timing



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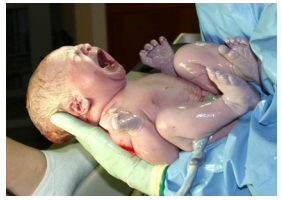
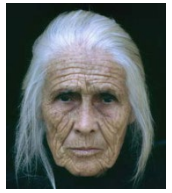


Maturation

years – decades



Age

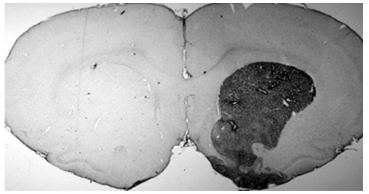
ms vs human ~2-3x

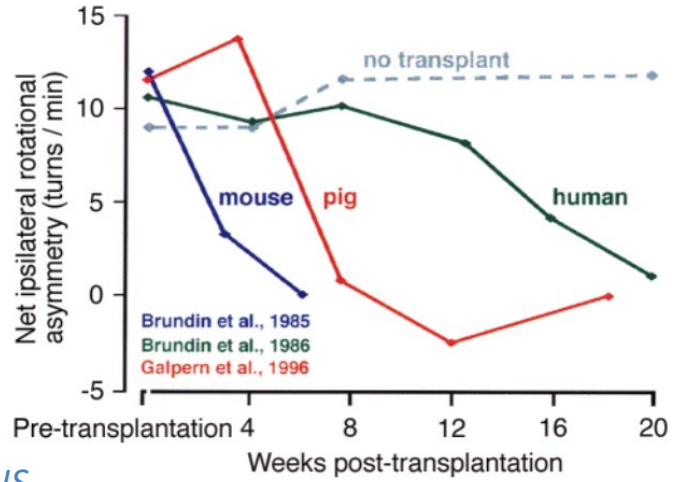
ms vs human ~10x

ms vs human ~ 30x

Xenografting studies: species-specific recovery rates

6OHDA lesion

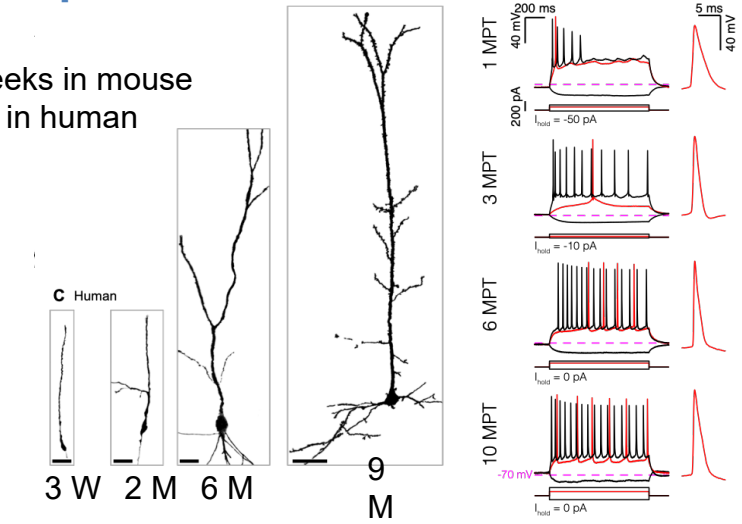




Deacon & Isacson (1997) TINS

Species-specific cortical neuron maturation

~ 3-4 weeks in mouse
> 1 year in human



Espuny-Camacho et al (2013); Linaro et al (2019) Neuron

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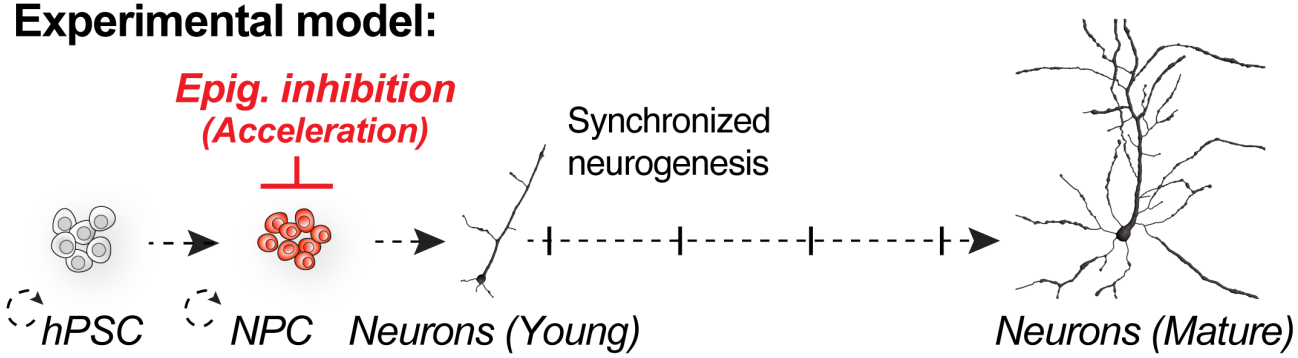
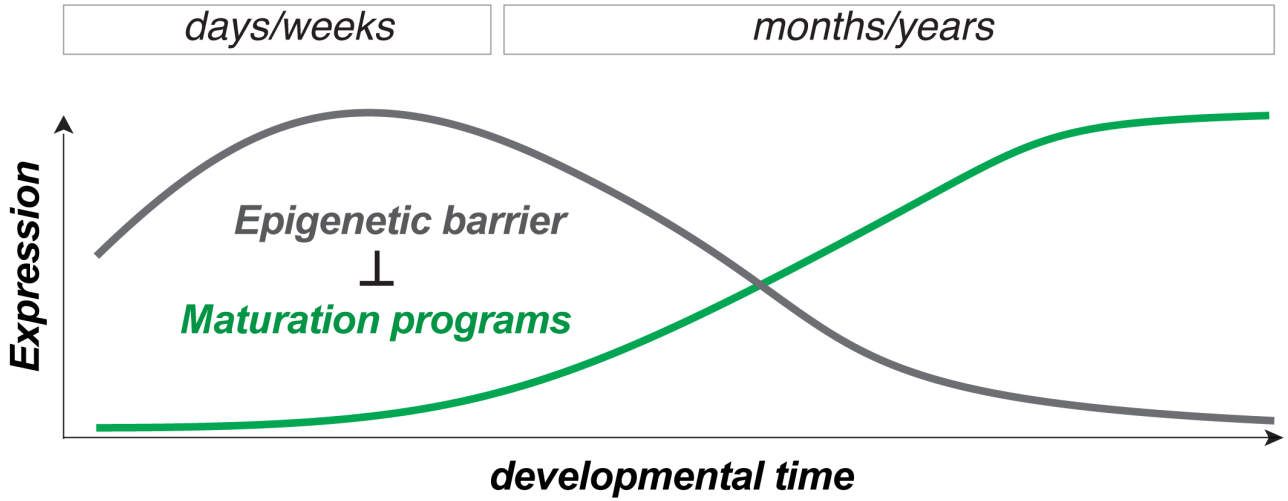
An epigenetic barrier sets the timing of human neuronal maturation

Experimental model:

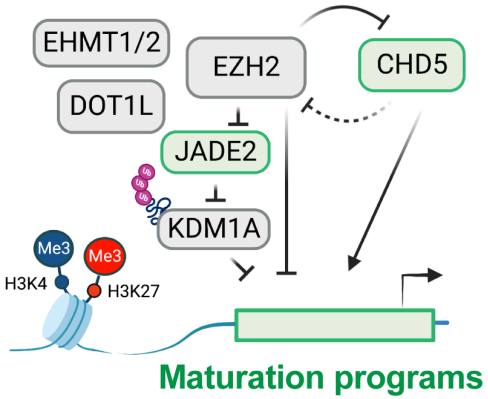


Maturation atlas:

- Morphology
- Functionality
- Transcription
- Chromatin accessibility



Mechanisms:



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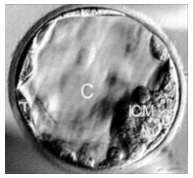
Ciceri et al., Nature (2024)

Challenge of species-specific Developmental Timing and Age

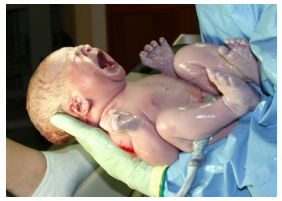


Pluripotency

weeks-months →

Cell Fate

months-years →



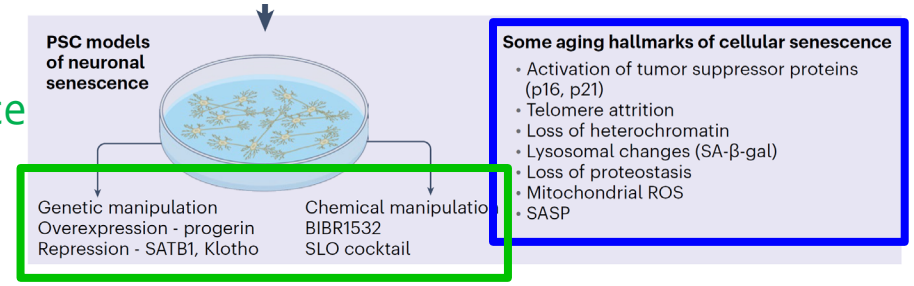
Maturation

years – decades →

Age

"induced aging"

Manipulating age & senescence



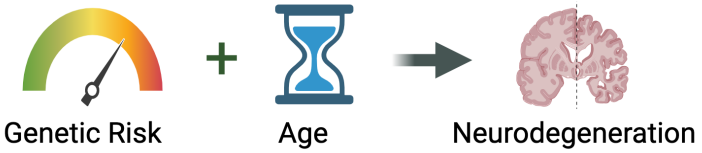
Measuring age & senescence

DeLuzy et al., Nature Aging (2024)

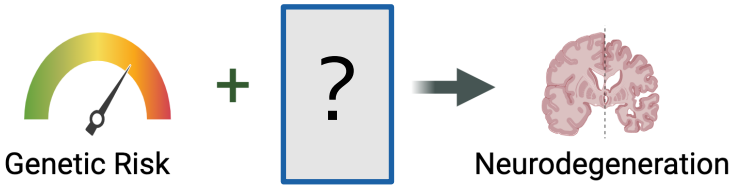
How to use genetic approach to identify age regulators?

Hypothesis: **Genetic risk** + **Age** = late-onset **phenotype**

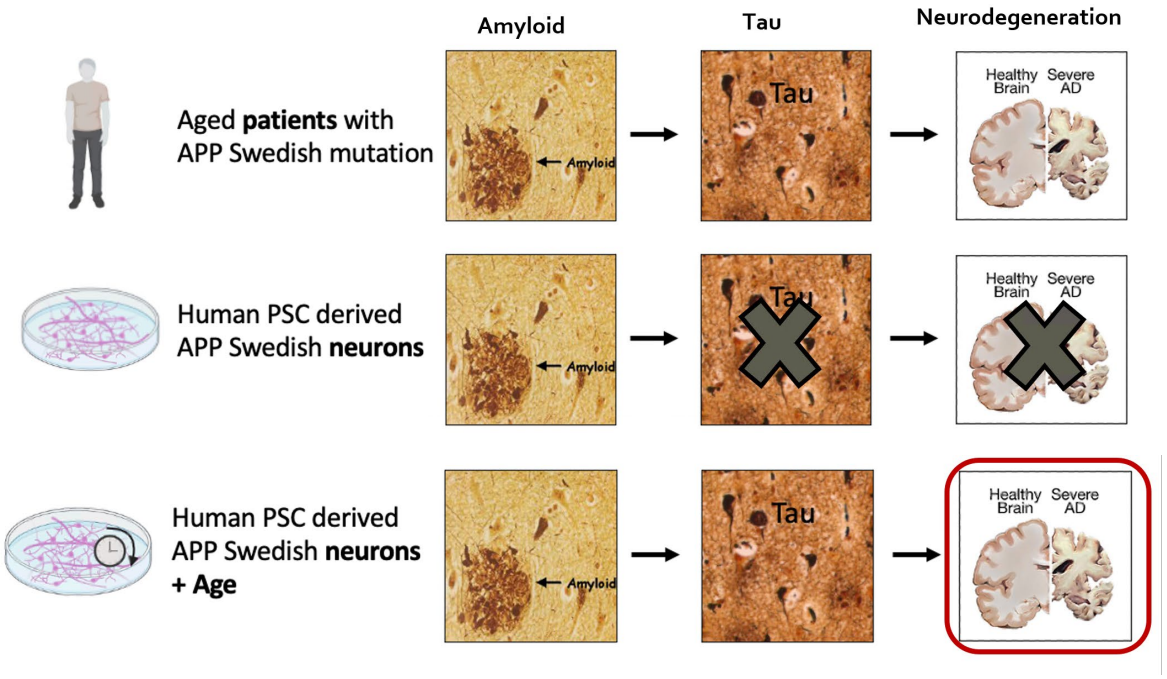
Nathalie Saurat



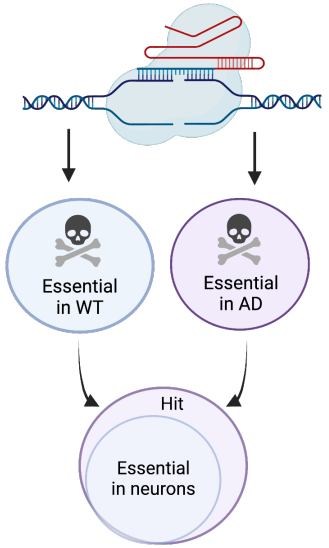
Can we screen for late-onset phenotype ?



Example: familial AD



Genome-wide CRISPR screen



Conditional **LOF in d25** cortical neurons
dropout at day 65

Saurat et al. Cell Stem Cell (2024)

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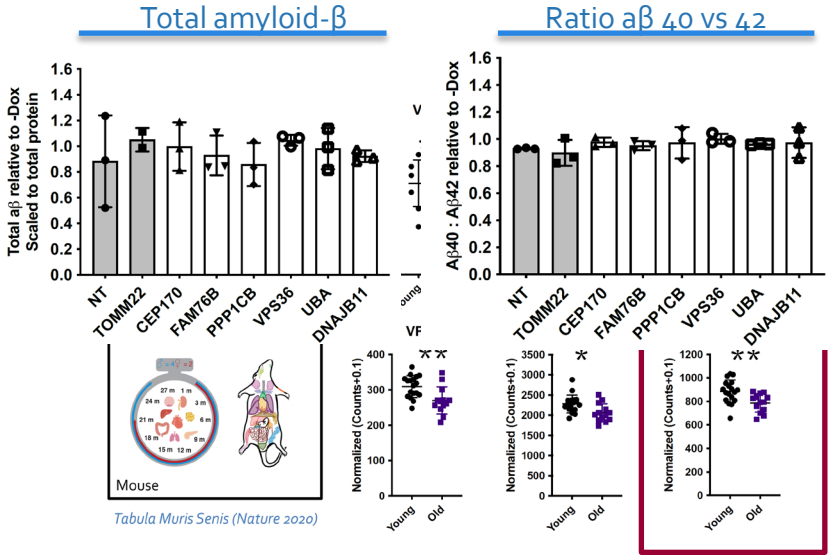
How to define age-related vs gene-gene interaction hits?

1. NOT essential in WT neurons / **not directly affecting A-beta**

Single gene validation in disease vs control neurons



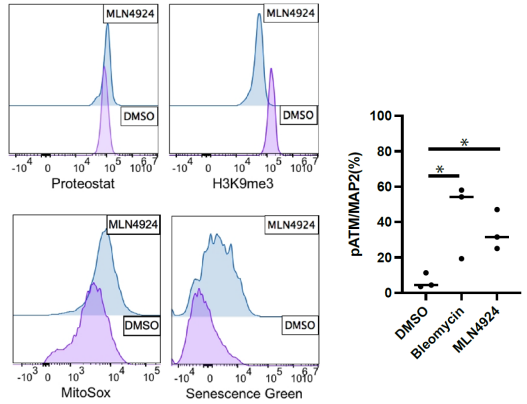
2. Decreased expression in aged human and mouse brain

Primary brain tissue expression atlases

3. Hallmarks of cellular age in both WT and disease neuron

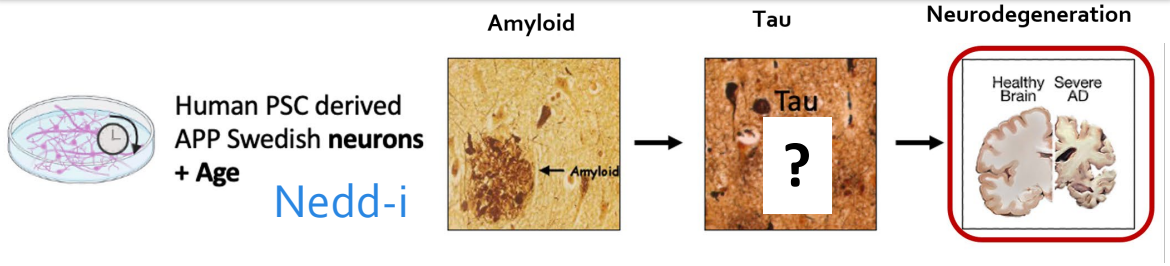
Cellular hallmarks of age adapted to neurons

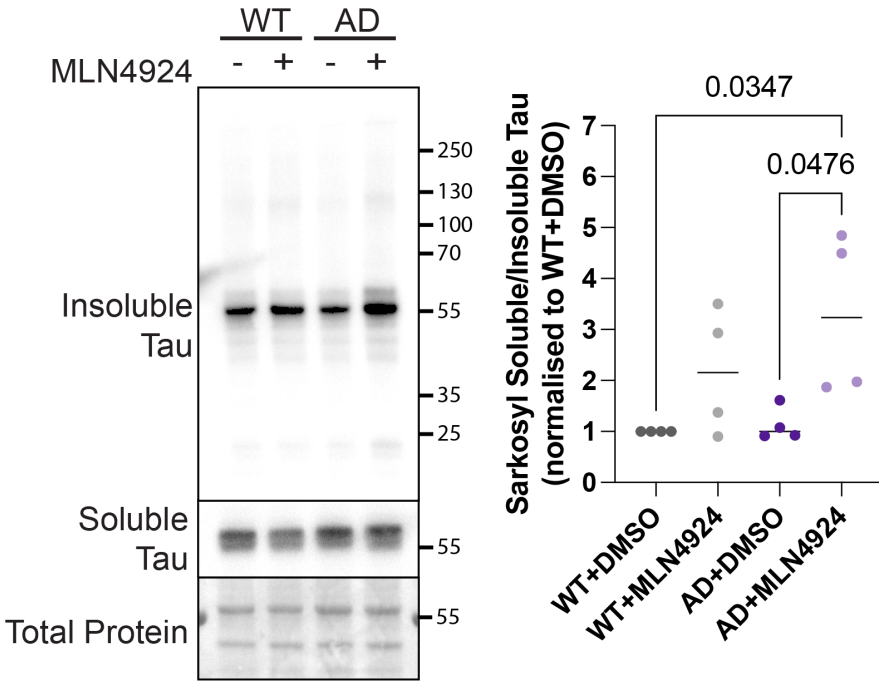
Cellular Aging Hallmark	Assay	Expect change in aged cell
Senescence	CellEvent Green (b-gal)	Increase
Proteostasis	Proteostat	Increase
Epigenetic	H3K9me3	Decrease
DNA Damage	Gamma H2AX	Increase
Mitochondria	MitoSox	Increase



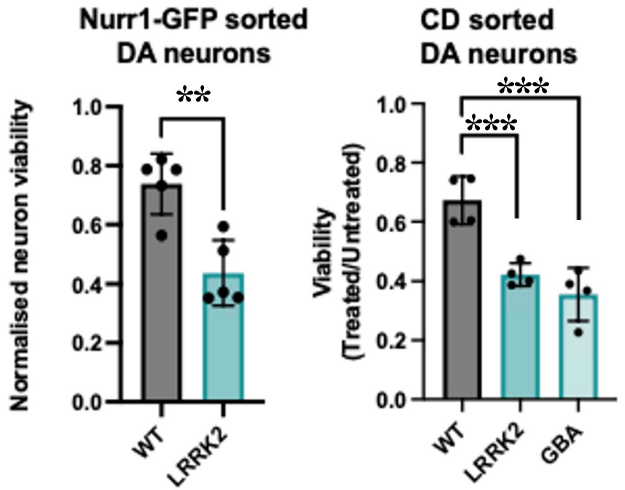
Nedd-i based induction of age-related phenotypes

Other age-related phenotype in AD model





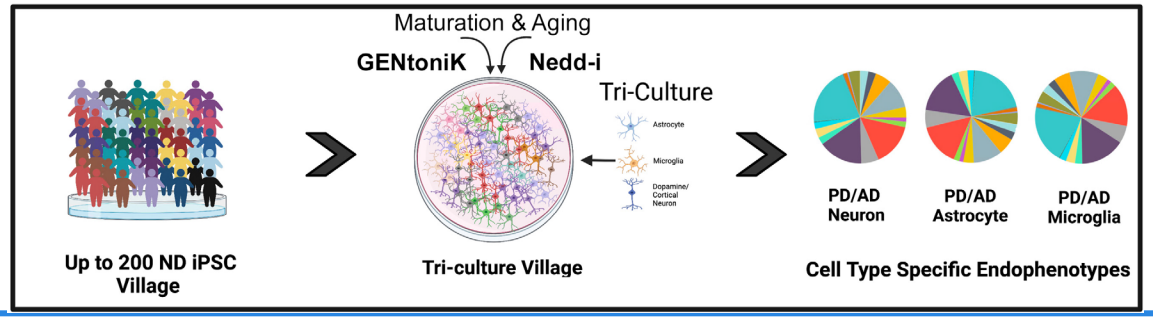
Nedd-i – triggering late-onset phenotypes in PD

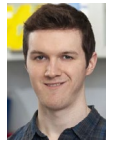


MLN4924 treated

Nathalie Saurat

→ Studies in ongoing in sporadic PD (PPMI cohort, village in dish)

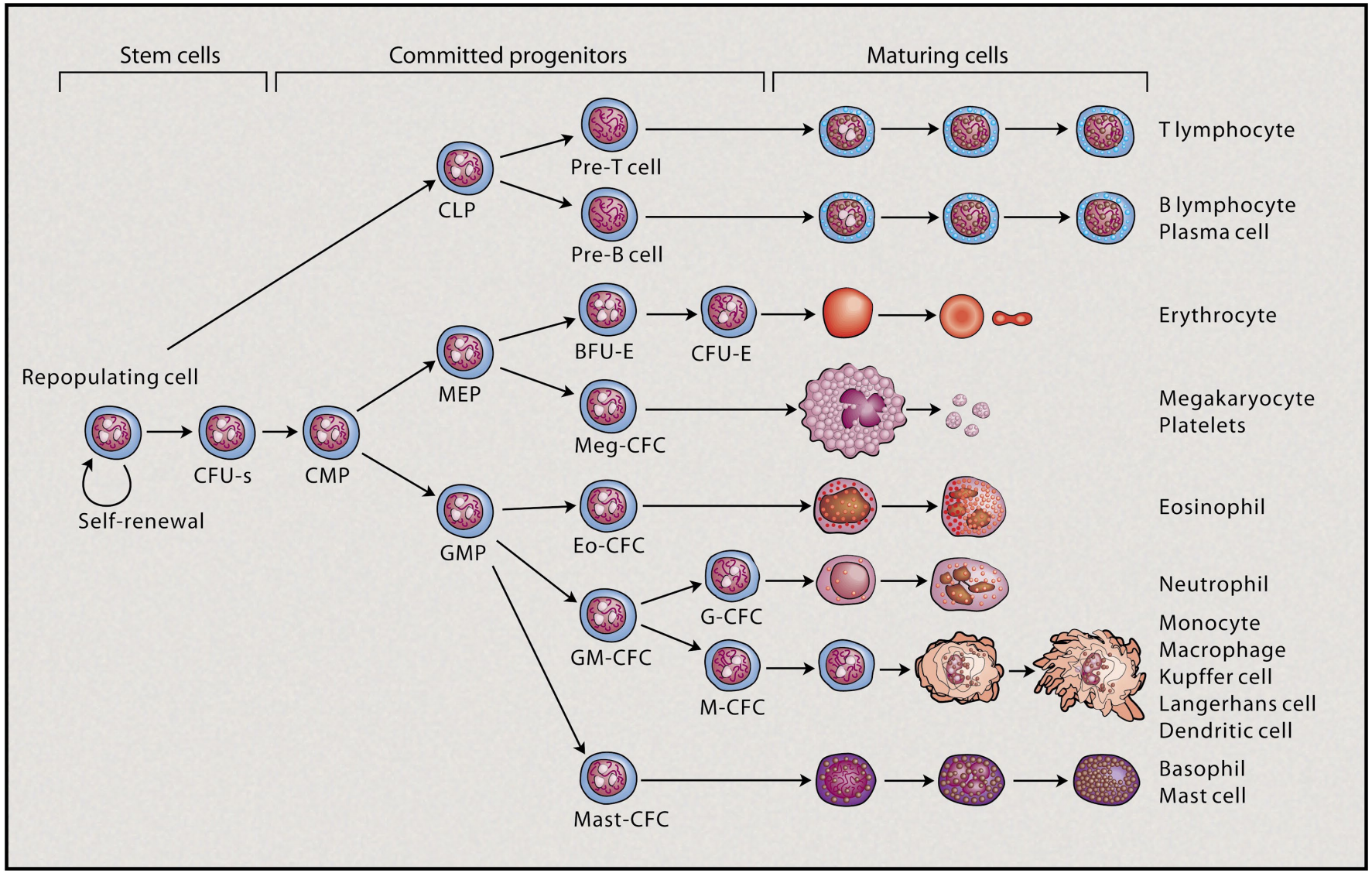




Andrew Minotti

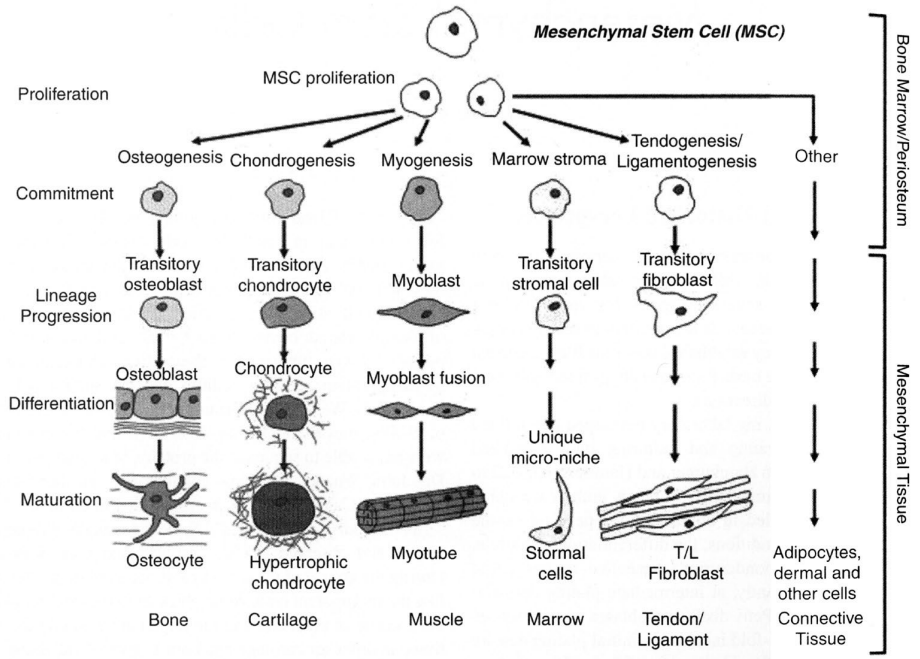
Memorial Sloan Kettering Cancer Center

Cell Sources – tissue specific stem cells (e.g. HSCs)

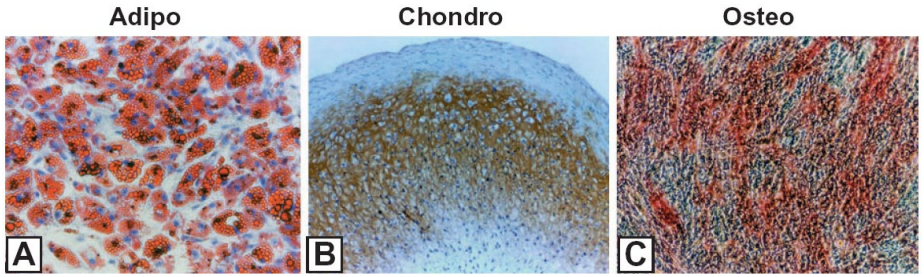


D. Metcalf; Immunity (2007)

Cell Sources – mesenchymal stem cells



Adapted From A. Caplan ?



Science. 1999 284(5411):143-7


COMMENT

DISASTERS Climate, fires and floods are linked — study them together p.458 | GENOMES Don't use genomics to excuse social inequality p.461 | AUTHORSHIP Follow the film industry and list contributions instead p.464 | CONFERENCES Do boring speakers talk for longer, or does it just feel that way? p.464

Scanning electron micrograph of what is called a human mesenchymal stem cell, which some say can develop into bone, cartilage or fat cells.

Clear up this stem-cell mess

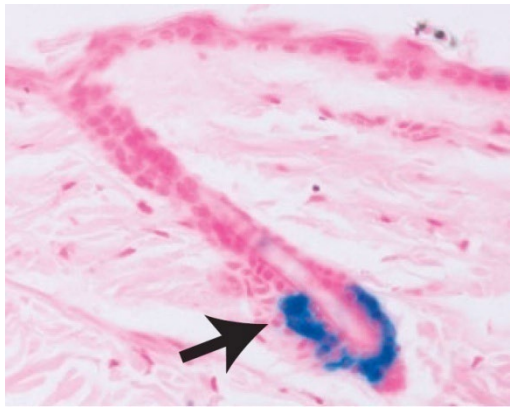
Confusion about mesenchymal stem cells is making it easier for people to sell unproven treatments, warn **Douglas Sipp**, **Pamela G. Robey** and **Leigh Turner**.

Nature. 2018 Sep;561(7724):455-457

D. Metcalf; Immunity (2007)

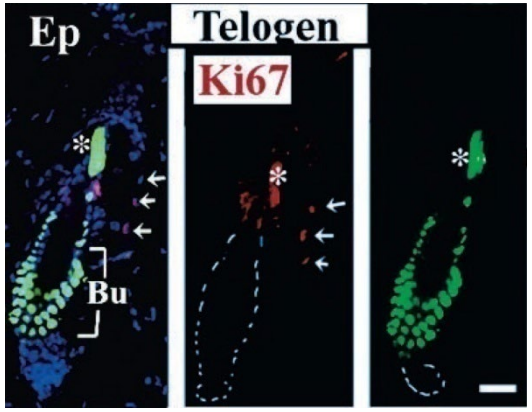
Cell Sources – skin and hair follicle stem cells

Specific Promoter



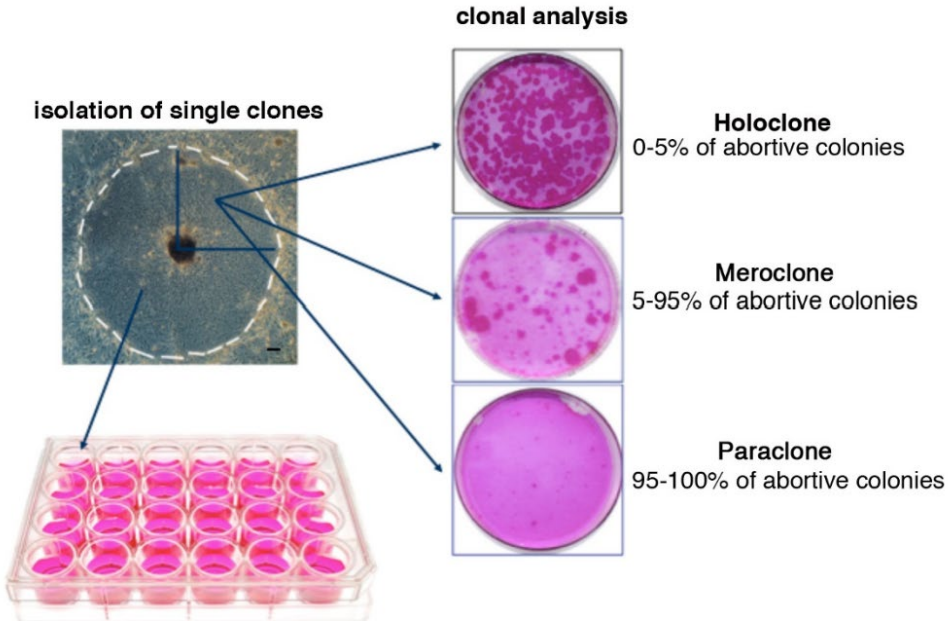
Nat Biotechnol. 2004 Apr;22(4):411-7

Label retaining cells



Science. 2004 Jan 16; 303(5656):359-63

Epidermal stem cell precursor cells

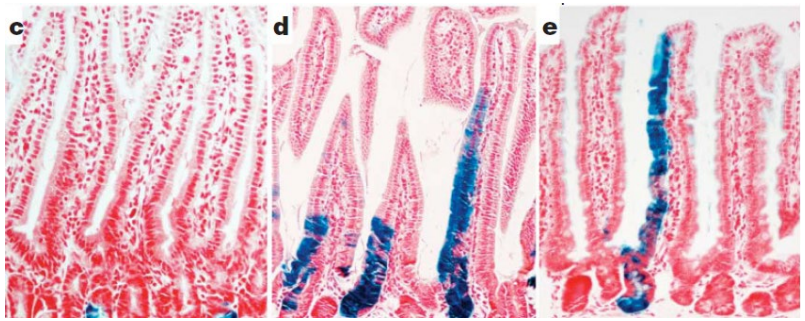


Hirsch et al., Nature 551, pages327–332 2017

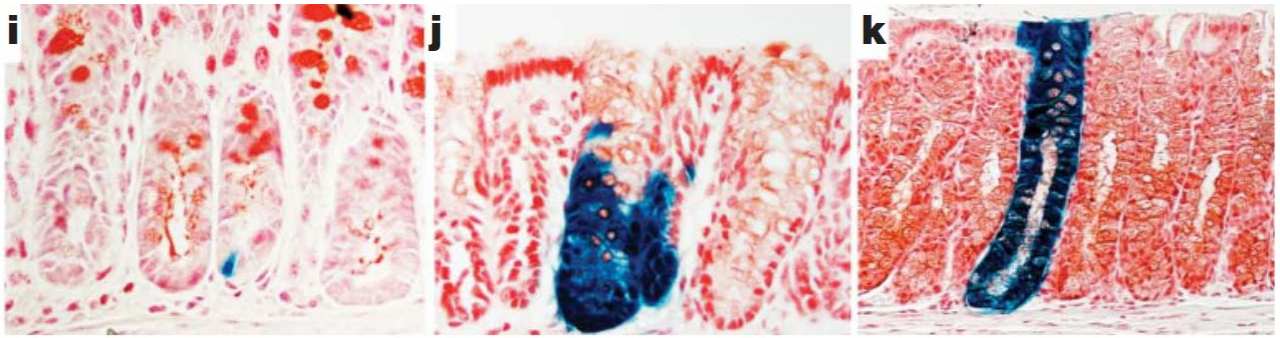
Cell Sources – LGR5+ stem cells in gut & in vitro organoids

Tamoxifen-inducible labeling of LGR5::lacZ

LGR5+ repopulating small intestine

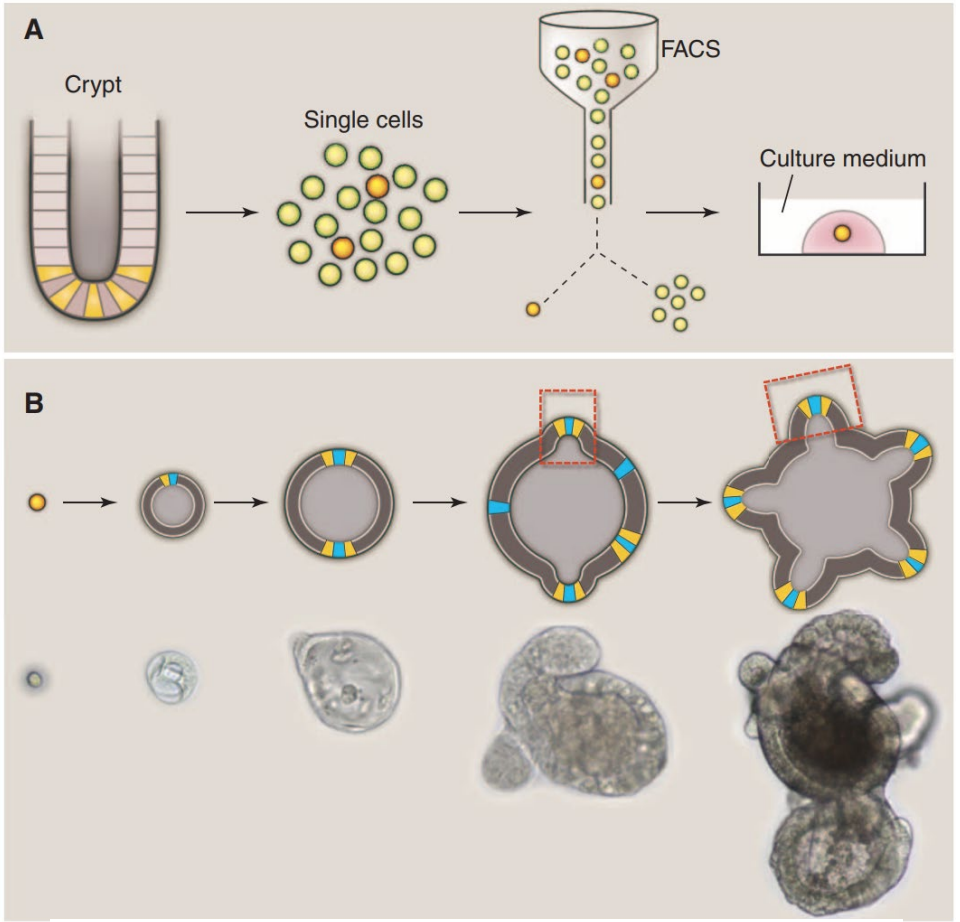


LGR5+ repopulating colon



Barker et al., Nature Vol 449 | 25 October 2007 | doi:10.1038/nature06196

WNT / R-spondin driven organoids

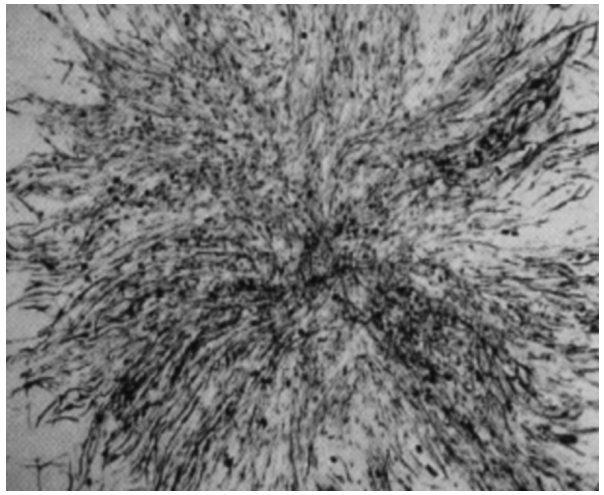


Sato & Clevers; Science (2013) Jun 7;340(6137):1190-4.

Cell Sources – neural stem cells (CNS vs PNS)

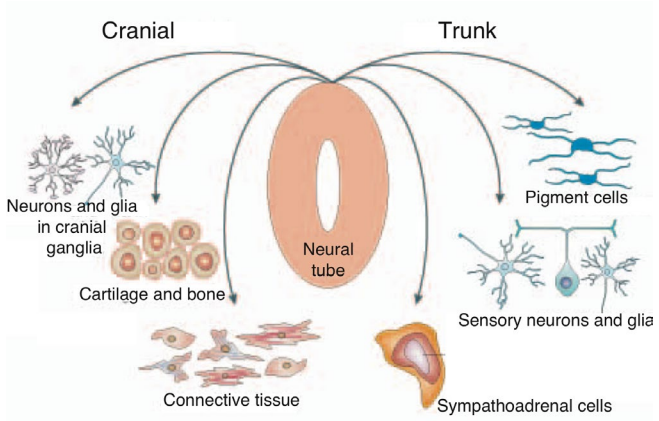
Central Nervous System

- Central Neurons
- Astrocytes
- Oligodendrocytes



PNS / Neural crest stem cells

- Peripheral Neurons + Glia (Schwann Cells)
- Endocrine Cells (Adrenal, carotid body, thyroid C cells..)
- Melanocytes
- Smooth muscle, mesenchymal, chondrocytes, bone (e.g. skull..)

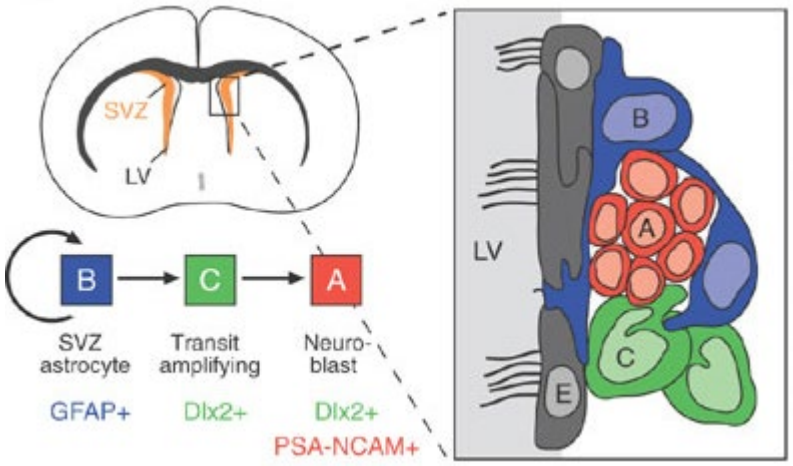


Cell Sources – neural stem cells (adult)

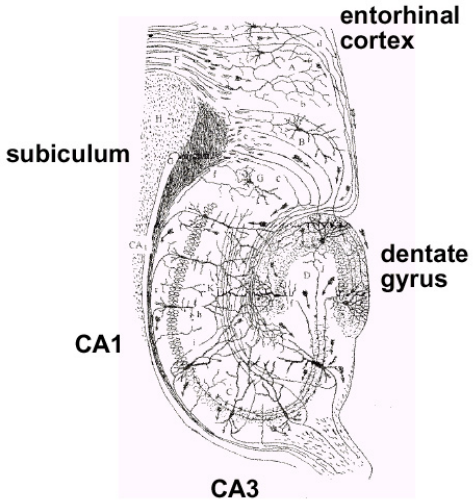
Mouse brain

Subventricular / Subependymal Zone

- Generation of olfactory bulb neurons
- "smell'

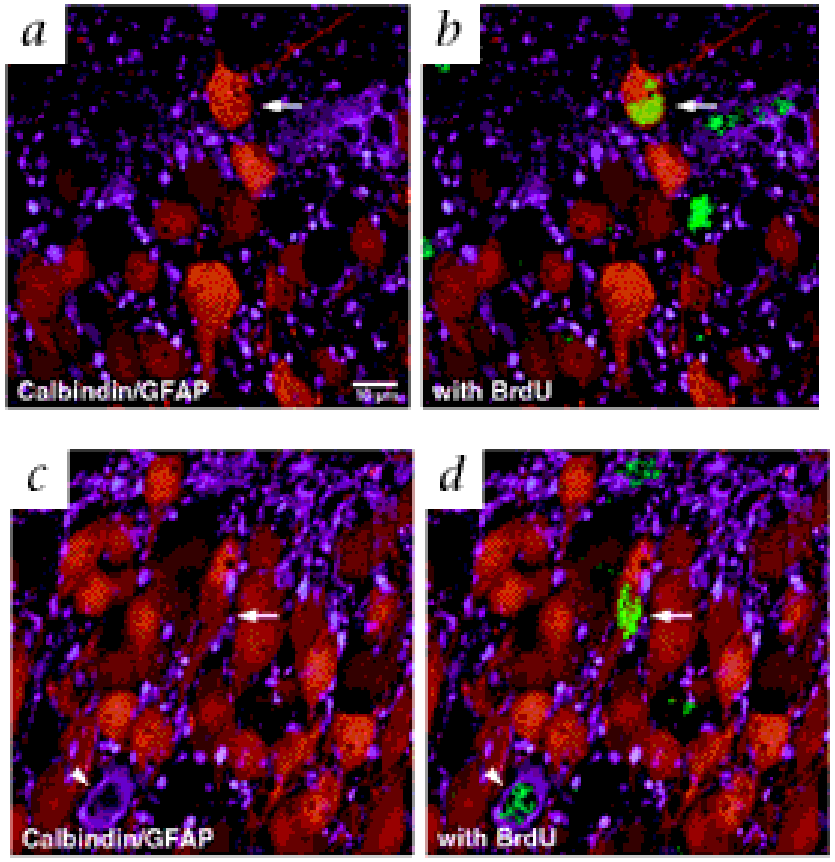


- Hippocampus; granule cells
- Generation of granule Neurons
- "memory?"



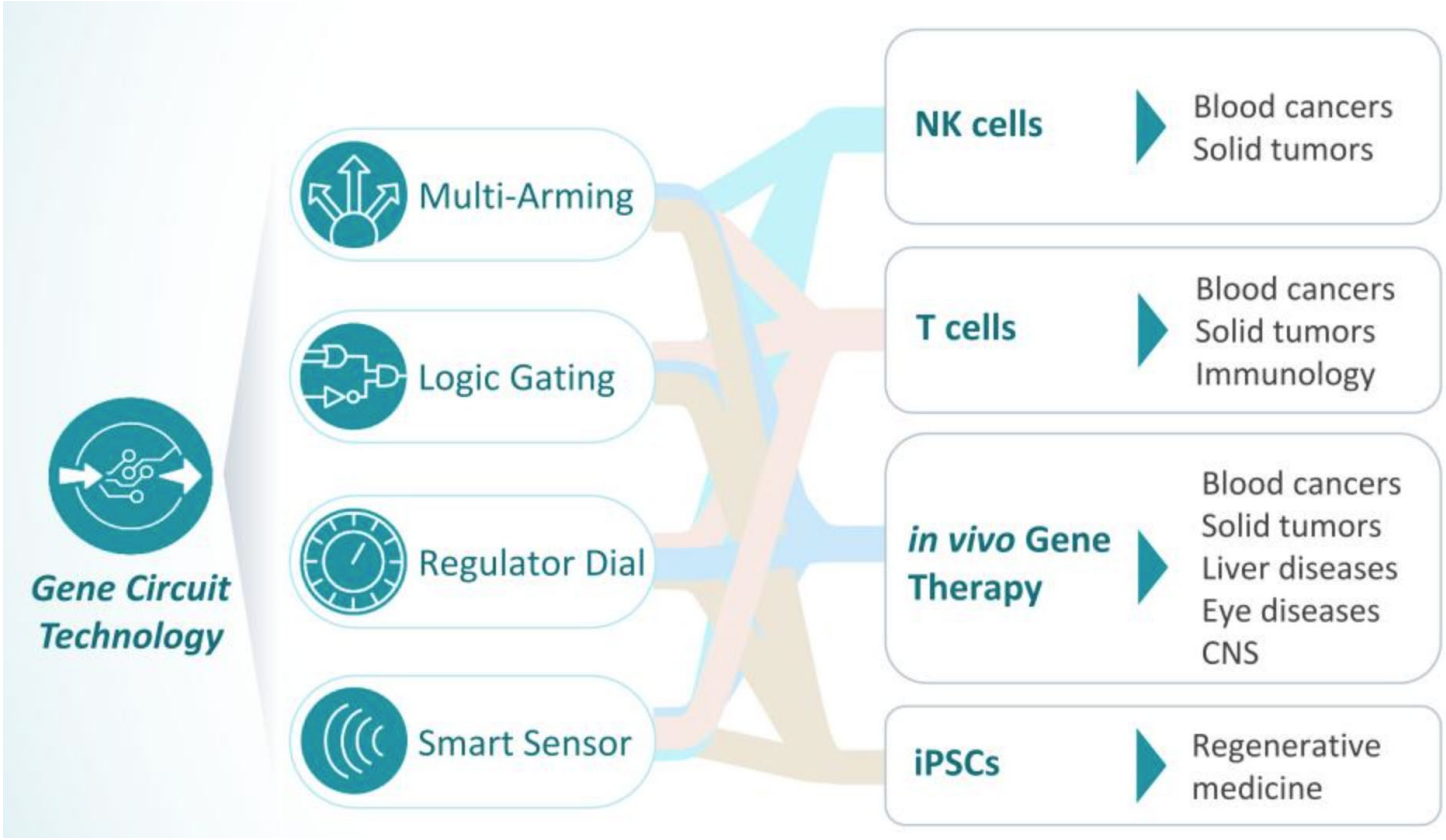
Human brain

Hippocampus dentate, granule cells



Nat Med 1998 Nov;4(11):1313-7

Cell Sources – engineered primary or iPSC-derived cells



e.g. Senti Bio

- Attacking on target and avoiding off-target cells
- Forcing cell fate transcriptionally (blocking alternative fates)
- Example of enhancing cell function to protect grafted cells or to modify disease progression & many others..

Outline – Cell Therapies

Cell sources

- Definition of stem cells and potency
- Pluripotent stem cells, directed differentiation, cell maturation and aging
- Other types of stem cells, tissue-specific stem cells, engineered cells (synthetic biology?)

Currently approved and investigational Cell therapies

- Approved cell therapies
- Investigational cell therapies
- Unproven cell therapies and stem cell tourism

Preclinical research

- Choice of disease and choice of candidate cell type
- In vivo model systems for preclinical research

Product development and clinical grade manufacturing

- Clinical grade manufacturing, GMP compliance, Critical quality attributes
- IND enabling studies, Device and cell delivery

✓ Case study**:** Manufacturing a **dopamine neuron cell product for Parkinson's disease**

✓ Case study: Manufacturing an **enteric neural precursor cell product for Hirschsprung's disease**

❑ **Design you own study (DIY):**

Cell Therapies in Clinical Use or Clinical Testing

Approved

Have undergone rigorous clinical testing and regulatory approval, demonstrating safety and efficacy.

Investigational

Currently being tested in clinical trials but have not yet been approved as effective and safe.

Unproven

Lack sufficient clinical evidence to support their safety and effectiveness.

Approved Cell Therapies

→ Backed by convincing evidence of efficacy and safety and **approved by the appropriate regulatory bodies.**

- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

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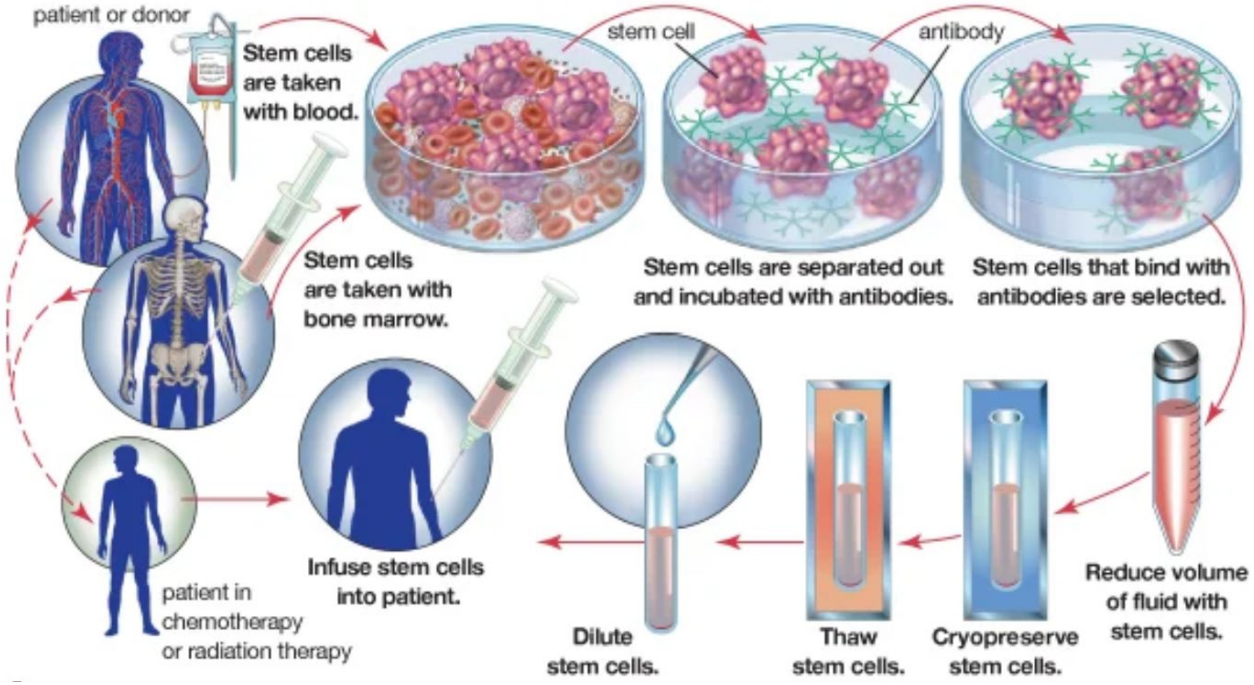
- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

Examples:

- **HSC transplantation**

(autologous, allogenic, engineered)



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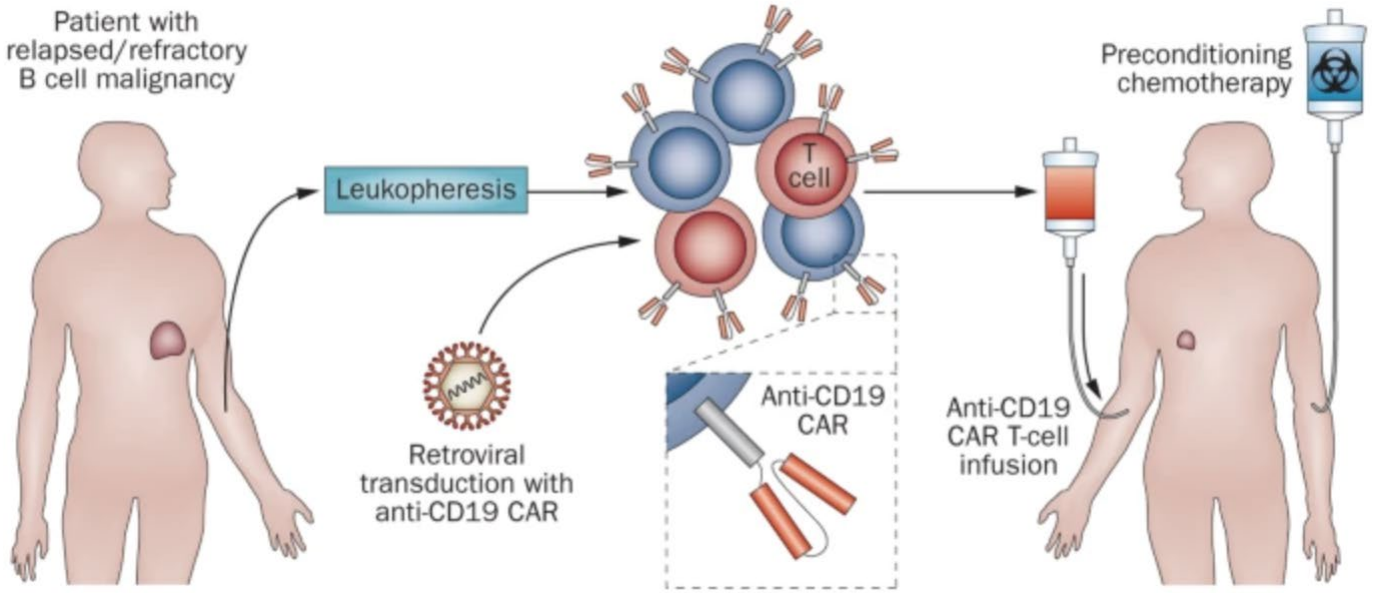
- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

Examples:

- **CAR-T** cell therapies

Figure 1: Treatment of patients with B-cell malignancies using anti-CD19 CAR T cells.



Approved Cell Therapies

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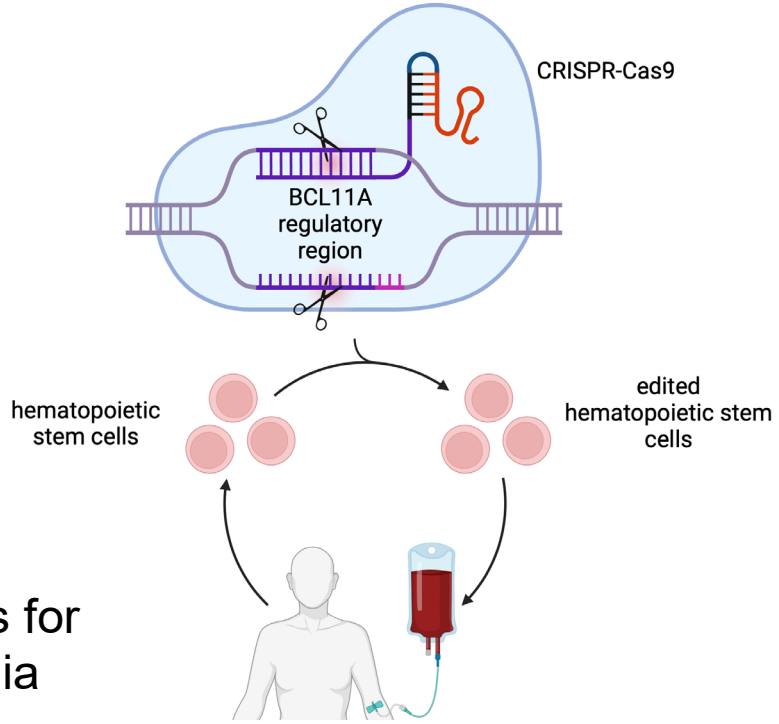
- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

Examples:

- HSC transplants for **sickle cell anemia**



Gene-Edited HSCs for
Sickle Cell Anemia

Approved Cell Therapies

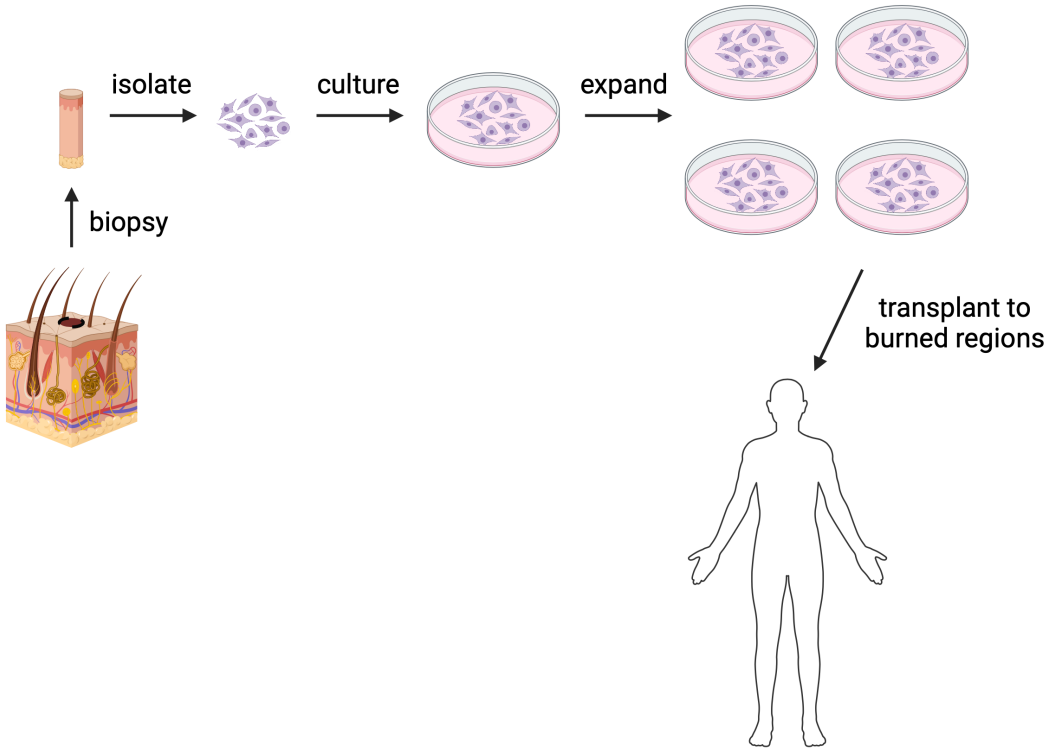
→ Backed by convincing evidence of efficacy and safety and **approved by the appropriate regulatory bodies.**

- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

Examples:

- **Skin** transplants in the case of **burn** injuries



Approved Cell Therapies

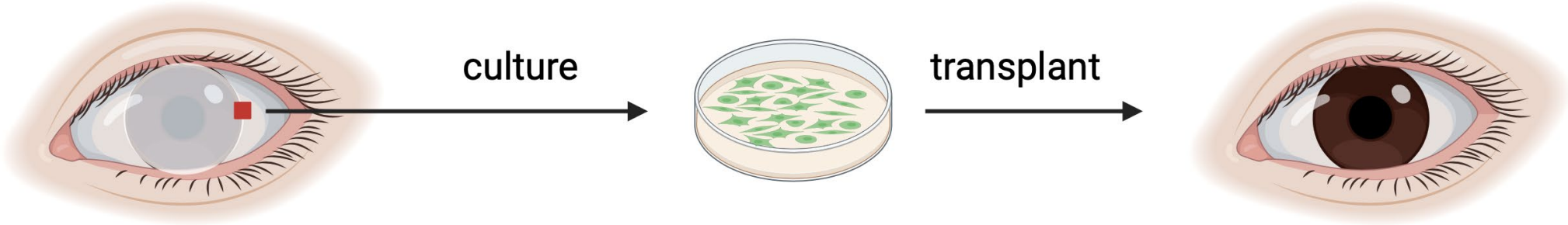
→ Backed by convincing evidence of efficacy and safety and **approved by the appropriate regulatory bodies.**

- Food and Drug Administration (**FDA**) in USA; (**EMA**) in Europe; (**PMDA**) in Japan; (**TGA**) in Australia

→ There are **only a very limited number of approved cell therapies:**

Examples:

- **Limbal stem** cell transplants (for chemical burns in eye)



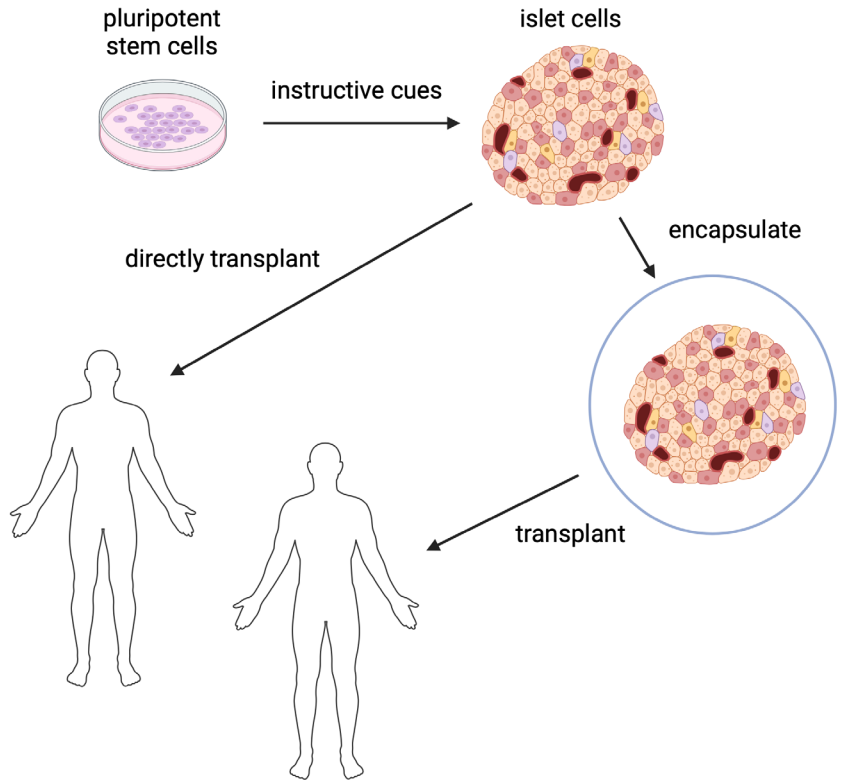
Investigational Cell Therapies

→Currently in **clinical trials,** but **not yet approved** as effective or safe.

Very large number of trials (for human PSC-derived cells there are an estimated 100+ ongoing trials)

Prominent Examples:

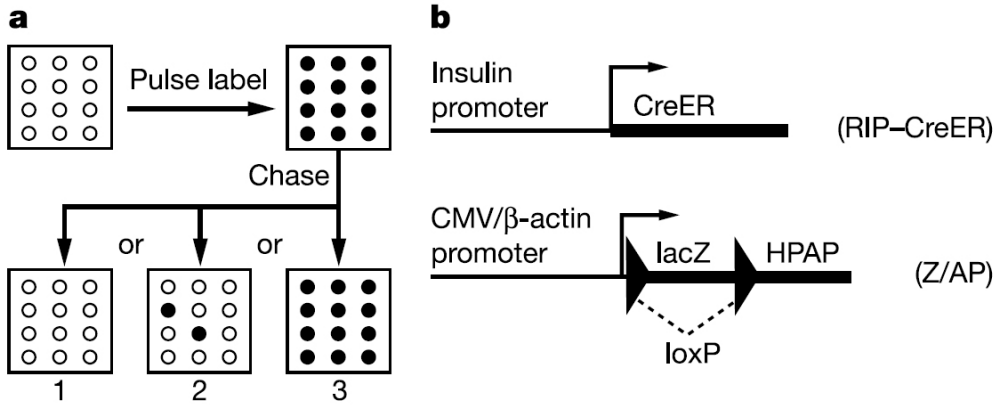
Human PSC-derived pancreatic islets (type I diabetes)



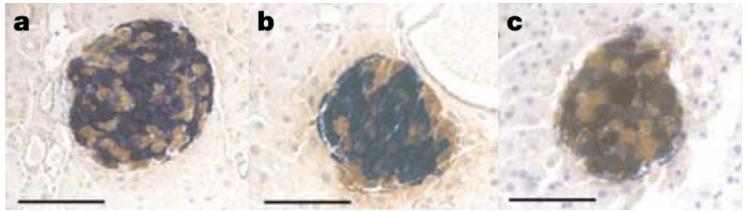
Why is type I diabetes might be good target?

- Are there Pancreatic Islet Stem Cells

Nature. 2004 May 6;429(6987):41-6.



Immedi-ately **4 mts** **1 yr**



A Cure for Type 1 Diabetes? For One Man, It Seems to Have Worked.

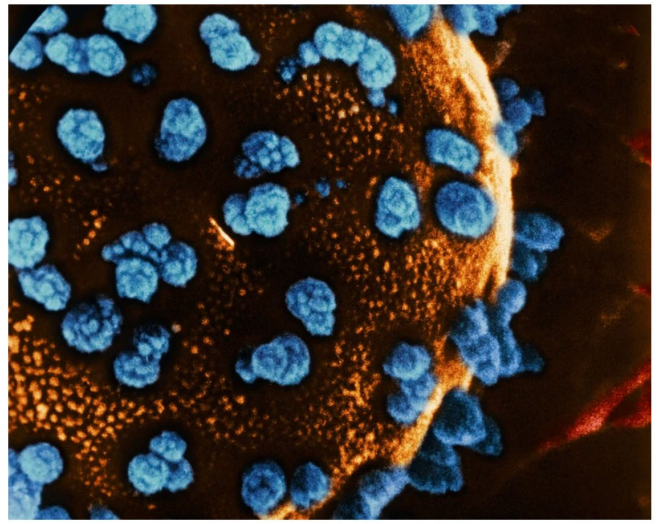
A new treatment using stem cells that produce insulin has surprised experts and given them hope for the 1.5 million Americans living with the disease.

NEWS | 26 September 2024

Stem cells reverse woman's diabetes – a world first

Patient is the first person with type 1 diabetes to receive this kind of transplant.

By Smriti Mallapaty



A woman with type 1 diabetes started producing insulin (blue) after a stem cell transplant. Credit: Lennart Nilsson, Boehringer Ingelheim International GmbH, TT/Science Photo Library

→ But what about the autoimmune disease?

Investigational Cell Therapies

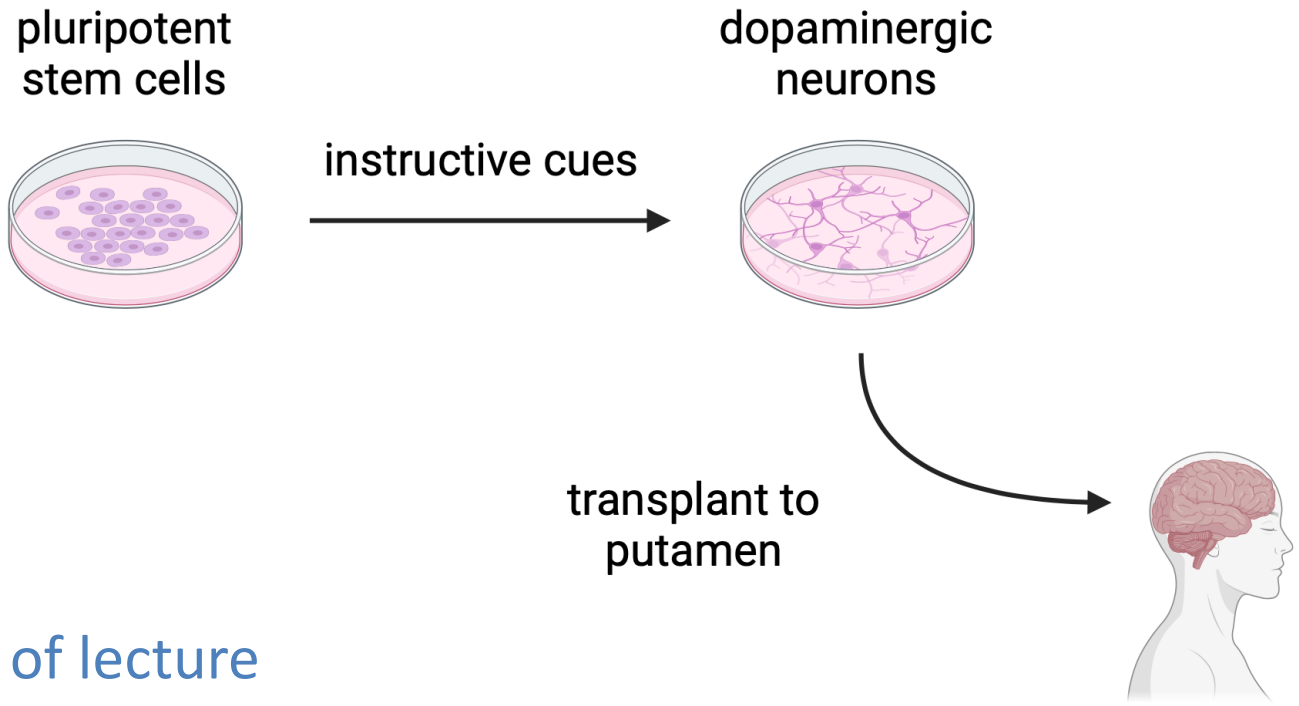
→Currently in **clinical trials,** but **not yet approved** as effective or safe.

Very large number of trials (for human PSC-derived cells there are an estimated 100+ ongoing trials)

Prominent Examples:

- **Human PSC-derived dopamine neurons**

  (Parkinson's disease)



→ Detailed case study in second part of lecture

Investigational Cell Therapies

→Currently in **clinical trials,** but **not yet approved** as effective or safe.

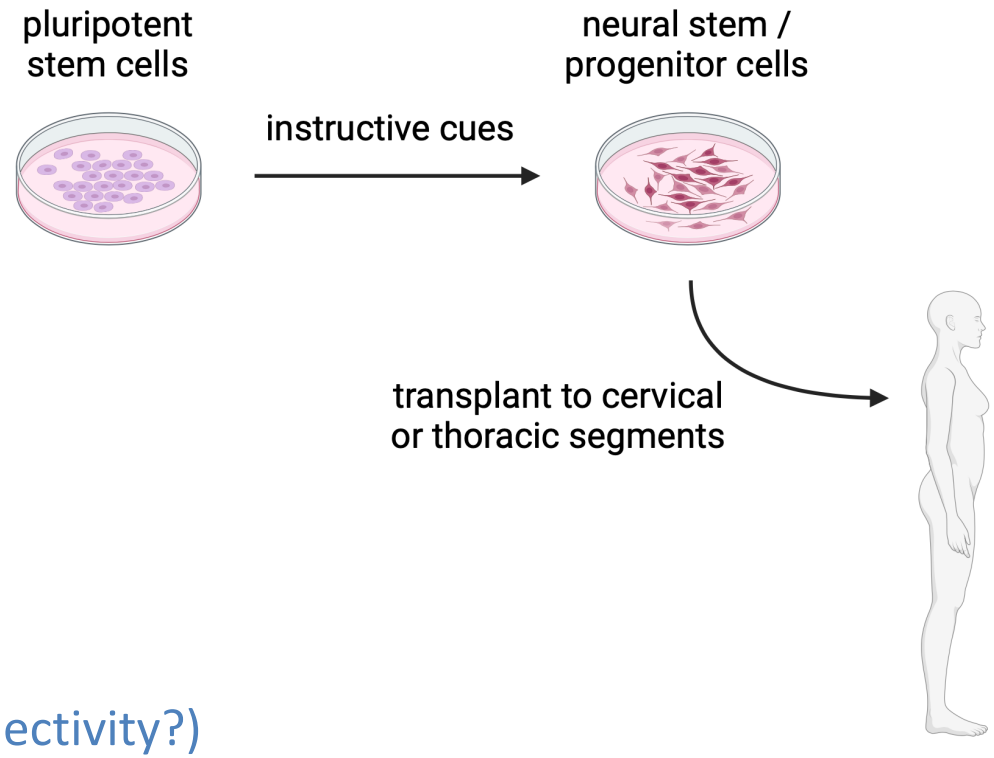
Very large number of trials (for human PSC-derived cells there are an estimated 100+ ongoing trials)

Prominent Examples:

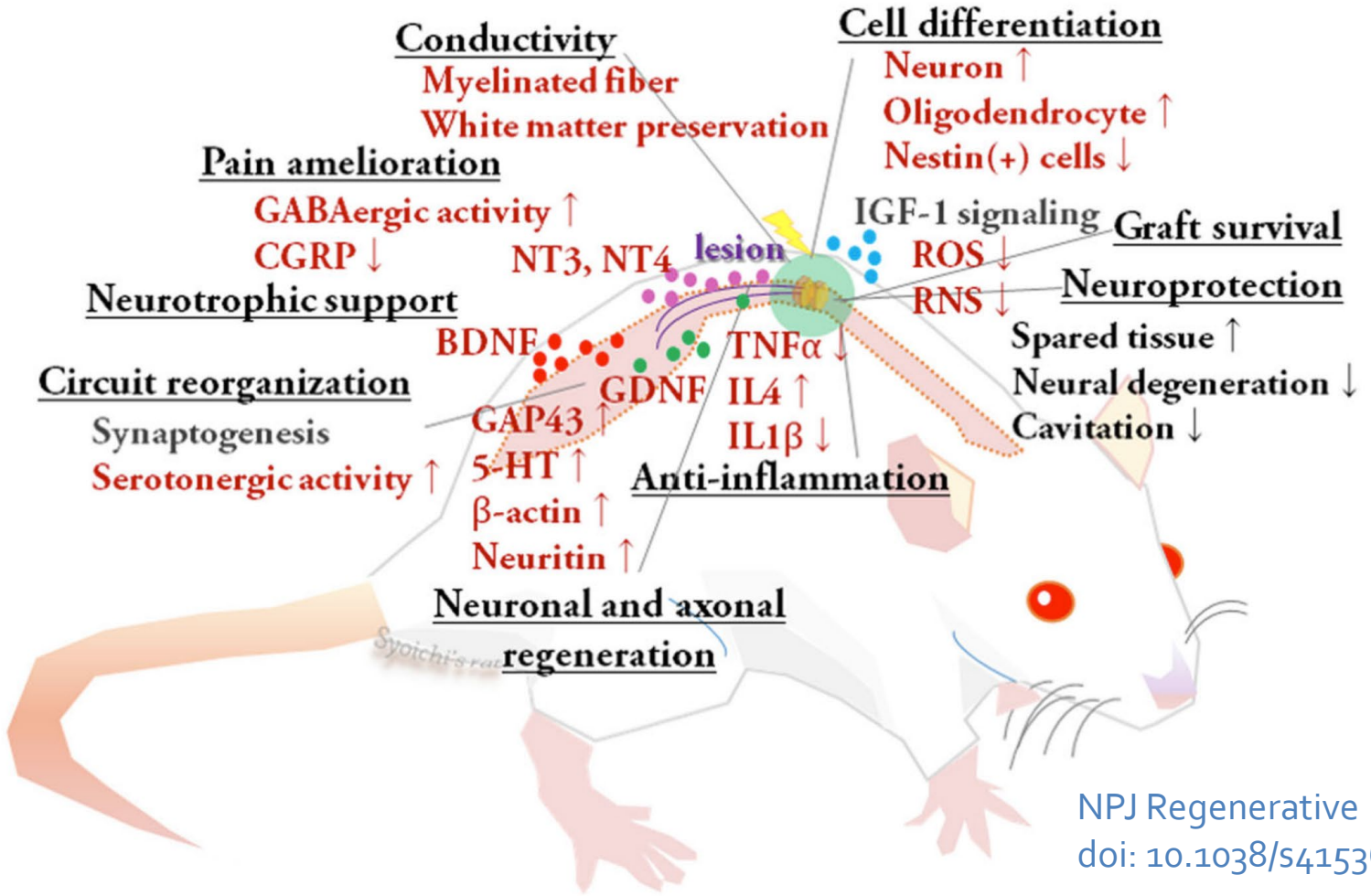
Spinal Cord Injury

- hESC-derived **Oliogdendrocyte precursor cells** (Geron/Asterias, USA) 2010~
- hESC-derived **Neural stem/ precursor** cells S. Biomedics, Korea) 2024~
- hiPSC-derived **Neural stem/ precursor** cells (Keio Univ, Japan) 2021~

→ Rationale for cell therapy is less obvious (reducing scar, myelination, re-routing neuronal connectivity?)



Why is spinal cord injury is a challenging target



NPJ Regenerative Medicine 2021 Nov 25;6(1):81. doi: 10.1038/s41536-021-00191-7.

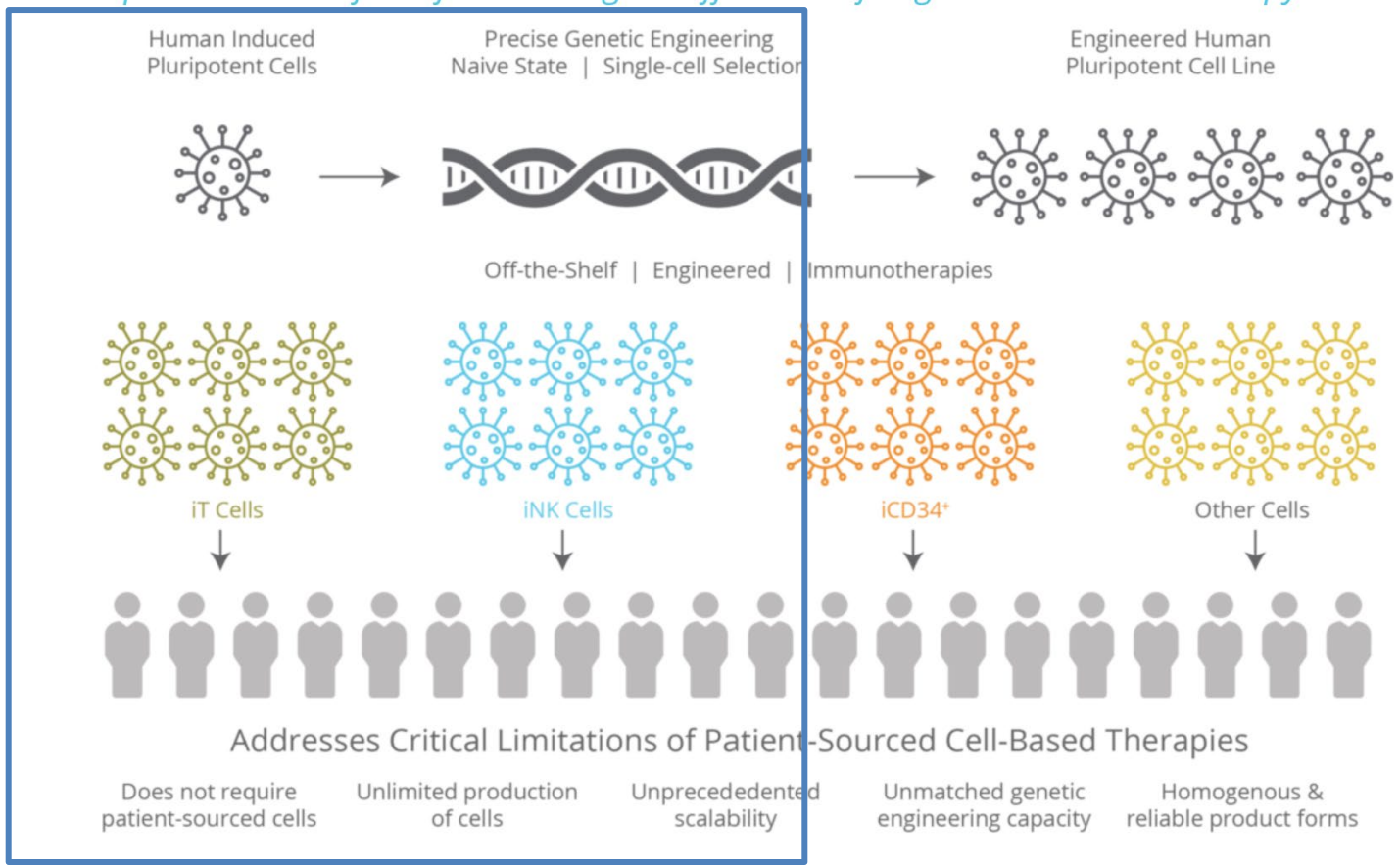
→ Many possible mechanisms proposed but **primary defect is an axonal loss and NOT cell loss**!

Investigational Cell Therapies

Prominent Examples:

- **Off-the-shelf CAR-T and NK cells in cancer**

A Pluripotent Cell Platform for Enabling an Off-the-Shelf Engineered Immunotherapy Revolutio



e.g. Fate Tx

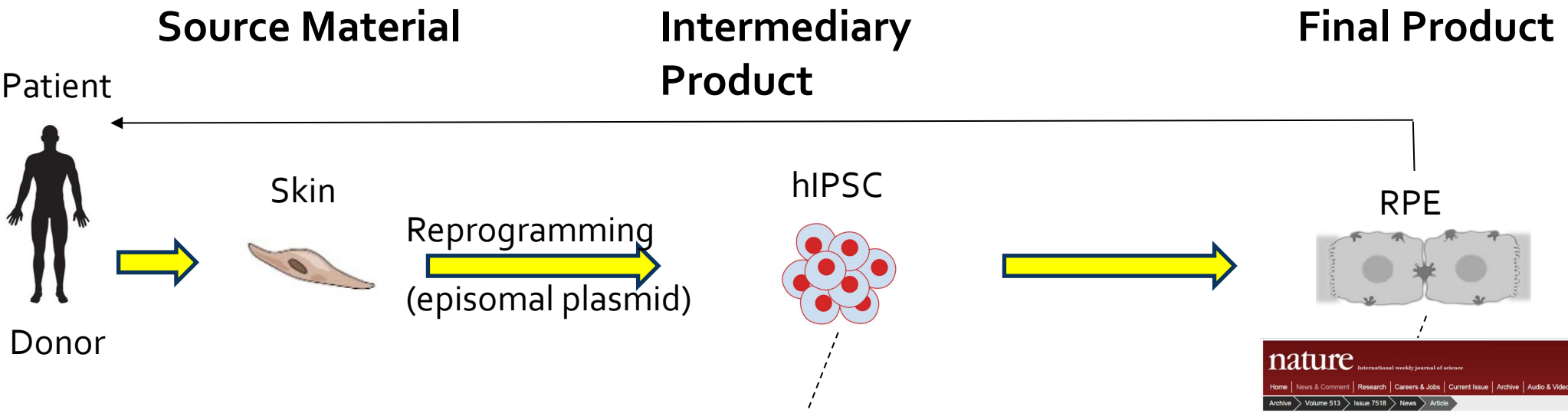
In clinical trials currently

Memorial Sloan Kettering Cancer Center

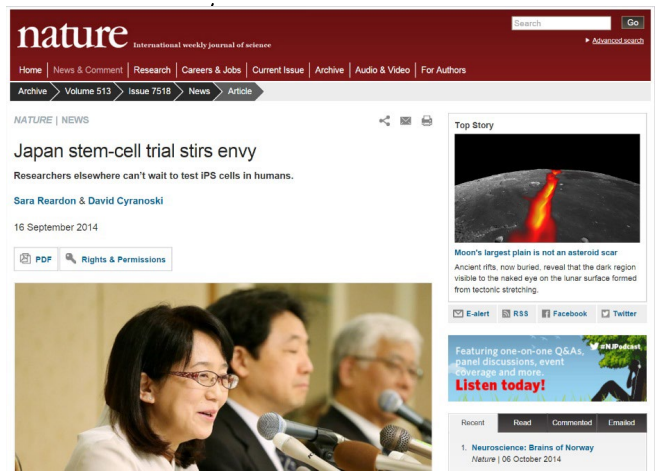
Investigational Cell Therapies

Prominent Examples:

- **Cell therapy in the eye – in particular PSC-derived retinal pigment epithelial cells**

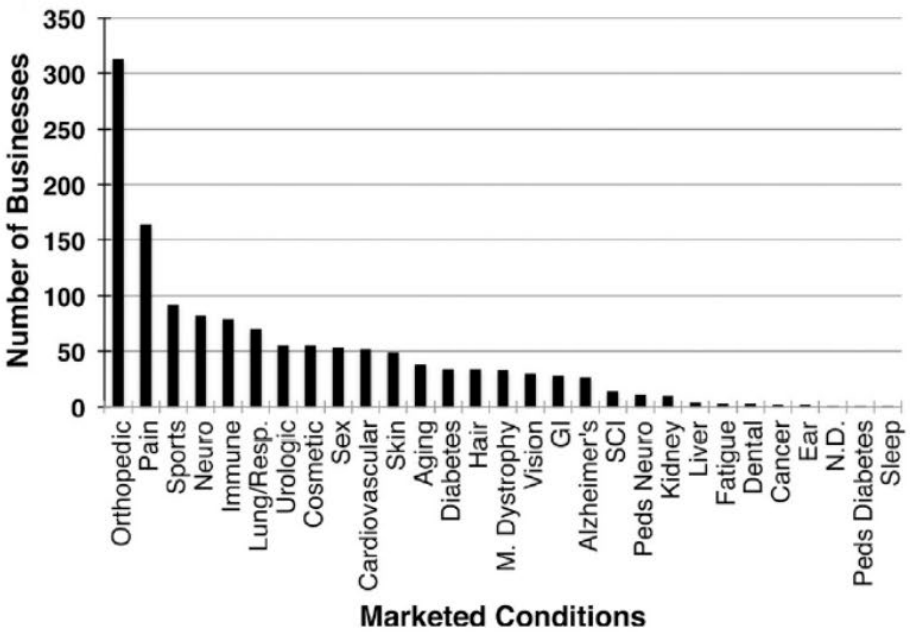


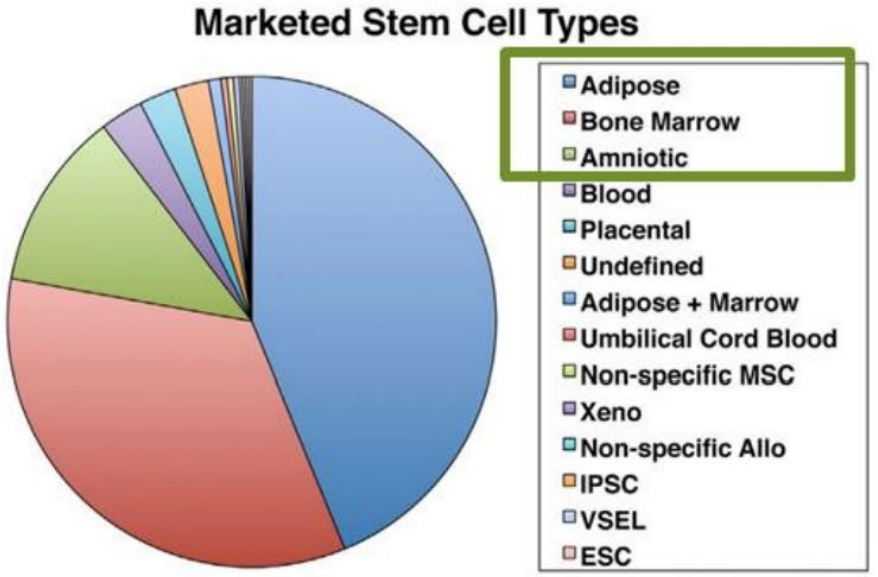
2014



→ Frist example of autologous trial using iPSCs (Macular Degeneration)

Unproven Therapies - Stem Cell Tourism





Kennedy's F.D.A. Wish List: Raw Milk, Stem Cells, Heavy Metals

Robert F. Kennedy Jr., one of President-elect Donald J. Trump's advisers on health, is taking aim at the agency's oversight on many fronts.

- Mode of administration:
  - Intravenous
  - Intrathecal
  - Intramuscular
  - Nebulized

Turner and Knoepfler
Cell Stem Cell 2016

Outline – Cell Therapies

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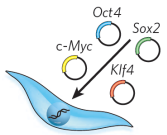
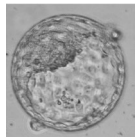
✓ Case study: Manufacturing an **enteric neural precursor cell product for Hirschsprung's disease**

❑ **Design you own study (DIY):**

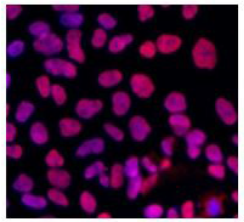
Stem Cells in Preclinical Research



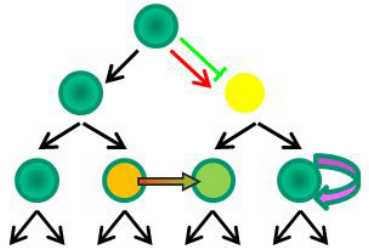
Cell reprogramming



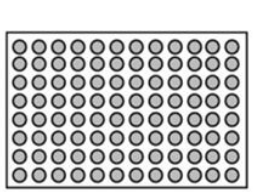
Embryonic cells (IVF)



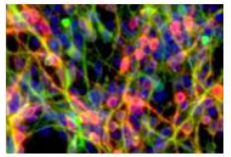
Human pluripotent Stem cells
(human **ES** or **iPS** cells)



Study Human Development



Modeling disease & Drug discovery



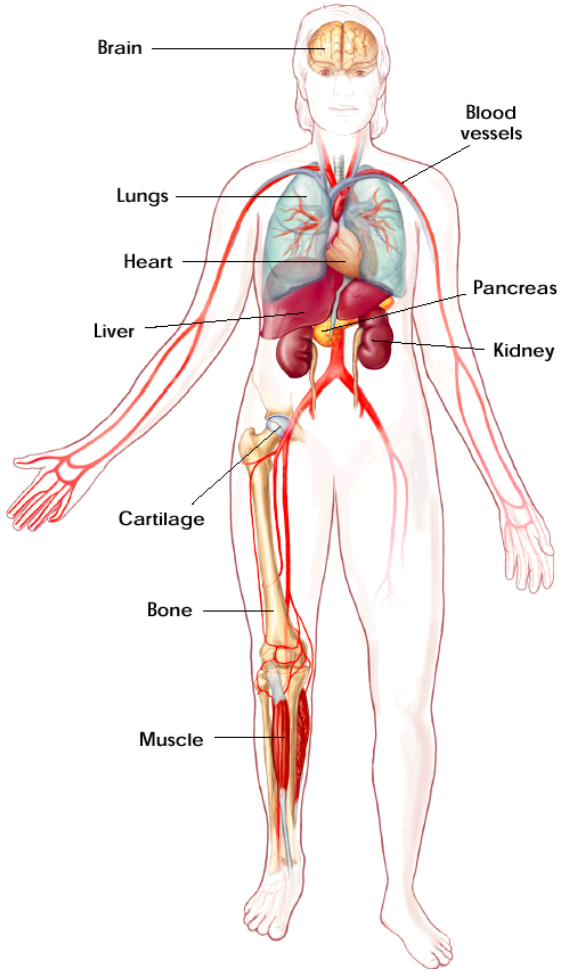
Cell therapy & Regenerative Medicine

Which disease to target by cell therapy – how to get proof of concept

Potential patient populations

• Cardiovascular disease	58 million
• Autoimmune disease	30 million
• Diabetes	16 million
• Osteoporosis	10 million
• Cancer	8.2 million
• Alzheimer's, Parkinson's	5.5 million
• Burns (severe)	0.3 million
• Spinal cord injury	0.25 million
• Birth defects	0.15 million/yr
Total	**128 Million**

(Perry, D. Science, 287; 1423, 2000)

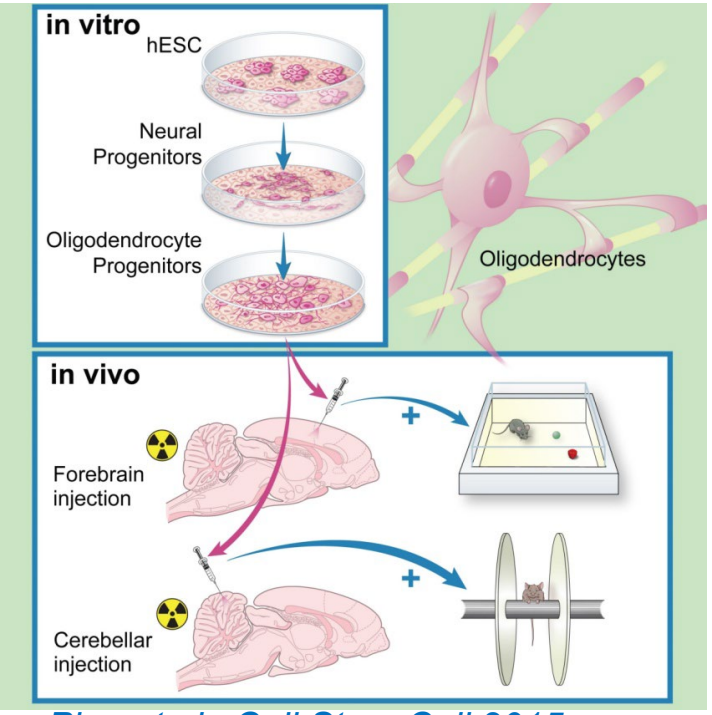


New regenerative medicine application in cancer?

Radiation & chemo are powerful tools in management of cancers such as brain or head & neck

However, those treatments are associated with long term irreversible sequelae:

- Drop in cognitive function and IQ and movement (brain tumors)
- Skeletal damage, salivary function loss, cranial nerve (head & neck)



Piao et al., Cell Stem Cell 2015

Can human PSC-derived cells repair the damage ?

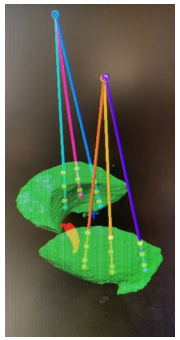
→ Repair of cognitive deficits

→ Repair of movement deficits

Preclinical Development – Impact of Graft host interface

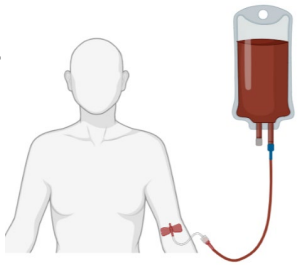
Injection route / homing

Intra-parenchymal



Intra-ventricular

Systemic delivery

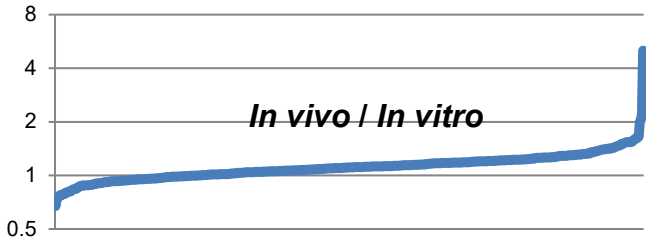


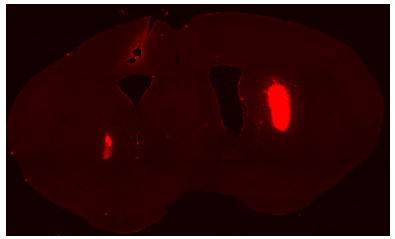
- Physiological mechanisms of cell migration and homing
- Engineered homing properties

→ **Safer & more efficient delivery**

Mechanisms of Graft survival

In vivo CRISPR/Cas9 screens



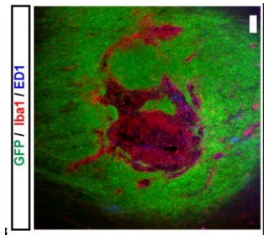


- Many cell types < 10% survival within 1 week (neural, muscle, cardiac..)
- Defining barriers of in vivo cell survival – scRNAseq, in vivo imaging,

→ **Improved survival** (intrinsic vs extrinsic strategies)

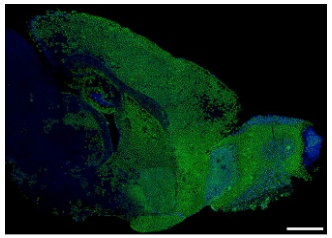
Host response / targeting niche

Reactive astroglial or microglial responses)



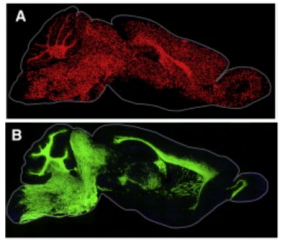
Kriks et al., Nature 2011

hPSC Microglia chimera (CSFR1-i)



Hasselmann et al., Neuron 2019

hPSC Oligodendrocyte (spontaneous / competition?)



Wang et al., Cell Stem Cell 2013

- Immunological, inflammatory, vascular host response
- Emptying or manipulating niche, cell competition

→ **Control cell/tissue organization**

Preclinical Research – General Challenges

How to obtain and validate authenticity of cell identity and assess safety

- Need to define every cell in final product (single cell-based approaches, vs traditional release assays by FDA)
- What is the level of scrutiny needed for genomic changes in the cells to be grafted

How to control maturation stage of cells (species specific maturation clock ?)

- Lag in maturation (e.g. coupling in cardiomyocytes, arrhythmia)
- Delayed efficacy in PD (6-12 months for effect, 2-3 years for optimal effect)

Need for Improved animal models

- Differences in physiology (e.g. cardiac repair – differences in heart rate)
- Differences in pathology (e.g. Huntington's disease: lesion models vs genetic models),

Generating immunologically compatible tissues

- Allogenic with transient immunosuppression
- Autologous tissue (patient-specific iPSCs; endogenous cells [HSCs, CAR-T, Skin,]
- Universal donor cells using gene engineering (hypo-immune, universal cells)

→ Which animal model to test hypo-immune strategies or comparing pros & cons of allo vs autologous strategies

Outline – Cell Therapies

Cell sources

- Definition of stem cells and potency
- Pluripotent stem cells, directed differentiation, cell maturation and aging
- Other types of stem cells, tissue-specific stem cells, engineered cells (synthetic biology?)

Currently approved and investigational Cell therapies

- Approved cell therapies
- Investigational cell therapies
- Unproven cell therapies and stem cell tourism

Preclinical research

- Choice of disease and choice of candidate cell type
- In vivo model systems for preclinical research

Product development and clinical grade manufacturing

- Clinical grade manufacturing, GMP compliance, Critical quality attributes
- IND enabling studies, Device and cell delivery

✓ Case study**:** Manufacturing a **dopamine neuron cell product for Parkinson's disease**

✓ Case study: Manufacturing an **enteric neural precursor cell product for Hirschsprung's disease**

❑ **Design you own study (DIY):**

Product development and clinical grade manufacturing

Choice of source material:

- Starting material (autologous vs allogeneic)
- Cell source and type
- Reprogramming method (if applicable)

These decisions should **prioritize patient safety** and aim for the **most effective** treatment.

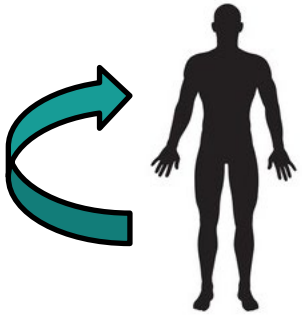
These decisions will impact the **design of the manufacturing process**, quality control requirements, and overall cost of the cell therapy program.

→ **Choice of cell type very much depends on disease and mechanism of action**

Product development and clinical grade manufacturing

Choice of source material (Pros- and Cons):

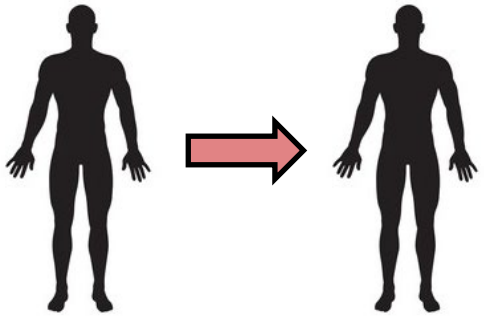
Autologous



Donor = Patient

- Personalized treatment
- Limited cell source choices
- Delayed access to treatment
- Higher manufacturing cost

Allogeneic



Donor Patient

- Off-the-shelf treatment
- More cell source choices
- Faster access to treatment
- Lower manufacturing cost
- Scalable production
- Additional QC required

Product development and clinical grade manufacturing

Requirements for manufacturing

- Adherence to **Good Manufacturing Practices (GMP)**
- **Quality control (QC)** measures, such as cell purity and potency (release criteria)
- **Safety** assessments, including sterility, genomic integrity, and stability

Product development and clinical grade manufacturing

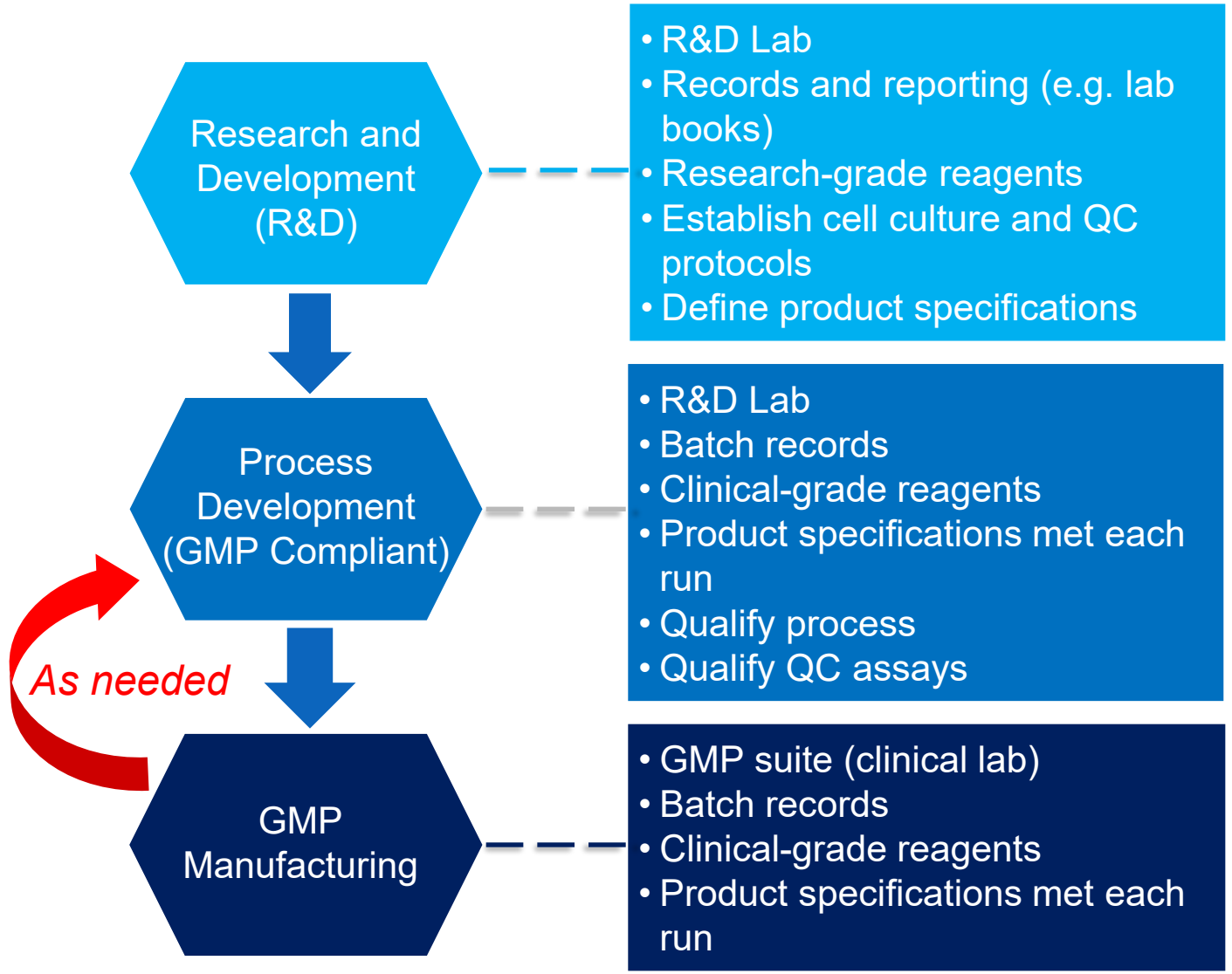
What does GMP manufacturing mean?

- Set of rules established by *regulatory agencies* that ensures medicinal products are **consistently produced** and controlled to the **quality standards** appropriate for their intended use and as required by the product specifications.
- Primary objective is to assure therapeutics are **safe** for patients.

.

→ Need to define GMP level of product / audit companies providing GMP qualified reagents etc

Product development and clinical grade manufacturing



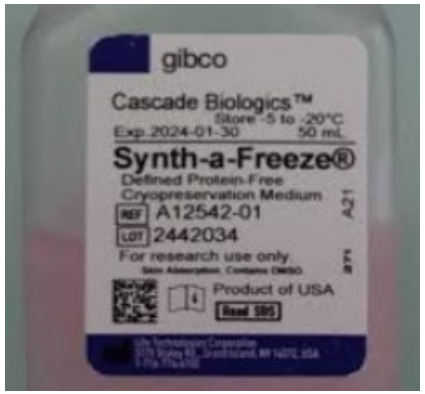
Consideration for reagent selection

Research Use Only (RUO) Reagents

- Can be derived from animals
- May contain serum
- No requirement to control lot-to-lot variability
- Not required to be manufactured in a GMP facility

GMP and Cell Therapy Systems (CTS) Reagents

- Serum-free or highly controlled serum
- Consistent batch-to-batch
- Manufacturing and QC in a GMP-compliant facility
- Stringent QC tests
- Drug master file available



Cryopreservation medium

Mouse Laminin

Cryopreservation medium

Human Laminin (recombinant)

Critical quality attribute of final cell product

Each stem cell-derived therapy product **must be tested** for:

- **Sterility** (absence of microbes)
- **Identity** (cell type)
- **Purity** (absence of contaminants)
- **Potency** (functional activity)
- **Viability** (cell health)
- **Genomic Stability** (chromosome integrity)
- **Stability** (ability to maintain quality over storage time)

Cell preparation, delivery and device

- Cellular **suspensions** versus Spheroids or **organoids**
- **Cryopreservation** versus **fresh**
- Combining cells with biocompatible **scaffolds**, such as hydrogels or other matrices

- **Administration Route**
  - **Intravenous** infusion
  - **Intrathecal** administration
  - **Topical** application
  - More **localized** treatments, direct injection or surgical implantation (often no optimized device available)

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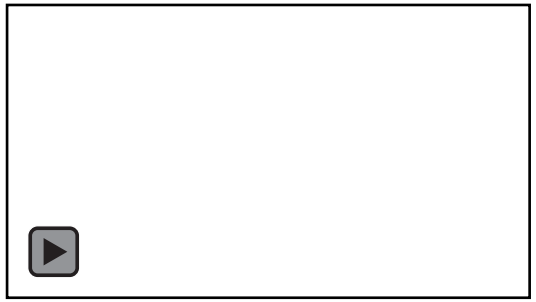
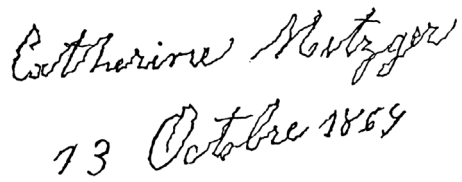
Parkinson's Disease

Instable posture / gait



"Tremor" = Shaking



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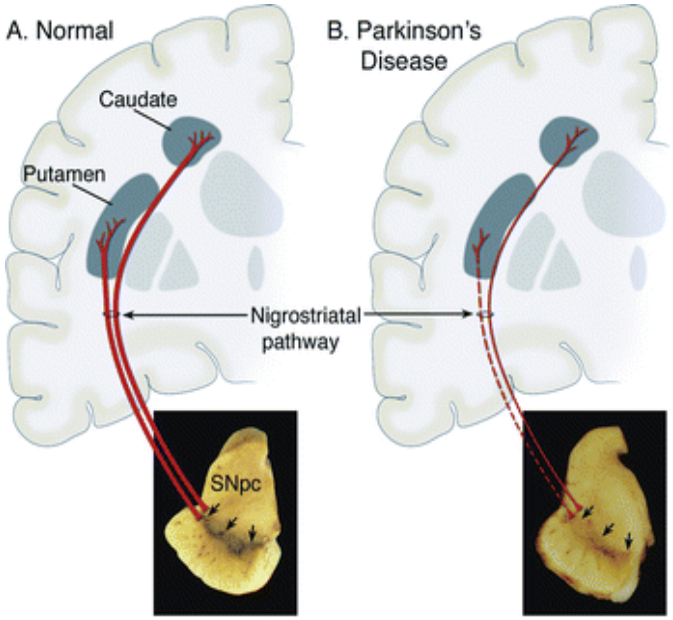
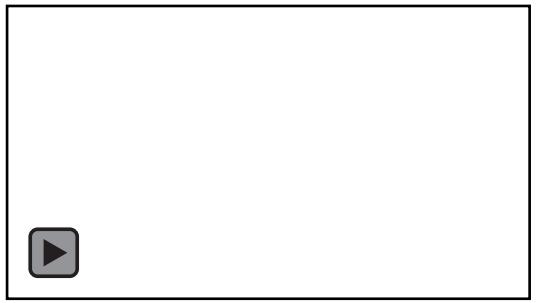


Loss of dopamine cells in midbrain
(300-400k in healthy individual)

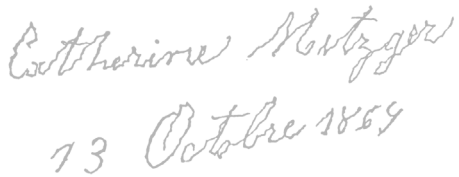
- Parkinson's disease affects about 1 million patients in US (> 10 million worldwide)
- Characterized by motor symptoms - due to the progressive loss of dopamine cells in the brain
- Annual health-related costs in US: $25 billion; economic burden: $52 billion (NPJ Parkinson's disease, Vol 6; 15 (2020)

Parkinson's Disease

Instable posture / gait



"Tremor" = Shaking



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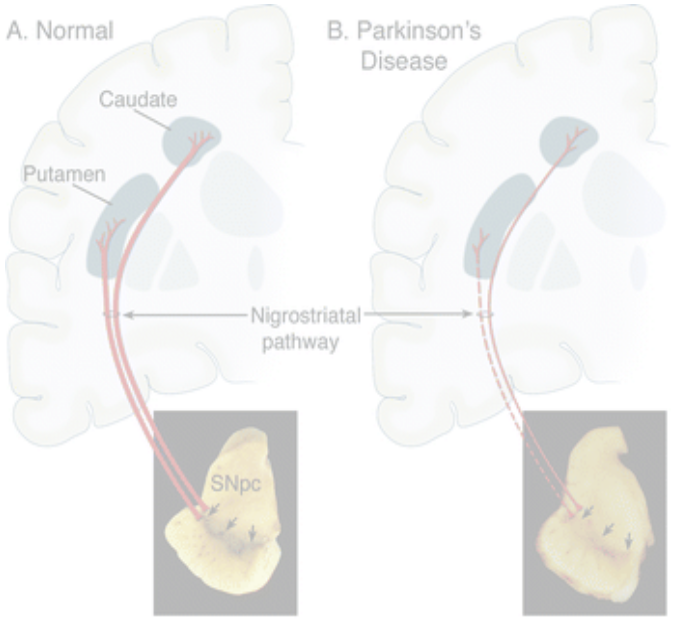
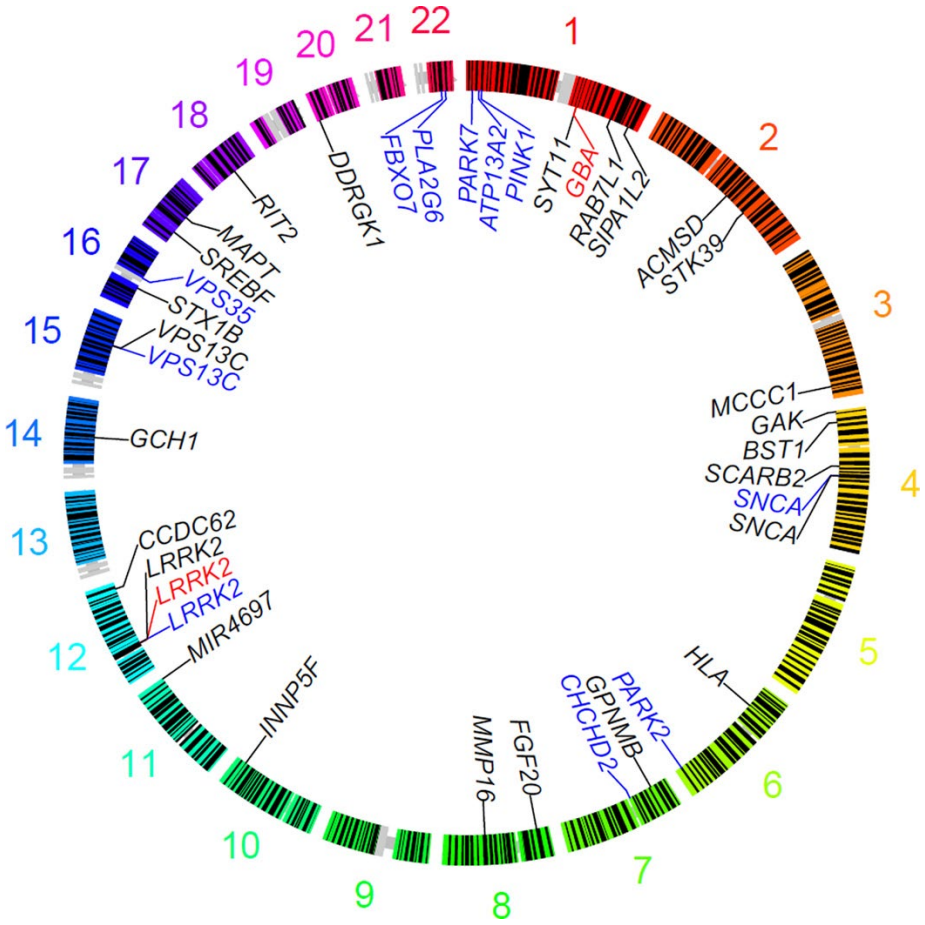


Loss of dopamine cells in midbrain
(300-400k in healthy individual)

PD is **not only a movement disorder** – other parts of brain and nervous system are also affected:

- Loss of **smell**, **restless leg syndrome**, **constipation**, …
- Loss of **cognitive function at later stages** of the disease (Lewy body disease)

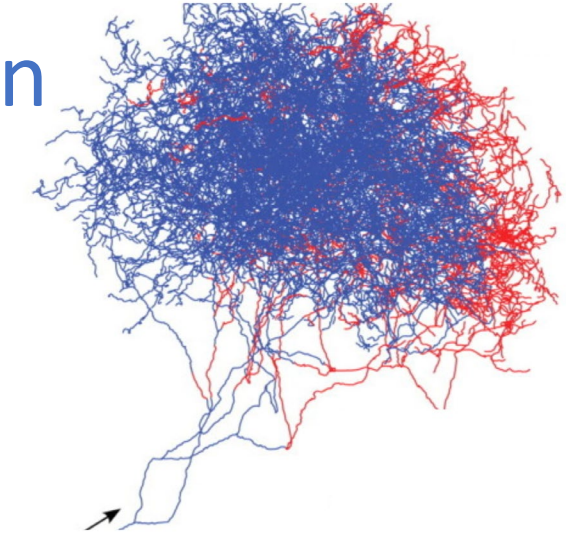
PD Genetics and Related Dysfunction



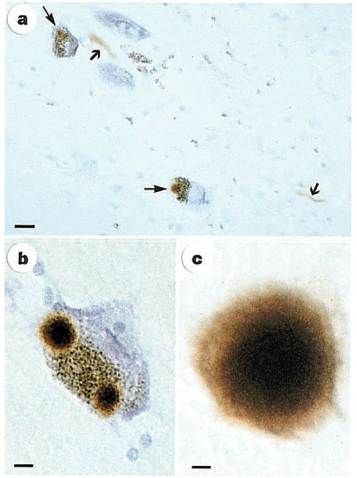
Andy Singleton & John Hardy; Neuron (2016)
Blauwendraat et al., Lancet Neurol (2020)

→ 90 risk variants across 78 genomic loci

- **Mitochondria / Mitophagy** Dysfunction (Cell energy usage)
- **Lyso/Endolysosomal –/ Proteasomal** Dysfunction
- **a-synuclein** production / aggregation
- **vesicle and synaptic** dysfunction
- Others…? (e.g. **Dopamine** metabolism, **inflammation, immunological** vulnerability)



Matsuda et al., J. Neurosci. (2009)



Spillantini et al., Nature (1997)

Memorial Sloan Kettering Cancer Center

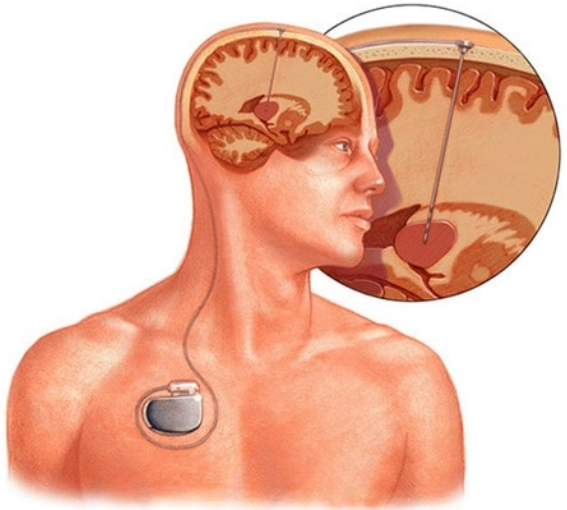
Therapeutic options (approved and experimental)

Medical Treatment

Dopamine as drug

- Highly effective initially
- less effective as disease progresses
- No disease-modifying drugs available

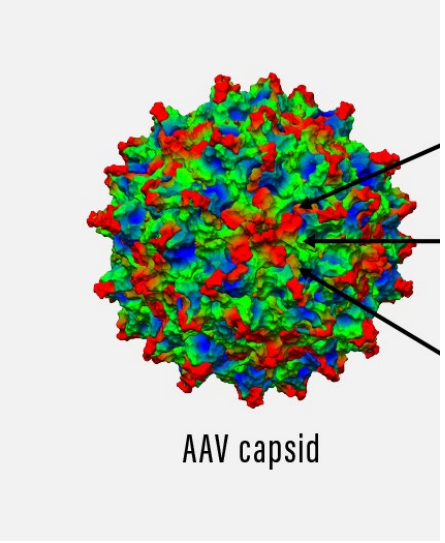
Surgical Treatment



Deep brain stimulation (DBS)

- Highly effective in tremor & dyskinesia
- Not suitable for all patients (<10%)
- Needs adjustments/battery change
- Speech problems, potential psychiatric symptoms can occur

Gene therapy (experimental)

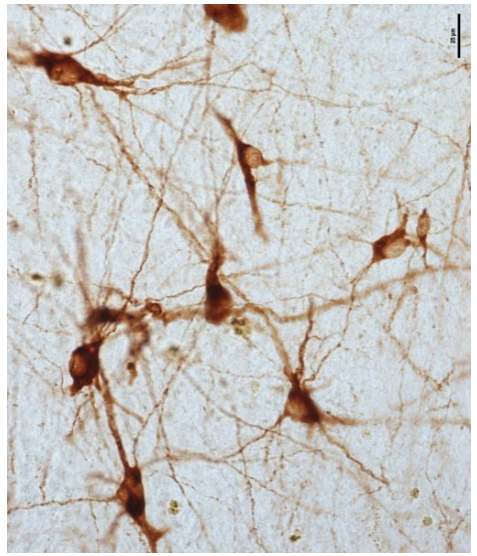


Boost dopamine production

<u>Not proven</u>

- Enhance dopamine production in remaining or force in non-dopamine cells
- Produce GDNF & other protective factors

Cell therapy (experimental)



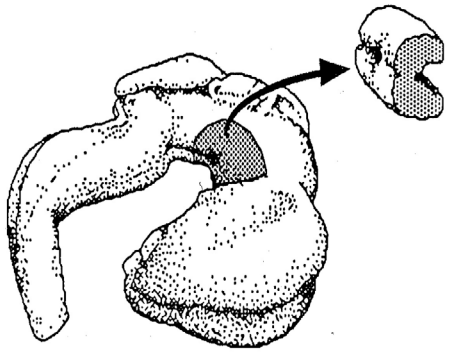
New dopamine cells

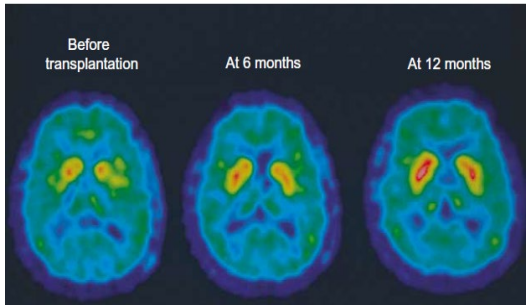
<u>Not proven</u>

- Implanting new dopamine cells
- Rebuilding circuits, long-term restoration of function?

Cell replacement as a Potential Therapy for PD – lessons from fetal grafting

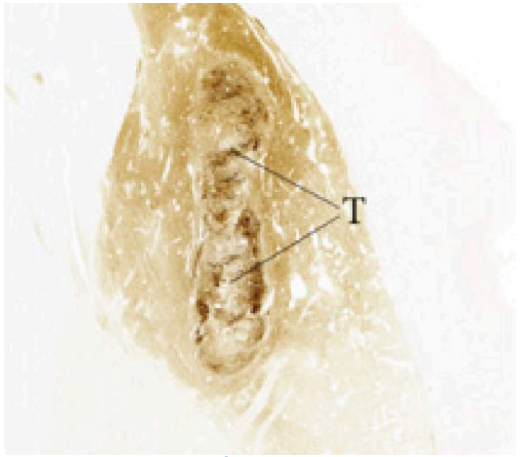
Fetal Grafting





N Engl J Med. 1995 Apr 27;332(17):1118-24.

Surviving fetal graft (24 yrs!)



Li et al., PNAS 2016

- > 300 patients were treated with fetal tissue world-wide
- Benefit at 12 months variable (e.g. placebo-controlled studies)
- Some patients showed graft-induced dyskinesia
- Long-term (>15 years !) follow-up showed benefits in subset of patients ("off L-Dopa" for decade)

→ **TRANSEURO trial (Cambridge UK & Lund)** 7 patients grafted with fetal tissue, challenges in consistency

Roger Barker

Nature Biotech (2025)

→ Need for a renewable source of dopamine neurons

Breakthrough in protocol development > 10 years ago - Derivation of Dopaminergic Neurons from hES or hiPS cells

nature biotechnology 2009

Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling

Stuart M Chambers[1], Christopher A Fasano[1], Eirini P Papapetrou[2], Mark Tomishima[1,2], Michel Sadelain[2,3] & Lorenz Studer[1,2,4]

Neural Tissue

Chambers et al., Nat Biotechnol (2009)

LETTER 2011

doi:10.1038/nature10648

Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease

Sonja Kriks[1,2*], Jae-Won Shim[1,2*], Jinghua Piao[1,3], Yosif M. Ganat[1,2], Dustin R. Wakeman[4], Zhong Xie[5], Luis Carrillo-Reid[5], Gordon Auyeung[1,3], Chris Antonacci[1,3], Amanda Buch[1,3], Lichuan Yang[6], M. Flint Beal[6], D. James Surmeier[5], Jeffrey H. Kordower[4], Viviane Tabar[1,3] & Lorenz Studer[1,2,3]

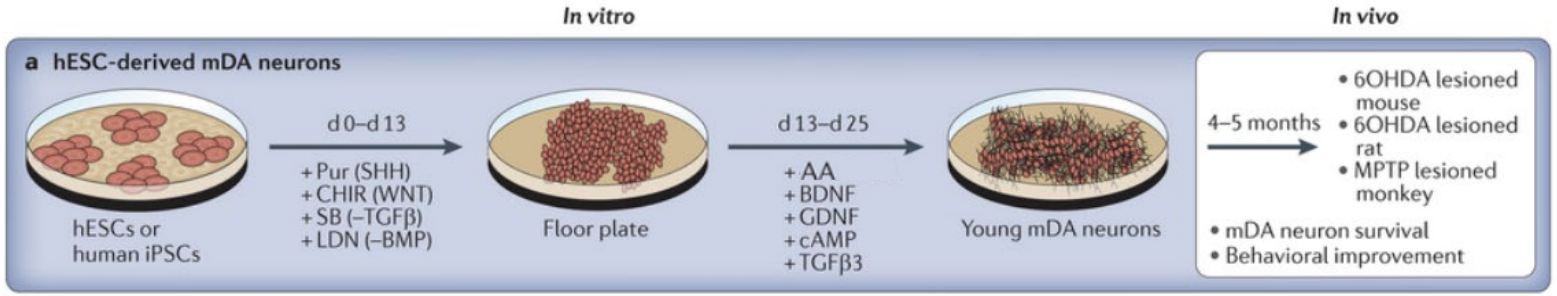
FP-derived **Dopaminergic neurons**

Kriks, Shim et al., Nature (2011)

Similar floor plate-based protocols by several groups:

- *Kirkeby et al., Cell Rep. 2012 Jun 28;1(6):703-14.*
- *Xi et al., Stem Cells. 2012 Aug;30(8):1655-63*

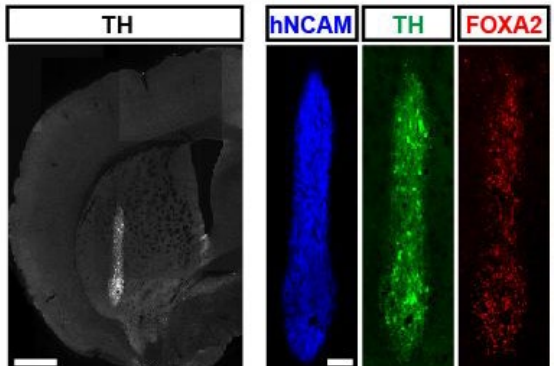
- *Sundberg et al., Stem Cells. 2013 May*
- *Doi et al., Stem Cell Reports 2014, 2, (3): 337-350*



Studer & Tabar, Nat Review Genet (2014)

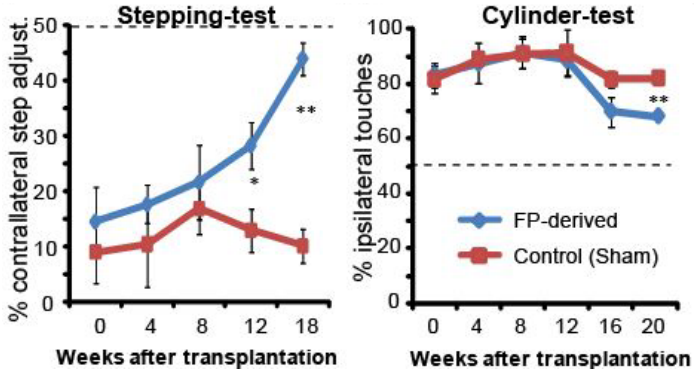
POC using hPSC-derived DA neurons in mouse, rat & monkey PD models

PD mouse (6OHDA)

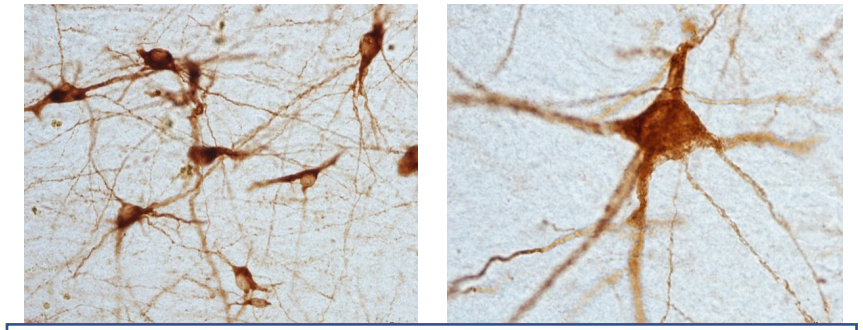


Kriks et al., Nature 2011

PD rat (6OHDA)

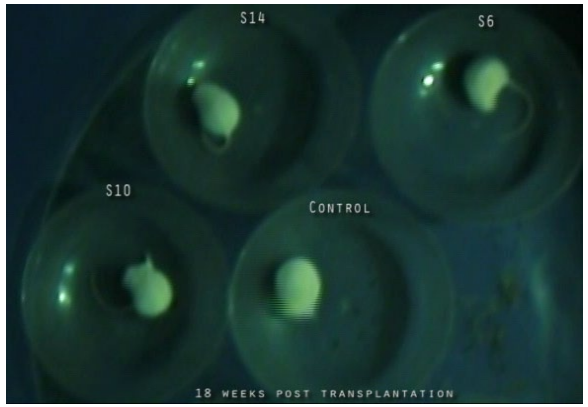


Rhesus monkey (MPTP)

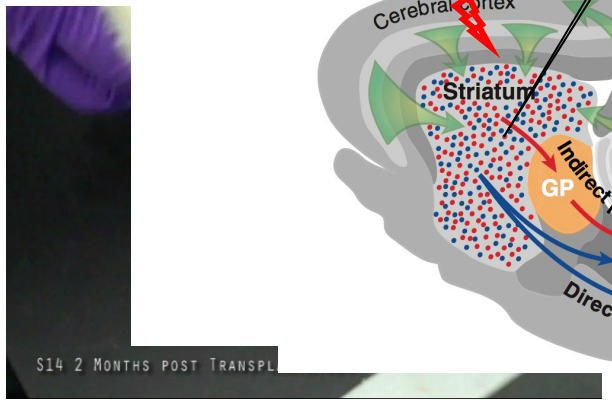


→ ~ 100,000 TH+ neurons in total

Amphetamine-induced rotations

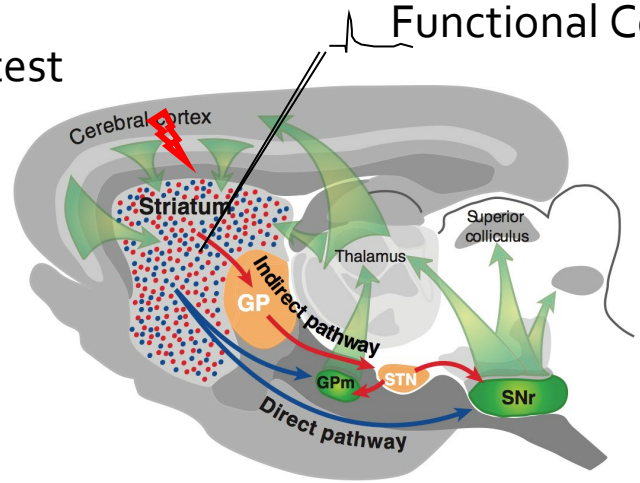


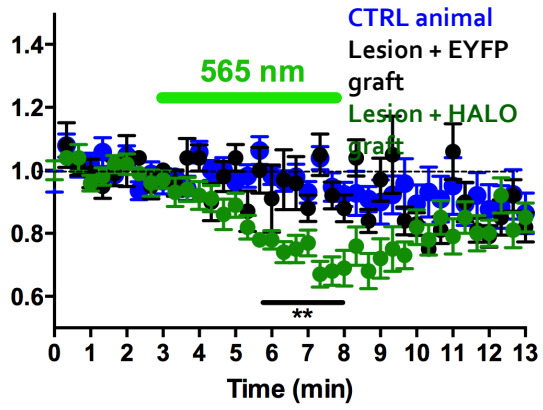
Pull test



Jinghua Piao

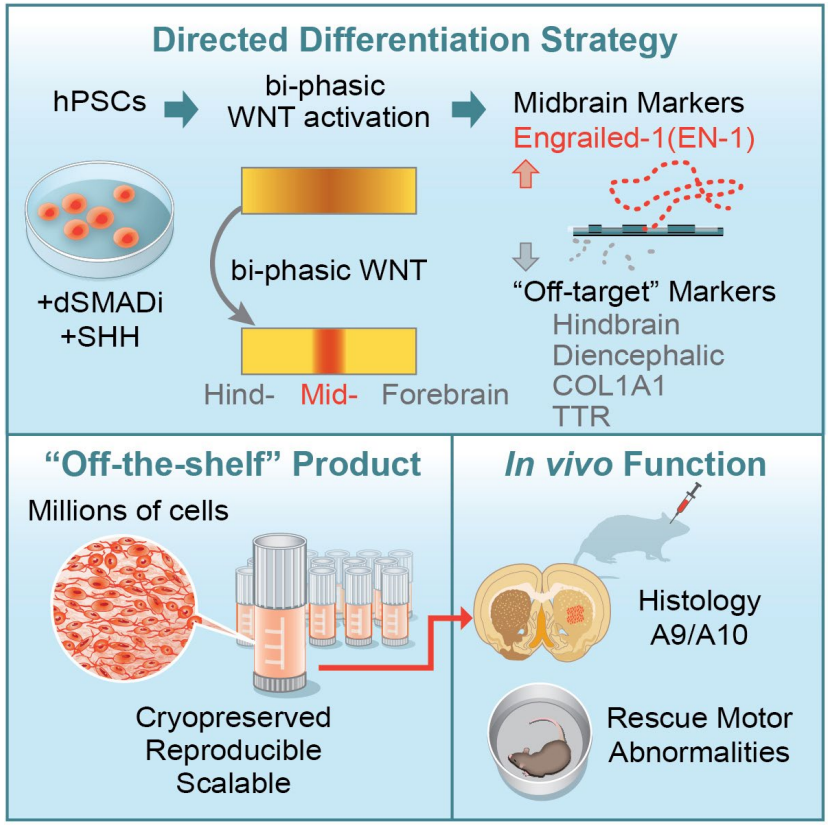
Functional Connectivity by Optogenetics





Steinbeck et al., Nat Biotechnol 2015 (w. Sulzer lab)

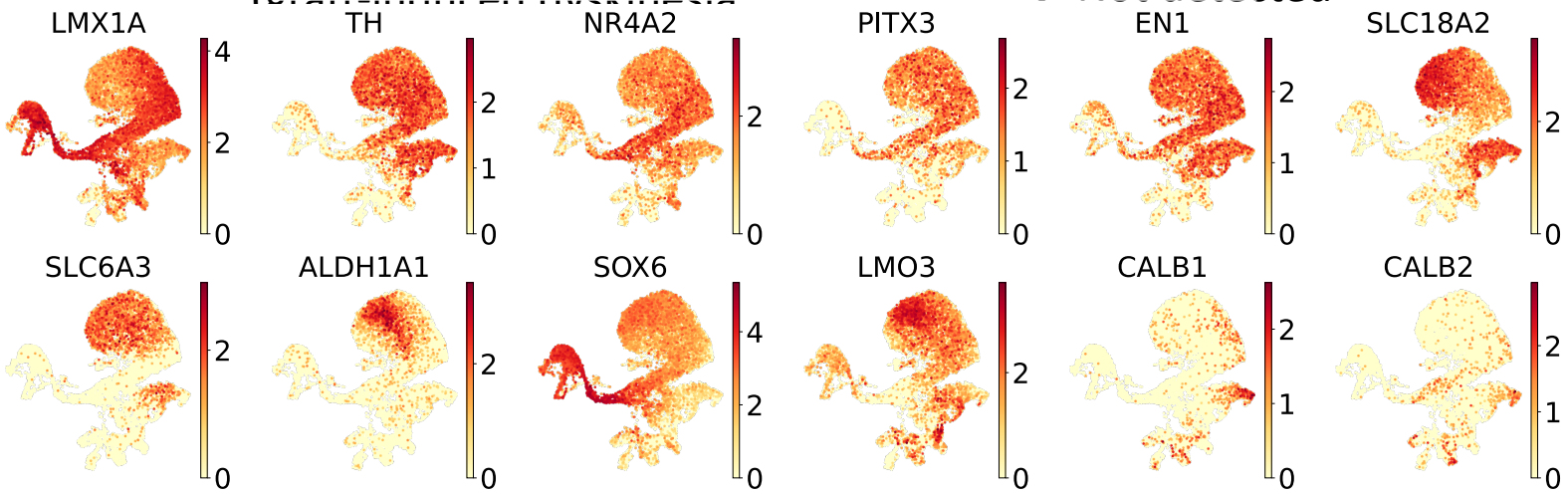
Next 10 years: Refining differentiation technology for clinical translation



(Kim TW et al., Cell Stem Cell, 2021)

- MSK-DA01 **manufactured in 2016** in GMP facility (**10 billon cells**)
- **Cryopreserved** at endpoint "off-the-shelf"
- Minimize unwanted "off target" cell types in the mix

s-nucSeq data (in vivo)



Kim,Piao, Bocchi et al., (BioRxiv & unpublished)

Memorial Sloan Kettering Cancer Center

IND enabling studies & Clinical trials – to be continued…. (Viviane Tabar lecture)

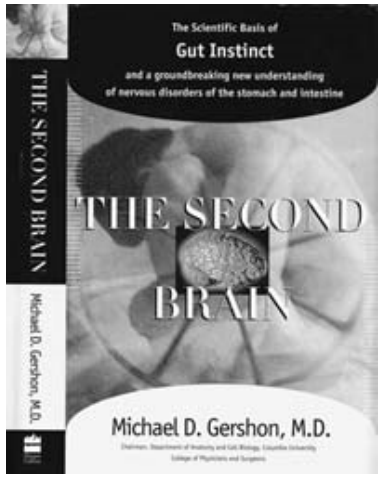
Tabar et al, Nature (2025)

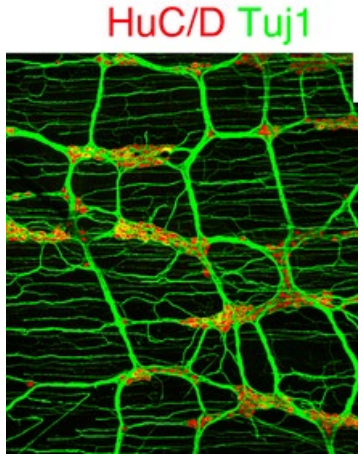
ENS function and disease – the "second brain" in the gut – defects in Hirschsprung's disease

ENS is **largest part of autonomic nervous system**
~ 500 mio neurons!

Essential for **motility** and **secretion in GI tract**

Many **subtypes of neurons in** ENS (transmitter diversity similar in complexity to brain!)



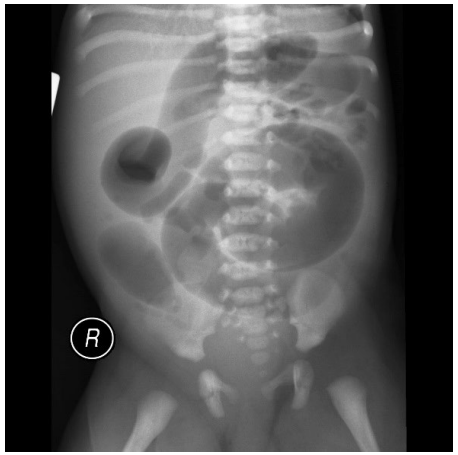


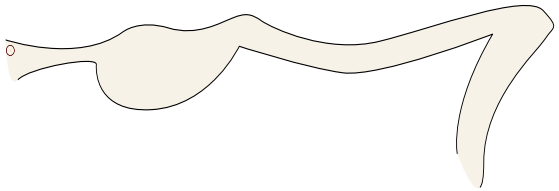
Ming Fu et. al., JCI, 2013

Hirschsprung's disease is a **congenital disorder** (1/5,000 children)

Most common mutations in **RET** and **EDNRB** receptor

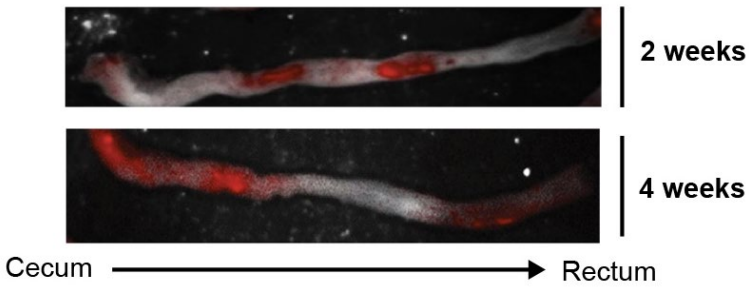
Surgical resection can be life saving. However:
- **functional problems** post surgery
- **total aganglionosis** cannot properly treated

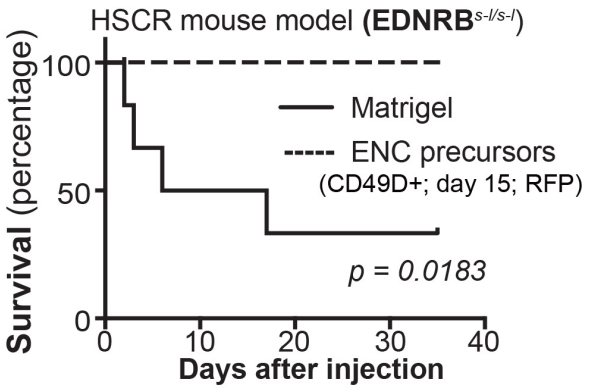




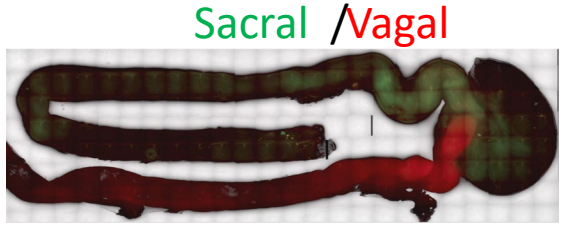
Potential cell therapy for Hirschsprung's disease

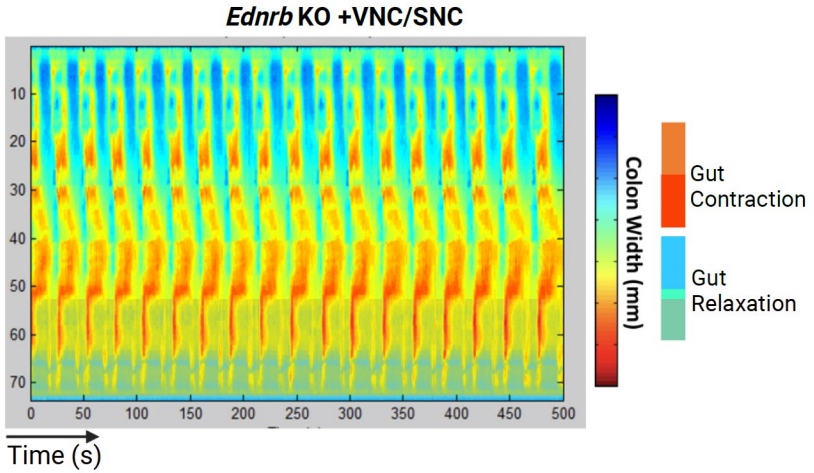
The Enteric Nervous System → Hirschsprung's disease



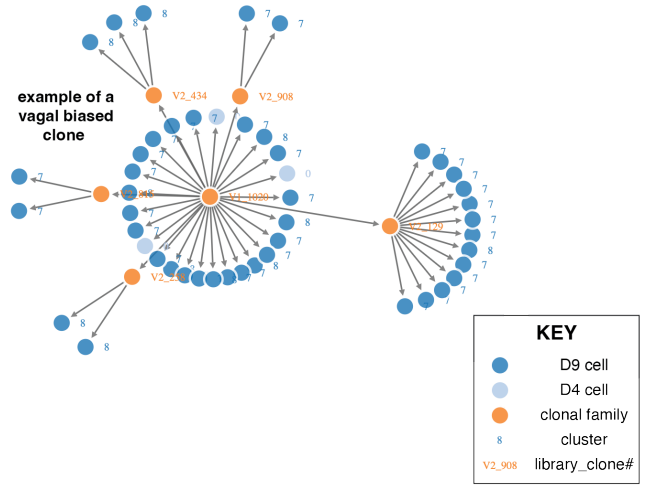


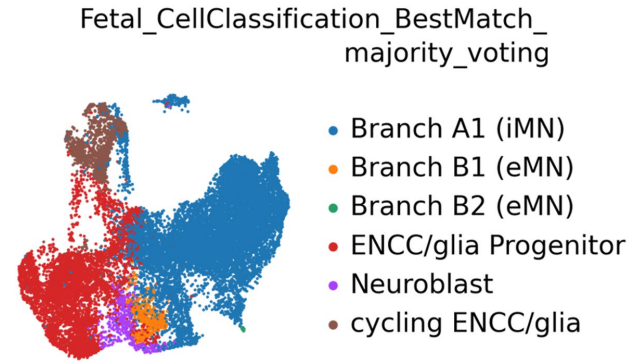
Fattahi et al., Nature (2016)





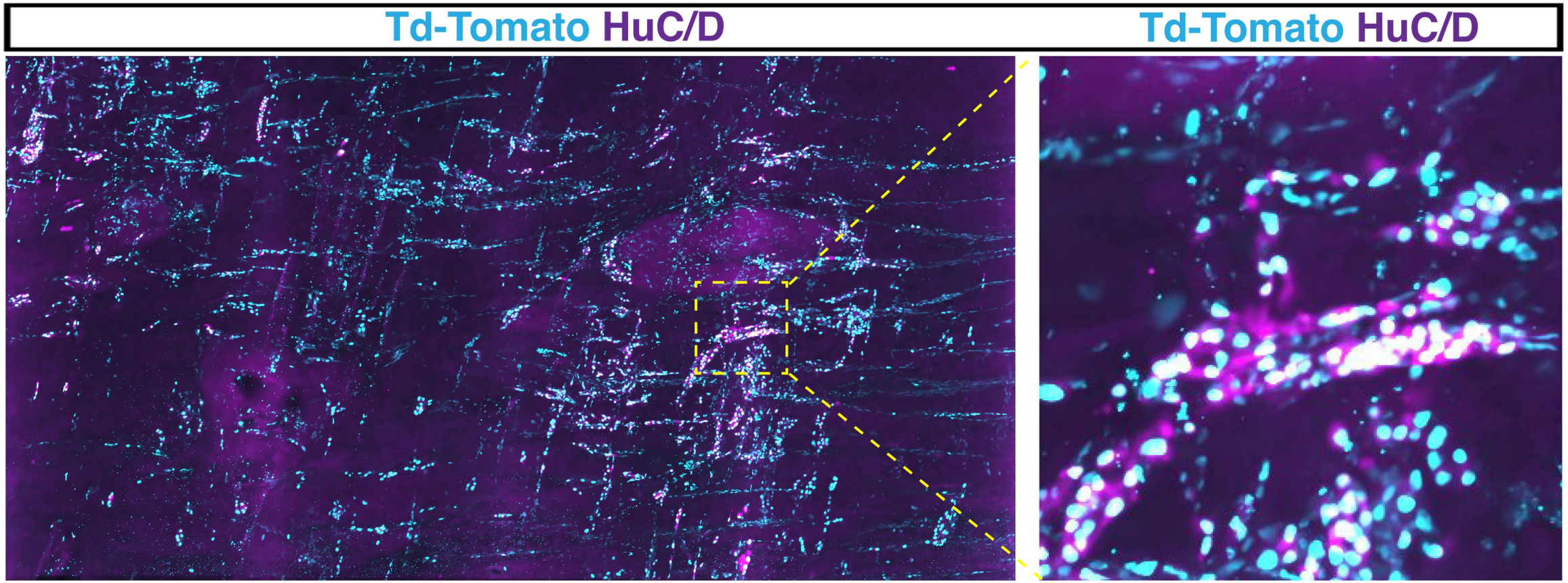
Fan et al., Cell Stem Cell (2023)





Jim Hackland (unpublished)

Beyond PD – The Enteric Nervous System → Hirschsprung's disease



→ **Challenge of funding** GMP development & IND enabling studies (abandoned by BlueRock, Takeda, Novo)

→ Founding of **REGEN-GI** (groups in Australia, UK, NYC, CA, Boston,..)

Design your own study – focus on preclinical work

What is the disease to target?

What is the cell source you choose to treat the disease?

What is the final product and how to qualify – do you need to engineer the cells and if so how?

What is your preclinical model – proof of concept data – IND enabling studies?

How many cells – how to manufacture and how deliver?

What are the main safety concerns?