

Core Concepts in Stem Cell Biology: Syllabus and Learning Guide

Annotated with
Foundational Literature

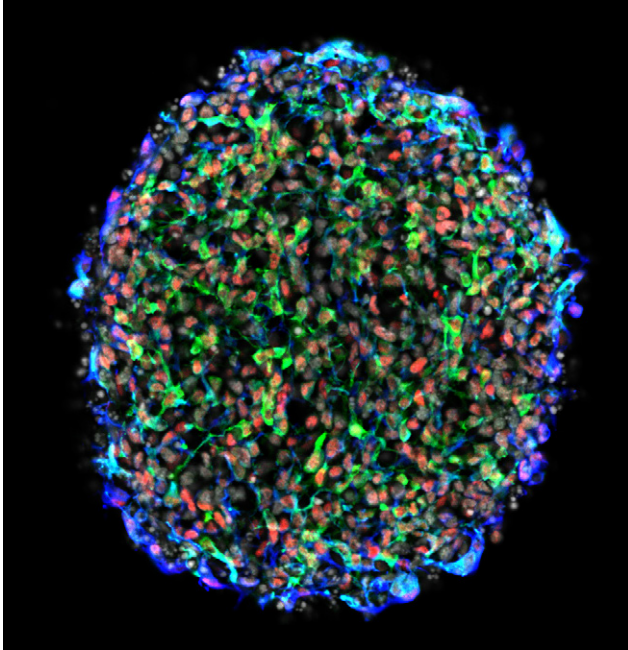


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ON THE COVER

Dopaminergic neurons derived from an embryoid body, a three-dimensional aggregation of pluripotent stem cells Achaete-scute homolog 1 (Ascl1) in red stains a pioneer transcription factor involved in neuronal differentiation, Tyrosine Hydroxylase in green plays an important role in neuronal physiology, and Tubulin Beta 3 Class III (Tubb3) in blue is expressed in axons. Credit: Begum Aydin, Mazzoni Lab, New York University, USA.

DISCLAIMER

The ISSCR is providing these educational resources to aid in course preparation. It is incumbent upon the individual user to ensure that the material they present is up-to-date and properly presented.

These resources are made possible due to the hard work and dedication of the ISSCR Education Committee. If you have any questions or feedback please contact Julie Perlin, PhD, Scientific Communications and Education Manager, at jperlin@isscr.org.

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Preface

Dear Stem Cell Community and Educators,

The first time I was tasked to put together a course on stem cell biology, I realized that there were insufficient resources available to help create a syllabus and identify key topics and materials. It became clear I was not the only one who felt this way. Moreover, the lack of resources has contributed to misinformation regarding stem cell biology and clinical treatments. To help address this need, the International Society for Stem Cell Research (ISSCR) Education Committee has prepared a syllabus and learning guide to aid instructors so that they have a place to start when building their own course.

We have identified eight key topics in stem cell biology and created corresponding teaching modules, each with learning objectives, core concepts, a bibliography of primary and secondary literature, and annotated foundational papers. Through these topic-centered modules, the course examines different types of stem cells found in the body and used in the lab, dissects the critical concepts of pluripotency, specification, and differentiation, and covers cutting-edge technologies used to study stem cells. Moreover, the course explores how stem cells are being translated for clinical application and the corresponding ethical questions that arise from this use.

This resource is intended for those who are teaching stem cell biology and related topics to undergraduate students, early graduate students, or medical students. The learning guide is designed to be flexible and adaptable. It can be used to plan a semester-long course that delves deep into each core topic or adjusted to teach an abbreviated overview.

This learning guide is a first step that we will build upon, incorporating new types of resources in the

future. Stem cell science is an incredibly vibrant and impactful field that is constantly changing. It is the responsibility of the instructor to make sure that the material is up to date and presented properly. As a committee, we will update the content regularly, and we welcome feedback to help us enhance these resources to best serve your needs as a teaching tool.

We know that often there is a lack of education about stem cells in universities or at medical schools, which may contribute to the proliferation of unproven stem cell treatments that put people at risk. We hope that by making stem cell education more accessible, we can help teach the next generation of researchers, medical professionals, and consumers worldwide.

Thank you to the hard work of the ISSCR Education Committee for making this resource possible, and for the support of the ISSCR Board of Directors. We hope that this learning guide enhances your teaching experience.

Sincerely,

Esteban Mazzoni, PhD

(Chair of the ISSCR Education Committee)

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Course Description

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What defines a stem cell? How many types of stem cells are there? How do cells that have the potential to become any cell in the body gain specific identity and function? How can stem cells be used to understand and treat diseases? What are the ethical considerations of stem cell science? These are the types of questions we will delve into during the “Introduction to Stem Cell Biology” course. Through topic-centered modules, the class examines the different types of stem cells found in the body and studied in the lab, dissects the key concepts of pluripotency, specification, and differentiation, and covers cutting-edge technologies used to study stem cells. Moreover, the course explores how stem cells are being translated for clinical application and the related ethical questions. The class emphasizes molecular genetic regulation of each step. At the end of the course, students will have a basic understanding of stem cell biology and be able to build upon this in upper-level courses. Because the syllabus is anchored by peer-reviewed primary literature, the material can be used to develop higher-level skills necessary for thinking like a scientist. Classic experiments can be used for conceptual knowledge or further dissected to understand hypothesis generation, experimental design logic, result interpretation, and discussion.

COURSE MODULES:

1. Introduction to Stem Cell Biology
2. Introduction to Development
3. Pluripotency and Reprogramming In Vitro
4. Adult Stem Cells and Regeneration
5. Directed Differentiation and Transdifferentiation
6. Leveraging Tools to Study Stem Cell Biology
7. Clinical Applications of Stem Cell Biology
8. Ethical Issues in Stem Cell Research

IMPORTANT DEVELOPMENTAL BIOLOGY CONCEPTS FOR THE COURSE IN GENERAL

1. Necessary vs. sufficient
2. Model systems used
3. Key signaling pathways
4. Key lab techniques (Thomas and Capecchi, 1987)
5. Mechanisms we can borrow from embryology to mimic differentiation in a dish
6. Disease modeling
7. Regenerative medicine

ADDITIONAL RESOURCES:

This course is intended for undergraduate students, early graduate students, or medical students interested in stem cell biology. This course assumes a working knowledge of basic cellular, molecular, and developmental biology.

Textbooks that cover pre-requisite material

- Developmental Biology, 12th edition, 2019 by Michael Barresi and Scott Gilbert, Oxford University Press.
- Molecular Biology of the Cell, 6th edition, 2017 by Bruce Alberts, Garland Science. Or similar introductory cell biology textbook.
- Stem Cells for Dummies, 1st Edition, 2010 by Lawrence S.B. Goldstein.

Online Resources that provide supplemental material for students and educators

- A Closer Look at Stem Cells: [Learn about stem cells](#).
- EuroStemCell: [Education resources](#).
- Society for Developmental Biology: [Education resources](#) and [Collaborative Resources for Learning Developmental Biology](#).
- Genetics Society of America: [Primer in genetics](#).
- HHMI: [BioInteractive resources](#).
- Michael Barresi lab: [Developmental Tutorials](#) and [Developmental Documentaries](#).
- iBiology Research Talks: [Development and Stem Cells](#).

Teaching resources for educators

- American Society for Cell Biology: [Getting started in teaching](#) and [Education Toolkit](#).
- EuroStemCell: [Stem Cell Teacher Tool Kit](#).
- Genetics Society of America: [Core concepts](#) and [Education resources](#).

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Module 1: Introduction to Stem Cell Biology

This image depicts neural cells that have differentiated from cells derived from the first human embryonic stem cell (hESC) line. Nuclei stained in blue and cytoskeleton proteins involved in axon generation and nerve cell development stained in green (Tubulin Beta 3 Class III) and red (Doublecortin).
Credit: Michael Schwartz and Chris Barry, Thomson Lab, Morgridge Institute for Research, USA.

Introduction and Learning Objectives:

The first module, “Introduction to Stem Cell Biology,” will provide students with the building blocks necessary for understanding, examining, and dissecting the dynamic field of stem cell research. This course will delve into the basic research that uncovers the molecular mechanisms and cell biology of stem cell properties and functions as well as translational research and applications of stem cell science to human health. This first module will cover the fundamentals of stem cell biology necessary for progressing to more advanced core concepts.

At the conclusion of this module students should be able to:

- Define what characterizes a stem cell;
- List and compare different types of stem cells;
- Describe the stem cell niche and its role on stem cell regulation;
- Explain stem cell differentiation in vivo and in vitro;
- Summarize different types of pluripotent stem cells and how they are induced;
- Analyze key experiments that define pluripotency;
- Design future experiments based on a data figure from a scientific paper.

1. What defines a stem cell? (Becker et al., 1963; Till and McCulloch, 1961)

- a. Stem cells can divide and renew through cell division for long periods of time;
 - b. Stem cells are an unspecialized population;
 - c. Stem cells can give rise to specialized cells after division in a process called differentiation.
-

2. Types of stem cells (Jaenisch and Young, 2008)

- a. Totipotent – cells can generate the full embryo plus the placenta and extraembryonic tissues;
 - b. Pluripotent – cells can generate all cells in the embryo (embryonic stem cell (ESC), induced pluripotent stem cell (iPSC)). Key related experimental techniques:
 - i. Tetraploid embryo complementation (Nagy et al., 1990);
 - ii. Embryo chimerism (Bradley et al., 1984);
 - c. Multipotent – can become more than one type of specialized cell (e.g., hematopoietic stem cell (HSC));
 - d. Unipotent – can only give rise to one type of specialized cell (e.g., muscle stem cell);
 - e. Progenitor – An intermediate cell type between stem cells and differentiated cells. Potential to give rise to a limited number or type of specialized cells and have a reduced capacity for self-renewal.
-

3. Stem cells in vivo

- a. Differentiation by tissue-specific stem cells: HSC (Orkin and Zon, 2008), germline stem cells (Spradling et al., 2011);
 - b. Dedifferentiation and regeneration in response to injury and tissue homeostasis (Brawley and Matunis, 2004);
 - c. Stem cell niche – what it is and how it regulates stem cells (Schofield, 1978);
 - d. Aging and senescence and stem cell biology (Conboy et al., 2005).
-

4. Stem cells in vitro

- a. Reprogramming (Gurdon et al., 1958);
- b. Experimental derivation of ESCs: [mouse (Martin, 1981; Evans and Kaufman, 1981); human (Thomson et al., 1998); Key related experimental techniques:
 - i. Teratoma formation;
 - ii. Embryoid bodies;
- c. Experimental derivation of iPSCs (Takahashi and Yamanaka, 2006);
- d. Directed differentiation into specialized cell types (Wichterle et al., 2002);
- e. Multipotent cells: Adult stem cells in culture such as Neuroprogenitor cells (NPCs), Intestinal Stem Cell (ISCs) Mesenchymal Stem Cells (MSCs) that give rise to restricted progeny (Sato et al., 2009).

► [View Module 1 Bibliography](#)



Module 2: Introduction to Development

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The transparent zebrafish, shown here at 22 hours post-fertilization, is a wonderful model system that allows researchers to study development as it is happening in an intact embryo. Credit: Keller Lab, Janelia Farm Research Campus, HHMI, USA and NIGMS.

Introduction and Learning Objectives:

This module introduces the underlying developmental biology principles from fertilization to differentiated cell types, starting from the archetypal totipotent cell - the fertilized embryo. Students will learn about *in vivo* pluripotency and the changes that take place, from fertilization and gastrulation to cell fate commitment in the early embryo. At the conclusion of the module, students will understand how a cell with the potential to become any cell type in the body differentiates into various tissues with unique identities and functions. There is a particular focus on organizing centers, signaling molecules, and transcription factors controlling differentiation trajectories. Throughout this module students will review landmark developmental processes during embryonic development to contextualize stem cells and differentiation strategies.

At the conclusion of this module students should be able to:

- Describe the early stages of fertilization and development across multiple species;
- Identify the source of mammalian ESCs and extra-embryonic tissues;
- Describe the importance and cell movements of gastrulation, and compare and contrast gastrulation in different model organisms (frog, zebrafish, chicken, mouse);
- Identify different germ layers in development and their derivatives;
- Recognize the distinctions between totipotency, pluripotency, and multipotency;
- Summarize mechanisms that regulate differentiation;
- Compare and contrast how embryonic organizers, morphogens, and transcriptional signaling pattern the early embryo;
- Apply how these phenomena learned in this module may be used *in vitro*.

1. Early development: fertilization, totipotency, and pluripotency (Mitalipov and Wolf, 2009)

- a. Mammalian fertilization (Clift and Schuh, 2013; Okabe, 2013);
- b. The totipotent zygote divides to form the blastocyst comprising the inner cell mass and trophectoderm (Niakan et al., 2012);
- c. The inner cell mass is the source of pluripotent embryonic stem cells (Boroviak et al., 2014; Martello and Smith, 2014).

2. Gastrulation and lineage commitment in the early embryo

- a. The three germ layers and body plan are established during gastrulation (Gadue et al., 2005; Solnica-Krezel and Sepich, 2012);
- b. Cell movements of gastrulation are conserved across vertebrates (Solnica-Krezel, 2005);
- c. Ectoderm specification (Li et al., 2013; Osteil et al., 2019);
- d. Mesoderm specification (Ciruna and Rossant, 2001);
- e. Endoderm specification (Lewis and Tam, 2006).

3. Mechanisms of cell fate determination in vivo

- a. Morphogens: graded positional cues driving cell specification (Ashe and Briscoe, 2006);
- b. Transcription factors and cell signaling in fate determination (Tam and Loebel, 2007);
- c. Organizers in development (Martinez Arias and Steventon, 2018; Martyn et al., 2018).

4. Specification and development of primordial germ cells

- a. Regulation of germ cell specification (Lawson et al., 1999; Ohinata et al., 2005);
- b. Germ stem cell-specific epigenetic reprogramming (Sasaki and Matsui, 2008);
- c. Primordial germ cell migration (Richardson and Lehmann, 2010).

► [View Module 2 Bibliography](#)

Module 3: Pluripotency and Reprogramming in Vitro

H1 pluripotent human embryonic stem cells (hESCs), the first hESC line ever derived, grown in culture. These stem cell colonies are pluripotent and can be coaxed to become a myriad of cell types. Credit: Chris Barry, Thomson Lab, Morgridge Institute for Research, USA.

Introduction and Learning Objectives:

Module 3 dives into different types of pluripotent stem cells. Students will learn what an embryonic stem cell is, how it is derived, and what makes them pluripotent. There is a special emphasis on defining pluripotency by molecular means and differentiation potential. Understanding pluripotency will unlock reprogramming and the generation of induced pluripotent stem cells. The module ends with an overview of the possible clinical application of pluripotent stem cells as a window into their medical and research potential. Additionally, this module contains an extra set of topics for advanced students interested in pluripotent stem cell biology.

At the conclusion of this module students should be able to:

- Identify defining pluripotent stem cell features;
- Define the principles of reprogramming;
- Design experiments to test if cells are pluripotent;
- Describe the embryonic stem cell origin;
- Understand cellular features that sustain pluripotency;
- Develop tools to evaluate stem cell potential;
- Compare different pluripotent stem cells and strategies for obtaining them;
- Evaluate which diseases and conditions can be modeled using iPS cells;
- Evaluate the use of different types of pluripotent stem cells in experiments or in the clinic.

1. Establishment of embryonic stem cells (ESCs)

- a. Establishment of ESCs from mouse and human (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1998);
 - b. Establishment of ESCs from other species: non-human primate, rat, pig, and fish (Li et al., 2008; Notarianni et al., 1991; Thomson et al., 1995; Wakamatsu et al., 1994);
 - c. Differences between ESCs of different species (Ginis et al., 2004).
-

2. Characterization of pluripotent stem cells (PSCs)

- a. Developmental potential: Teratoma/Chimerism/Germline transmission/Tetraploid complementation (Carpenter et al., 2003; Czechanski et al., 2014);
 - b. Cell-cycle and high telomerase activity (Marion et al., 2009; White and Dalton, 2005);
 - c. Cellular metabolism (Mathieu and Ruohola-Baker, 2017);
 - d. Transcriptome profiling (Richards, 2004);
 - e. DNA methylation: Hypomethylated imprinted genes/both X-chromosomes activated (Altun et al., 2010).
-

3. Molecular mechanisms underlying pluripotency

- a. Intrinsic core pluripotent transcription factors (TFs) and networks (Young, 2011);
 - b. Signaling and metabolic pathways essential for PSC maintenance (Ying et al., 2003);
 - c. “Open” epigenetic architecture (Meshorer and Misteli, 2006);
 - d. Bivalency and poised chromatin (Azuara et al., 2006).
-

4. Induction of pluripotency

- a. Cell fusion (Chad et al., 2005);
 - b. Somatic cell reprogramming using TFs (Stadtfield et al., 2010; Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007);
 - c. Induced pluripotency stem cells (iPSCs) vs ESCs (Bilic and Izpisua Belmonte, 2012; Stadtfield et al., 2010).
-

5. Potential of induced pluripotent stem cells (iPSCs) in basic and clinical applications

- a. Disease modeling (Robinton and Daley, 2012);
- b. Drug screening (Cohen and Melton, 2011);
- c. Gene-editing and regenerative medicine (Robinton and Daley, 2012).

6. Alternative PSCs

- a. Naïve and Ground state pluripotency (De Los Angeles et al., 2012; Ying et al., 2008);
- b. Epiblast PSCs (Brons et al., 2007; Tesar et al., 2007);
- c. Formative PSCs (Smith, 2017);
- d. Differences between the “alternative” and “primed” states of pluripotency (Liu et al., 2017; Tonge et al., 2014; Wu et al., 2015).

7. Reprogramming using defined factors

- a. Reprogramming cocktails (Cohen and Melton, 2011; González et al., 2011);
- b. Delivery modes: Integrating vs non-integrating, virus, mRNA, proteins or chemicals (González et al., 2011; Plath and Lowry, 2011);
- c. Reprogramming of other species (Ezashi et al., 2016; Wunderlich et al., 2014);
- d. Epigenetic memory (Kim et al., 2010; Kim et al., 2011; Lister et al., 2011; Polo et al., 2010).

8. Mechanisms of reprogramming

- a. Kinetics of reprogramming: multi-step process, erasure of somatic transcriptional program and establishment of pluripotency (Buganim et al., 2013; Cacchiarelli et al., 2015; Chronis et al., 2017; Maherali et al., 2007; O'Malley et al., 2013; Polo et al., 2012; Theunissen and Jaenisch, 2014);
- b. Stochastic vs deterministic reprogramming (Guo et al., 2014; Liu et al., 2016);
- c. Efficiency and barriers of reprogramming (Toh et al., 2016).

► [View Module 3 Bibliography](#)



Module 4: Adult Stem Cells and Regeneration

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The *Drosophila* ovary, in purple, contains three types of adult stem cell (germline stem cell (GSC), somatic stem cell (SSC) and escort stem cell (ESC)), which work together to form the oocyte. Credit: Hogan Tang and Denise Montell, Johns Hopkins University and University of California, Santa Barbara, USA, NIGMS.

Introduction and Learning Objectives:

Module 4 concentrates on adult stem cells and their function. Students will be exposed to adult stem cells and how they maintain tissues during homeostasis and in response to injury, contextualizing the role of endogenous adult stem cells. The module will explore differences in adult stem cell differentiation potential across the animal kingdom and various tissues. Moreover, students will appreciate how, depending on their potency, adult stem cells play diverse functions across species. Finally, the module introduces transdifferentiation and *in vitro* and *in vivo* mechanisms to change cell fate.

At the conclusion of this module students should be able to:

- Describe different types of adult stem cells and how they were experimentally identified;
- Design an experiment to test whether a newly identified cell is an adult stem cell;
- Explain the role of adult stem cells in tissue homeostasis;
- Categorize adult stem cell potency across different types of mammalian tissues;
- Compare regenerative potential across species;
- Hypothesize why the regenerative capacity of adult stem cells varies widely among different tissues and species;
- Design experiments to test whether unique characteristics of highly regenerative adult stem cells could be applied to enhance regeneration of adult stem cells with little ability to regenerate;
- Develop testable hypotheses around how adult stem cells could be used in the clinic.

1. Tissue regenerative capacity

- a. Homeostasis by progenitor cell division:
 - Pancreas (Dor et al., 2004);
 - Liver (Yanger et al., 2014);
- b. Homeostasis by stem cell proliferation and differentiation:
 - Intestine (Barker et al., 2007);
 - Skin (Blanpain et al., 2004);
 - Sperm (Brinster and Avarbock, 1994);
 - Skeletal (Chan et al., 2018);
 - Brain (Kempermann et al., 2018).

2. Regeneration in planaria, zebrafish, axolotl, and mammals

- a. Planaria:
 - Polarity (Gurley et al., 2008; Morgan, 1904; Petersen and Reddien, 2008);
 - Functional unit of regeneration (Wagner et al., 2011; Zeng et al., 2018);
 - Positional information and regeneration (Scimone et al., 2017);
- b. Zebrafish
 - Fin (Johnson and Weston, 1995; Singh et al., 2012);
 - Heart (Gupta et al., 2012; Poss et al., 2002);
 - Brain (Kizil et al., 2012);
 - Spinal cord (Becker et al., 1997);
- c. Axolotl:
 - Initiating signal (Mescher, 1976; Sugiura et al., 2016);
 - Dedifferentiation (Gerber et al., 2018; Kragl et al., 2009; Sandoval-Guzman et al., 2014);
 - Developmental program is redeployed for regeneration (Nacu et al., 2016);
 - Immune system (Godwin et al., 2013);
- d. Mammals:
 - Heart (Porrello et al., 2011);
 - Digit tip (Lehoczky et al., 2011; Takeo et al., 2013; Yu et al., 2010);
 - Hippocampus (Toda and Gage, 2018).

3. Facultative stem cells

- a. Muscle (Moss and Leblond, 1971).

4. Transdifferentiation

- a. Heterokaryons (Blau et al., 1983);
- b. Reprogramming with single transcription factor (Davis et al., 1987);
- c. In vivo reprogramming (Zhou et al., 2008).

5. Dedifferentiation and plasticity

- a. Intestinal stem cells and dedifferentiation (Buczacki et al., 2013; Van Es et al., 2012);
- b. Lung stem cells and dedifferentiation (Tata et al., 2013).

▶ [View Module 4 Bibliography](#)

Module 5: Directed Differentiation and Transdifferentiation

Two human iPSC-derived neurospheres send out neuronal processes to contact each other. Neurons in green and nuclei in red. Credit: Muotri Lab, University of California, San Diego, CA, USA.

Introduction and Learning Objectives:

Building on basic developmental biology knowledge, students will delve more deeply into how cells differentiate, both in vivo (during development and in the adult) and in vitro. Students will be introduced to different in vitro differentiation strategies with specific examples. Specifically, students will learn about directed differentiation by signaling molecules and direct programming by genetic means. This module will also explore using directed differentiation in regenerative medicine and the application of techniques such as organoids, chimeras, and drug screening. The examples will also convey different strategies for benchmarking differentiation success.

At the conclusion of this module students should be able to:

- Define differentiation and programming;
- Compare and contrast specification, commitment, and differentiation;
- Define a cell progenitor;
- Understand differentiation trajectories in vivo and in vitro;
- Extrapolate developmental biology concepts to stem cell differentiation in vitro;
- Compare differentiation strategies;
- Critique differentiation outcomes;
- Evaluate whether in vitro cell differentiation is comparable to in vivo counterparts.

1. In vivo differentiation

- a. How stem cells give rise to many specialized cells in the body during development;
- b. How stem cells give rise to tissue-specific cells in fetal and adult development;
- c. Differentiation and the stem cell niche.

2. Specification during development

- a. From a single cell to all cells;
- b. Progenitor cells - An intermediate cell type between stem cells and differentiated cells. Potential to give rise to a limited number or type of specialized cells and have a reduced capacity for self-renewal;
- c. Signaling and gradient during development (see also module 2).

3. Specification in adults

- a. Tissue-specific stem cells and differences among different tissue;
- b. Dedifferentiation and regeneration (see also module 4);
- c. Aging and senescence.

4. In vitro differentiation (Thomas and Capecchi, 1987)

- a. How to measure potency: teratoma, embryoid bodies, chimeras (Höpfl et al., 2004; Mascetti and Pedersen, 2016);
- b. Looks like, smells like, seems like a neuron, but is this a neuron (Wichterle et al., 2002);
- c. Transcription factors and directed differentiation (Eiraku et al., 2008);
- d. Regenerative medicine, ex vivo and in vivo (Cohen and Melton, 2011);
- e. Applications: disease modeling (2D vs 3D vs Chimeras), drug screening (Kroon et al., 2008).

5. Transdifferentiation and direct programming

- a. Differentiated cells can be transdifferentiated into other cell types (Aydin and Mazzoni, 2019; Srivastava and DeWitt, 2016) (see module 7).

► [View Module 5 Bibliography](#)



Module 6: Leveraging Tools to Study Stem Cell Biology

A cross-section of a human brain organoid, showing different cell populations, neurons (green), progenitor cells (red), radial glia cells (white), nuclei in blue. Credit: Muotri Lab, University of California, San Diego, CA, USA

Introduction and Learning Objectives:

Module 6 reviews the cutting-edge technologies that can be applied to interrogate stem cells to further their experimental use and clinical potential. Students are presented with genetic and cellular tools that enhance cell differentiation strategies. This module will explore different mechanisms for gene editing and cell engineering and discuss chimeric embryo models and their uses. Finally, the module stresses the promise of omics technologies, single-cell assays, and bioinformatics tools that elevate the use and analysis of stem cell-based platforms for the clinical setting and fundamental discoveries. After this module, students will be equipped to analyze stem cells experimentally and better understand how they can be used to further understand basic biology or enhance regenerative medicine.

At the conclusion of this module students should be able to:

- Compare different methods of genome engineering;
- Synthesize omics technologies and how they help to dissect cellular heterogeneity;
- Appreciate the power and limitations of computational methods in analyzing omics data;
- Explain animal chimerism and define its importance as a model system;
- Couple genetic and cellular technologies to solve experimental queries;
- Weigh the justification of genetic modifications in different experimental settings;
- Use these tools to revamp classic developmental biology experiments and design new testable hypotheses.

1. Editing the stem cell genome

- a. Gene editing basics, focusing on Cas technologies (Knott and Doudna, 2018);
- b. Editing pluripotent or adult stem cells (Deuse et al., 2019);
- c. Cell engineering for in vitro disease modeling (Arias-Fuenzalida et al., 2017; Zhang et al., 2017);
- d. Germ cell editing (Chapman et al., 2015).

2. In vivo tools in stem cell biology

- a. Humanized mouse models (Zhao et al., 2015);
- b. Animal chimeras (Masaki and Nakauchi, 2017);
- c. Genetic modification and chimeras (Kobayashi et al., 2010; Matsunari et al., 2020).


3. Computational tools to dissect stem cell heterogeneity

- a. Single-cell omics methodologies (Kumar et al., 2017);
- b. Single-cell imaging (Rompolas et al., 2012);
- c. Computational methods (Trapnell et al., 2014).

4. In vitro cultures of adult stem cells to analyze differentiation capacity

- a. Adult stem cell-derived organoids (Sato et al., 2009);
- b. Directed differentiation of adult stem cell-derived organoids (Yin et al., 2014);
- c. Modelling the stem cell lineage selection and plasticity using organoids (Kretzschmar and Clevers, 2016).

► [View Module 6 Bibliography](#)



Module 7: Clinical Applications of Stem Cell Biology

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Stem cell-derived motor neurons and glia, stained for the neuronal cytoskeleton. These cells are used to study why neurons die during neurodegeneration. Credit: Disi An, Mazzoni Lab, New York University, USA.

Introduction and Learning Objectives:

Module 7 discusses how stem cell technologies can be leveraged for clinical applications. The module builds upon developmental biology concepts applied to stem cell differentiation, transdifferentiation, and direct reprogramming for therapeutic purposes. It reinforces the advantage of embryonic and adult stem cell technologies for in vitro and in vivo disease modeling. Specific examples are provided for stem cell applications, from using them to compare normal and pathological states to uncovering novel treatments to cell replacement therapies. Finally, students will be presented with an introduction to ethical, business, and legal frameworks related to stem cells.

At the conclusion of this module students should be able to:

- Define different methods for using stem cells to study and treat disease;
- Distinguish disease modeling from cell replacement;
- Compare the use of pluripotent or adult stem cell strategies to treat different disease scenarios;
- Calculate potential risks of utilizing stem cells as a therapeutic strategy;
- Formulate a workflow for how one would apply these techniques to a new disease;
- Identify what tests must be completed before a new cell therapy can be considered for clinical application;
- Debate whether there are some diseases for which cell replacement therapy would not work.

1. Overview of the clinical application of stem cell technologies (Fowler et al., 2020; Shi et al., 2017)

- a. Stem cells are ideal cells for clinical applications since they can regenerate and differentiate;
- b. The clinical translation process of stem cell technologies;
- c. Cell replacement therapies;
- d. Disease modeling;
- e. Drug screening;
- f. Personalized medicine.

2. Pluripotent stem cell differentiation

- a. ESCs and iPSCs can be differentiated into patient-affected cell types (Dimos et al., 2008; Velasco et al., 2019);
- b. Comparison of health and pathogenic phenotypes: e.g., direct differentiation (ALS) and organoids (autism spectrum disorder) (Kiskinis et al., 2018; Mariani et al., 2015);
- c. Cell replacement therapies from pluripotent cells: dopaminergic neurons or age-related macular degeneration (Barker et al., 2017; Kikuchi et al., 2017).

3. Transdifferentiation and direct programming

- a. Differentiated cells can be transdifferentiated into other cell types (Aydin and Mazzoni, 2019; Srivastava and DeWitt, 2016);
- b. In vitro transdifferentiation: fibroblast to neuron (diseases and aging) (Li et al., 2015);
- c. In vivo transdifferentiation: cardiac fibroblasts into cardiomyocytes (or dopaminergic neurons) (Qian et al., 2012; Rivetti Di Val Cervo et al., 2017).

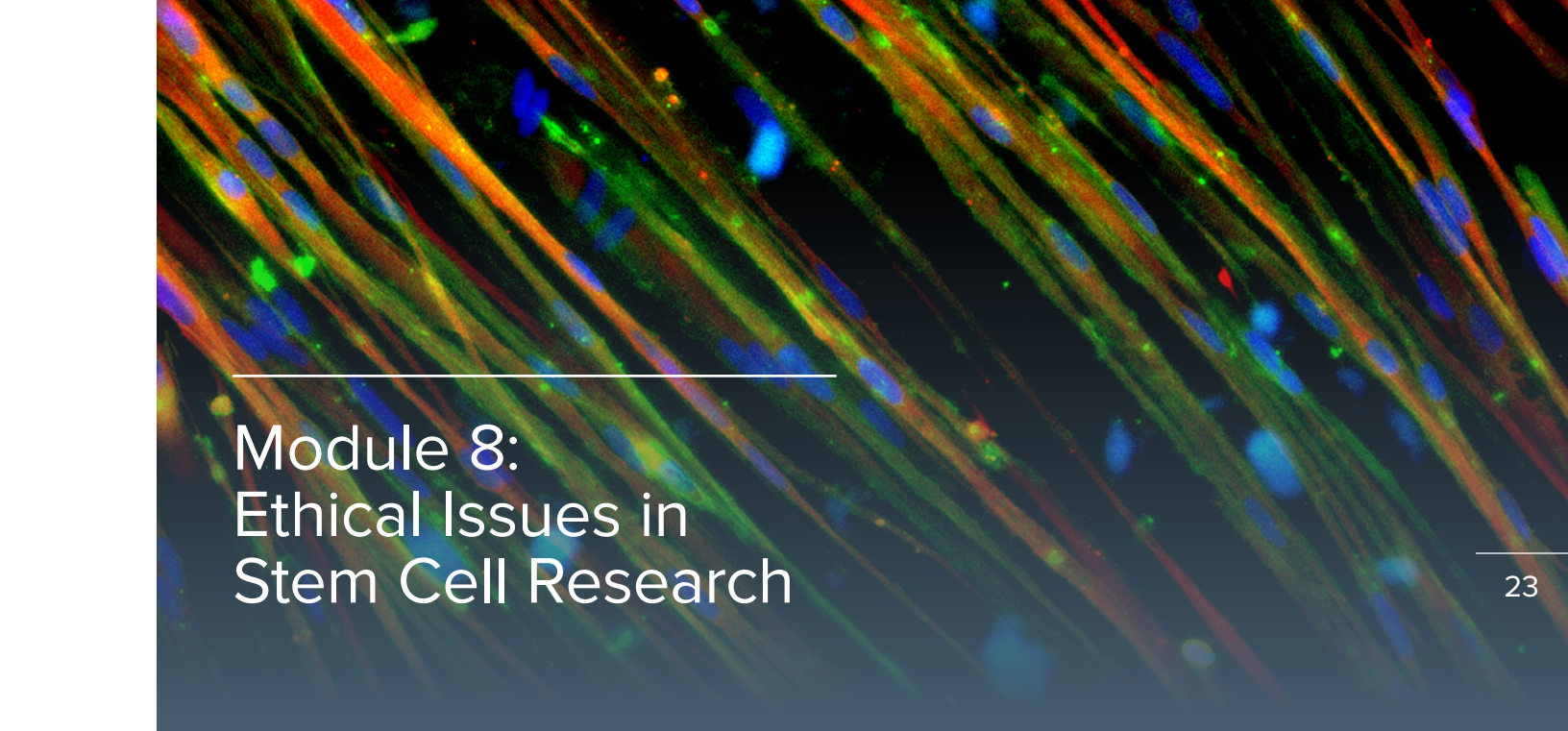
4. Adult stem cell disease modeling and therapy

- a. Adult stem cells as a source of differentiated cells for modeling (Pellettieri and Alvarado, 2007);
- b. Modeling tissues: Intestinal organoids for cystic fibrosis (Dekkers et al., 2013; Sugimoto and Sato, 2017; Vlachogiannis et al., 2018);
- c. Modeling tissues-pathogen: Brain organoids for Zika infections (Cugola et al., 2016; Zhou et al., 2017);
- d. Bone Marrow Transplant ([Bone marrow transplant](#), Mayo Clinic);
- e. Cell replacement strategy: cornea replacement (Rama et al., 2010).

5. Stem cell therapy guidelines and clinical trials

- a. [Guidelines](#) (Cossu et al., 2018, ISSCR 2016);
- b. Stem cell clinical trials ([EuroStemCell Getting involved: Clinical Trials and Patient Registries](#); A database of privately and publicly funded clinical studies conducted worldwide: [ClinicalTrials.gov](#); [Stem Cell Clinical Research DB](#)).

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Module 8: Ethical Issues in Stem Cell Research

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CRISPR/Cas9 gene editing technology was used to restore a mutation in muscle cells derived from human iPS cells. Dystrophin (in green) was restored in muscle cells with Duchenne muscular dystrophy (DMD), a technology that could help up to 60% of patients with DMD. Myosin is stained in red and nuclei in blue. Credit Spencer lab, University of California, Los Angeles, USA, and NIAMS.

Introduction and Learning Objectives:

Module 8 introduces the critical ethical, legal, and social implications of stem cell and regenerative-based technologies. As the ethical, legal, and social issues have received significant attention from the public, professionals, and policy makers, it is important for learners to recognize and consider these issues as part of their research and training. Understanding these issues will help each scientist appreciate the ethical, legal, and social aspects of their research and their place within it. Students will discuss the ethics and policies surrounding human embryos, egg donation, gene editing, unproven stem cell interventions, fetal tissue research, and the responsible communication of science to the public. Given the rapid pace at which the field is evolving, the overarching goal of this module is to provide the building blocks on which to evaluate new technologies as they are developed.

At the conclusion of this module students should be able to:

- Appreciate the nuances between ethical theories surrounding the moral status of human embryos;
- Describe ethical and social concerns in egg donation;
- Distinguish the ethics of somatic versus germline gene editing for therapeutic and enhancement purposes;
- Evaluate the individual risks associated with premature commercialization of stem cell clinical applications;
- Consider the equitable access to stem cell treatments for patients with serious diseases;
- Discuss the implications of novel stem cell technologies to the public in a sensible way balancing its potential and avoiding hype.

1. Embryo ethics (Master, 2018)

- a. Human embryonic stem cells (hESCs) are derived from human embryos, which leads to the destruction of the embryo. There are several theoretical viewpoints on the moral status of human embryos, which may confer them certain protections.
 - Consideration 1: Embryos have full moral status (personhood) at the moment of conception and would be provided full protection from destruction.
 - Consideration 2: Embryos obtain personhood gradually throughout development to be a member of the moral community and appreciate a life similar to ours (sometimes known as a “future like ours” argument) and thus could be afforded protection from being destroyed.
 - Consideration 3: Persons need at least some cognitive capacities, e.g., sentience or reasoning, to be considered a person with full moral protections. Based on this argument, embryos may be used to derive hESCs. Some of these debates are now applied in the context of neural organoid technology.
 - Consideration 4: Embryos do not need moral status to have some protections. Human embryos are symbolic of human life and deserve respect. This may mean they could be used for socially laudable goals, i.e., hESC derivation, but may not be used for frivolous activities e.g., making jewelry.
- b. Research Ethics Issues Surrounding iPSCs and Other Stem Cells.
 - Protecting donors’ privacy and ensuring adequate informed consent.
 - Manufacturing and ensuring safety of iPSC lines for clinical applications

2. Ethics of egg donation (Bracewell-Milnes et al., 2018; Haines et al., 2013) ([Guidelines for the Conduct of Human Embryonic Stem Cell Research, 2006](#); [ISSCR Sample Informed Consent: Egg donation for stem cell research: provided directly and solely for stem cell research.](#))

- a. Derivation of hESCs from human embryos or deriving hESCs after somatic cell nuclear transfer requires eggs from women.
- b. Physical harms: If a large supply of eggs is needed, there may be physical harm to women from ovarian stimulation and egg procurement.
- c. Social harms: There may also be social harms to egg donors, including the solicitation and exploitation of women to exchange money for ova. This may be especially challenging for economically disadvantaged women.
- d. Egg donor perceptions: Present the dominant themes from studies examining the perceptions of egg donors.

integrity
ethics germline
responsible communications
misinformation gene editing responsible research
hype stem cell
regenerative medicine therapy
technology policy enhancement

It is critical for stem cell scientists to understand the ethical, legal, and social implications of stem cell and regenerative-based technologies.

3. Ethics of gene editing

- a. Under what circumstances can gene editing be performed? Two major concepts in gene editing are enhancement vs therapy distinction and performing somatic vs. germline interventions (Meagher et al., 2020).
- b. Enhancement vs. Therapy: Can gene editing be used to enhance human capabilities, or should it be used only for therapeutic purposes? The distinction between therapy and enhancement is not always clear (Human genome editing: Science, ethics, and governance, 2017; Juengst, 2017).
- c. Somatic vs. Germline: Similarly, should only somatic gene editing be performed given the safety considerations of changing a gene in a single person as opposed to germline modification, which would be passed onto future generations? Here, issues surrounding intergenerational justice deserve consideration (Human genome editing: Science, ethics, and governance, 2017; Juengst, 2017).
- d. Public perceptions and policies: Present the dominant themes from studies examining public perceptions on gene editing (Human genome editing: Science, ethics, and governance, 2017; Isasi et al., 2016; Juengst, 2017).

4. Premature translation of stem cell interventions

- a. Features of the unproven stem cell intervention (SCI) industry: describe the market size and growth, marketing practices of clinics, patient perspectives, clinician qualifications, and harms to patients and society (Fu et al., 2019; ISSCR, 2016; Tanner et al., 2017; Turner and Knoepfler, 2016, Turner, 2017).
- b. Misinformation and showcasing scientific legitimacy of unproven SCIs: Many marketing practices attempt to showcase unproven SCIs as scientifically legitimate, including the use of low quality or irrelevant scientific publications, celebrity testimonials, registering unproven SCIs as pay-to-participate trials in ClinicalTrials.gov, association with reputable scientific institutions, and seeking research ethics approval (Hawke et al., 2019; Marcon et al., 2017; Sipp et al., 2017) ([Stem Cell-Based Clinical Trials: Practical Advice for Physicians and Ethics/Institutional Review Boards](#), 2018; [Informed Consent Standard for Stem Cell-Based Interventions Offered Outside of Formal Clinical Trials](#), 2019).
- c. Efforts to curtail the unproven SCI industry: There have been many proposals to better inform patients about the unproven SCI industry and efforts to better enforce providers (ISSCR, 2016; Sipp et al., 2017).

5. Fetal tissue (Fox and Cohen, 2019; Goldstein, 2020; [Human Fetal Tissue: a Critical Resource for Biomedical Research, 2017](#)).

- a. Ethics of using fetal tissue from abortuses have similar moral concerns to the use of human embryos, and many are divided on the use of fetal tissues.
- b. Outline the scientific value of fetal tissue research for studying the detrimental effect of Zika virus on brain development, developing therapies to treat HIV, using fetal ventral mesencephalic tissue to treat Parkinson's disease, and using fetal tissue for ALS research. In addition, outline the pros and cons of current alternatives for fetal tissue research.

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6. Access to future stem cell therapies (Chan, 2017; Hogle, 2017).

- a. A potential role for scientists? Should scientists be cognizant of and consider attempts at reducing costs when developing stem cell interventions? Current cellular therapies may be quite costly for patients and, depending on their efficacy, may not be covered by private insurers.
- b. Balancing scientific evidence and the needs of patients: Should premature access of unproven SCIs be permitted under certain conditions to out-of-options patients? And if so, what would these conditions be? There is a notable social movement worldwide of reducing regulations to speed up the development and market clinical interventions and ensuring preapproval access to potentially helpful products, e.g., the Right-to-Try movement.

7. Responsible communication of science to the public (ISSCR, 2016) ([AAAS Communication Kit](#)).

- a. Hype and stem cell research: There has been considerable discussion regarding the hype surrounding stem cell research, fueling ethical debates. Some of this hype has been created by scientists. The public's understanding of science comes primarily from various media and news sources, including social media.
- b. Public communication of research: Scientists should ensure that their communication surrounding stem cell science is based on the data. How the science is applied to the clinic and prediction of scientific or clinical futures of the research, need to be tempered appropriately to prevent hype. Communication strategies to emphasize that studies were performed in animal models and/or that greater clinical testing is needed before interventions can be marketed is important to convey when discussing scientific discoveries to the public.

► [View Module 8 Bibliography](#)

Accessing Referenced Literature

Where possible we have included scientific articles that have Open Access availability. Unfortunately, some of the foundational literature that supports the core concepts of stem cell biology are not freely available. We have compiled the following suggestions for obtaining access to these papers.

HOW TO SEARCH FOR SCIENTIFIC LITERATURE:

There are multiple search engines that you can use to look for scientific papers. We recommend using [PubMed](#) to look up papers of interest. Copy the article title into the search bar or hit advanced to add additional search terms.

If the paper is Open Access there will be a button that says “Open Access” – press this button to be directed to a free version of the paper. Many papers must have a publicly available copy. Look for a button that says “PMC Full Text Free.” This version may be slightly different than the version that is ultimately published but should contain the core content. You can also go directly to the journal that published the paper to search for the article.

IF THERE IS NO FREE VERSION OF THE PAPER AVAILABLE:

Many institutions and libraries have journal subscriptions. Check with institutions with which you have an affiliation, local academic libraries, or local public libraries to see if they have access to the journal article through their journal subscriptions. If an electronic version is not immediately available, you can often request one through inter-library loan.

Alternatively you can directly contact the corresponding author and ask them to share the manuscript.

ADDITIONAL RESOURCES:

- [How to Get a Journal Article](#), which includes suggestions for finding articles for free.
- [Free Biomedical Literature Resources](#)
- Add the [Google Scholar extension](#) to your Chrome browser. This tool will search for all available versions of the article, including freely available versions.
- [Unpaywall.org](#) harvests Open Access journal articles from publishers and repositories and also offers a browser extension to search for free journal articles.
- [Guide to Open Access journals](#)
- [Resources for Finding and Accessing Scientific Papers](#)

Bibliography and Selected Foundational Literature Annotations

MODULE 1: INTRODUCTION TO STEM CELL BIOLOGY

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Becker, A. J., McCulloch, E. A. and Till, J. E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*.

Bradley, A., Evans, M., Kaufman, M. H. and Robertson, E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature*.

Brawley, C. and Matunis, E. (2004). Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science*.

Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weismann, I. L. and Rando, T. A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*.

Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature*.

Gurdon, J. B., Elsdale, T. R. and Fischberg, M. (1958). Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*.

All cells in the body derive from a single cell, the zygote. As development proceeds, cells undergo progressive restrictions in their fate. It was unclear whether these restrictions were due to differential gene expression or irreversible changes that occur in the DNA. Following work from Briggs and King, John Gurdon modified their experimental techniques and used somatic cell nuclear transfer (or cloning) to challenge the nucleus of a differentiated cell to re-direct development in *Xenopus laevis*. This is one of his earlier papers in which he used nuclei from cells derived from the three different embryonic germ layers taken from different stages of development up to the tadpole stage. Later studies used donor nuclei from a variety of adult cell types. Gurdon's work demonstrated the constancy of the genome, namely that most fully differentiated cells retain the ability to re-direct development. This work on nuclear reprogramming set the stage for cloning efforts by others in different species and inspired later work on induced pluripotent stem cells. John Gurdon shared the Nobel Prize in Physiology or Medicine with Shinya Yamanaka in 2012 for his accomplishments.

Jaenisch, R. and Young, R. (2008). Stem Cells, the Molecular Circuitry of Pluripotency and Nuclear Reprogramming. *Cell*.

Martin, G. R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U. S. A.*

Nagy, A., Gocza, E., Merentes Diaz, E., Prideaux, V. R., Ivanyi, E., Markkula, M. and Rossant, J. (1990). Embryonic stem cells alone are able to support fetal development in the mouse. *Development*.

Orkin, S. H. and Zon, L. I. (2008). Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. *Cell*.

Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., Van Es, J. H., Abo, A., Kujala, P., Peters, P. J., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*.

Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. A hypothesis. *Blood Cells*.

Raymond Schofield first presented the idea of the stem cell niche as a specialized microenvironment that regulates stem cell function in 1978. Schofield described a theory in which stem cells were not autonomous, but instead relied on a specific physical location that regulated the stem cell, restricted entry into the cell cycle and differentiation, maintained a state of self-renewal, and was protected from mutation. Schofield studied this in the context of hematopoietic stem cells in the bone marrow niche after Till and McCulloch first described these blood stem cells in another landmark paper (see Till and McCulloch, 1961). Schofield's work set the stage for decades of work exploring the stem cell niche in many other contexts.

Spradling, A., Fuller, M. T., Braun, R. E. and Yoshida, S. (2011). Germline stem cells. *Cold Spring Harb. Perspect. Biol.*

Takahashi, K. and Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*.

The ability to generate patient-specific pluripotent stem cells has great applications for regenerative medicine. Many attempts were made to reprogram adult cells to a pluripotent state. For example, many tried using human somatic cell nuclear transfer to generate a cloned embryo, allowing it to develop to the blastocyst stage and then isolating an embryonic stem cell line. Another attempt involved heterokaryons made from fusing an embryonic stem cell with a somatic cell from the patient. Both methods had major drawbacks and challenges. Kazutoshi Takahashi and Shinya Yamanaka reported a breakthrough to reprogram the nucleus of a fully differentiated cell by forcing the expression of key transcription factors (Klf4, Oct4, Sox2, and c-Myc). They called these reprogrammed cells induced pluripotent stem cells or iPSCs. Subsequent work tweaking the method led to a cell line that was reasonably equivalent to a pluripotent embryonic stem cell line based on a variety of different pluripotency assays. These cells could be used for various potential applications, including disease modeling, drug screening, and cellular therapy. Shinya Yamanaka shared the Nobel Prize in Physiology or Medicine with John Gurdon in 2012 for his accomplishments.

J. A. Thomson 1, J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, J. M. Jones (1998). Embryonic stem cell lines derived from human blastocysts. *Science*.

Till, J. E. and McCulloch, E. A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.*

Wichterle, H., Lieberam, I., Porter, J. A. and Jessell, T. M. (2002). Directed differentiation of embryonic stem cells into motor neurons. *Cell*.

Because of their ability to differentiate into any cell type found in the embryo or adult body, pluripotent stem cells promise to be transformative tools in medical research and clinical applications. A critical experimental step was determining how to make these stem cells exit pluripotency and differentiate into specific cell types for cellular studies and cell replacement therapies. Before this study by Wichterle et al., it was unclear how differentiating pluripotent stem cells would respond to signaling cues. This work demonstrated three critical aspects of pluripotent stem cell differentiation. First, it was determined that like differentiating cells in the embryo, differentiating pluripotent stem cells are patterned by responding to signaling molecules in a concentration-dependent manner. Second, these stem cells acquire terminal motor neuron fate progressively, further recapitulating embryonic development. Third, embryonic stem cell-derived motor neurons integrate into developing circuits when transplanted into a developing spinal cord, demonstrating that in vitro produced motor neurons are functional. These three principles that were uncovered in this landmark paper guide the vast majority of embryonic stem cell differentiation protocols.

MODULE 2: INTRODUCTION TO DEVELOPMENT

Ashe, H. L. and Briscoe, J. (2006). The interpretation of morphogen gradients. *Development*.

Boroviak, T., Loos, R., Bertone, P., Smith, A. and Nichols, J. (2014). The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. *Nat. Cell Biol.*

Embryonic stem cells (ESC) are commonly derived from the inner cell mass, but they can be propagated from any pre-implantation stage. The inner cell mass becomes segregated into primitive endoderm and preimplantation epiblast. Before this study, the exact origin of ESCs in the embryonic inner cell mass was unclear. This work presented transcriptional data demonstrating that cultured ESCs are distinct from the early inner cell mass and instead more closely resemble the preimplantation epiblast. Moreover, the authors showed that pre-implantation epiblast cells could be injected into blastocysts and successfully result in chimeric mice with germline transmission, confirming that these cells are functionally equivalent to whole ICM-derived ESCs. Thus, this work defined the epiblast as the in vivo counterpart of pluripotent ESCs.

Ciruna, B. and Rossant, J. (2001). FGF Signaling Regulates Mesoderm Cell Fate Specification and Morphogenetic Movement at the Primitive Streak. *Dev. Cell*.

Clift, D. and Schuh, M. (2013). Restarting life: Fertilization and the transition from meiosis to mitosis. *Nat. Rev. Mol. Cell Biol.*

Gadue, P., Huber, T. L., Nostro, M. C., Kattman, S. and Keller, G. M. (2005). Germ layer induction from embryonic stem cells. *Exp. Hematol.*

Lawson, K. A., Dunn, N. R., Roelen, B. A. J., Zeinstra, L. M., Davis, A. M., Wright, C. V. E., Korving, J. P. W. F. M. and Hogan, B. L. M. (1999). Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.*

Lewis, S. L. and Tam, P. P. L. (2006). Definitive endoderm of the mouse embryo: Formation, cell fates, and morphogenetic function. *Dev. Dyn.*

Li, L., Liu, C., Biechele, S., Zhu, Q., Song, L., Lanner, F., Jing, N. and Rossant, J. (2013). Location of transient ectodermal progenitor potential in mouse development. *Dev.*

The ectoderm is one of the three germ layers of the gastrulating embryo, and it gives rise to the central nervous system and the epidermis. Fate-mapping studies in mice had previously shown that discrete clusters of cells located at the defined regions in the epiblast give rise to either neurectoderm (that forms the central nervous system) or surface ectoderm (that gives rise to the epidermis). However, it was unclear whether a transient ectodermal progenitor gave rise to both neurectoderm and surface ectoderm tissues. In this study, the authors isolated anterior ectodermal tissue from defined points during and after mouse gastrulation and studied differentiation potential in culture. They found that anterior ectoderm at embryonic day (E) 7.0 had the capacity to differentiate into neurectoderm or surface ectoderm in the presence or absence of BMP4, respectively. Moreover, this potential was restricted to this embryonic stage, as the ability to become both ectodermal lineages was abrogated by E7.5. Thus, this work identified the molecular determinants that specified the ectoderm lineage and demonstrated for the first time the presence of a transient shared ectodermal progenitor pool.

Martello, G. and Smith, A. (2014). The Nature of Embryonic Stem Cells. *Annu. Rev. Cell Dev. Biol.*

Martinez Arias, A. and Steventon, B. (2018). On the nature and function of organizers. *Dev.*

Martyn, I., Kanno, T. Y., Ruzo, A., Siggia, E. D. and Brivanlou, A. H. (2018). Self-organization of a human organizer by combined Wnt and Nodal signaling. *Nature.*

In 1924, Hans Spemann and his graduate student Hilde Mangold published the remarkable discovery that a small cluster of cells in the developing amphibian embryo is capable of inducing the formation of an entirely new body plan when transplanted into a different embryo. This ability of a group of cells (collectively known as the ‘organizer’) in the developing embryo to autonomously instruct specific fates and morphogenesis in their surrounding tissue is evolutionarily conserved and has been subsequently identified in fish, birds, and rodents. However, this functional property had not been demonstrated in human embryos due to ethical limitations of working with early human embryos. This study leveraged the ability of human embryonic stem cells (hESCs) to form embryo-like ‘gastruloids’, concentric rings of each embryonic germ layer when grown on micropatterned geometric discs and stimulated with BMP4 to demonstrate human organizer activity in vitro. They found that Wnt and Activin signaling induced organizer activity and expression of the organizer-specific transcription factor GSC. Incredibly, when transplanted into chick embryos, these Wnt- and Activin-treated hESCs induced and contributed to a secondary axis and the formation of new neural tissue in the chick embryo. This discovery and the chick transplantation model set the stage for further interrogating the mechanisms of early human embryonic development.

Mitalipov, S. and Wolf, D. (2009). Totipotency, pluripotency and nuclear reprogramming. *Adv. Biochem. Eng. Biotechnol.*

Niakan, K. K., Han, J., Pedersen, R. A., Simon, C. and Pera, R. A. R. (2012). Human pre-implantation embryo development. *Development.*

Ohinata, Y., Payer, B., O’Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S. C., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A., et al. (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature.*

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Osteil, P., Studdert, J. B., Goh, H. N., Wilkie, E. E., Fan, X., Khoo, P. L., Peng, G., Salehin, N., Knowles, H., Han, J. D. J., et al. (2019). Dynamics of wnt activity on the acquisition of ectoderm potency in epiblast stem cells. *Dev.*

Richardson, B. E. and Lehmann, R. (2010). Mechanisms guiding primordial germ cell migration: Strategies from different organisms. *Nat. Rev. Mol. Cell Biol.*

Sasaki, H. and Matsui, Y. (2008). Epigenetic events in mammalian germ-cell development: Reprogramming and beyond. *Nat. Rev. Genet.*

Solnica-Krezel, L. (2005). Conserved patterns of cell movements during vertebrate gastrulation. *Curr. Biol.*

Solnica-Krezel, L. and Sepich, D. S. (2012). Gastrulation: Making and shaping germ layers. *Annu. Rev. Cell Dev. Biol.*

Tam, P. P. L. and Loebel, D. A. F. (2007). Gene function in mouse embryogenesis: Get set for gastrulation. *Nat. Rev. Genet.*

MODULE 3: PLURIPOTENCY AND REPROGRAMMING IN VITRO

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Azuara, V., Perry, P., Sauer, S., Spivakov, M., Jørgensen, H. F., John, R. M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M., et al. (2006). Chromatin signatures of pluripotent cell lines. *Nat. Cell Biol.*

Bilic, J. and Izpisua Belmonte, J. C. (2012). Concise review: Induced pluripotent stem cells versus embryonic stem cells: Close enough or yet too far apart? *Stem Cells.*

Brons, I. G. M., Smithers, L. E., Trotter, M. W. B., Rugg-Gunn, P., Sun, B., Chuva De Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A., et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature.*

Buganim, Y., Faddah, D. A. and Jaenisch, R. (2013). Mechanisms and models of somatic cell reprogramming. *Nat. Rev. Genet.*

Cacchiarelli, D., Trapnell, C., Ziller, M. J., Soumillon, M., Cesana, M., Karnik, R., Donaghey, J., Smith, Z. D., Ratanasirintrao, S., Zhang, X., et al. (2015). Integrative Analyses of Human Reprogramming Reveal Dynamic Nature of Induced Pluripotency. *Cell.*

Carpenter, M. K., Rosler, E. and Rao, M. S. (2003). Characterization and differentiation of human embryonic stem cells. *Cloning Stem Cells.*

Chad, A. C., Jocelyn, A., Douglas, A. M. and Kevin Eggan (2005). Nuclear Reprogramming of Somatic Cells After Fusion with Human Embryonic Stem Cells. *Science.*

Chronis, C., Fiziev, P., Papp, B., Butz, S., Bonora, G., Sabri, S., Ernst, J. and Plath, K. (2017). Cooperative Binding of Transcription Factors Orchestrates Reprogramming. *Cell*.

Cohen, D. E. and Melton, D. (2011). Turning straw into gold: Directing cell fate for regenerative medicine. *Nat. Rev. Genet.*

Czechanski, A., Byers, C., Greenstein, I., Schrode, N., Donahue, L. R., Hadjantonakis, A. K. and Reinholdt, L. G. (2014). Derivation and characterization of mouse embryonic stem cells from permissive and nonpermissive strains. *Nat. Protoc.*

De Los Angeles, A., Loh, Y. H., Tesar, P. J. and Daley, G. Q. (2012). Accessing naïve human pluripotency. *Curr. Opin. Genet. Dev.*

Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature*.

Mouse embryonic stem cells (ESCs) display high pluripotency and can proliferate indefinitely without entering senescence. Before 1981, it was not possible to establish mouse embryonic stem cells (ESCs) that could proliferate indefinitely in vitro. At that time, cell lines had only been obtained after teratocarcinoma formation in vivo. Successful isolation and propagation of ESCs depends on the ability to maintain them. Evans and Kaufman were the first to establish mouse ESCs in culture in 1981. These ESCs were isolated directly from mouse blastocysts by culturing inner cell masses on feeder layers of mouse fibroblasts. These ESCs were shown to possess a normal karyotype and to differentiate either in vitro or after inoculation into a mouse as a tumor in vivo and possess a normal karyotype. It was demonstrated that these mouse ESCs displayed high pluripotency and could proliferate indefinitely without entering senescence. As mouse ESCs are commonly used in stem cell and developmental biology research, this is a pivotal study that has since led to the establishment of additional mouse ESC lines in stem cells research.

Ezashi, T., Yuan, Y. and Roberts, R. M. (2016). Pluripotent stem cells from domesticated mammals. *Annu. Rev. Anim. Biosci.*

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González, F., Boué, S. and Belmonte, J. C. I. (2011). Methods for making induced pluripotent stem cells: Reprogramming à la carte. *Nat. Rev. Genet.*

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Stadtfeld, M., Apostolou, E., Akutsu, H., Fukuda, A., Follett, P., Natesan, S., Kono, T., Shioda, T. and Hochedlinger, K. (2010). Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature.*

Takahashi, K. and Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell.*

In this landmark paper, Takahashi and Yamanaka derived pluripotent cells from somatic cells, rather than from embryos. This breakthrough allows scientists to bypass the ethical issues of deriving pluripotent human embryonic stem cells from human embryos and the technical issues of tissue rejection following transplantation of cells that are not immunologically matched between donor and recipient. Here, Takahashi and Yamanaka were the first to generate induced pluripotent stem cells from mouse embryonic fibroblasts. They reprogrammed somatic stem cells by transferring defined factors that play essential roles in the maintenance of embryonic cells to induce pluripotency in somatic cells. This study found that the combination of 4 factors, Oct3/4, Sox2, Klf4, and c-Myc, is able to generate pluripotent cells directly from mouse embryonic or adult fibroblast cell cultures. It is the first mouse reprogramming study that served as an important step in inducing pluripotency, which eventually allowed the creation of pluripotent cells directly from somatic cells of patients.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell.*

Takahashi and Yamanaka demonstrated for the first time that human somatic cells could be reprogrammed into induced pluripotent stem cells (iPSCs) using the same four factors (Oct3/4, Sox2, Klf4, and c-Myc) identified in their 2006 mouse reprogramming study. Further, they determined that these iPSCs could differentiate into cell types from the three germ layers in vitro and form teratomas. These important findings demonstrated that iPSCs can be generated from adult human fibroblasts. Contemporaneously, Jamie Thomson's lab found that four factors (Oct4, Sox2, Nanog, and Lin28) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem cells. Thomson's study (Yu et al., 2007) was published in *Science* and garnered international attention for potentially ending the ethical controversy surrounding human embryonic stem cell research. *Science* later featured induced pluripotent stem cells in its "Scientific Breakthrough of the Year" article, 2008.

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Theunissen, T. W. and Jaenisch, R. (2014). Molecular control of induced pluripotency. *Cell Stem Cell.*

J. A. Thomson 1, J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, J. M. Jones (1998). Embryonic stem cell lines derived from human blastocysts. *Science.*

Human embryonic stem cells (hESCs) are undifferentiated cells that can self-renew or differentiate into most or all cell types found in the adult human body. In 1998, Thomson's lab developed the techniques for deriving and culturing stable hESC lines in cell culture. The inner cell mass cells were isolated from human blastocysts and cultured using an immunosurgery technique. The seminal work was published in

Science in 1998 and featured in its “Scientific Breakthrough of the Year” article the following year. The authors predicted that these cell lines could be useful in human developmental biology, drug discovery, and transplantation medicine and the prediction is playing out today. The breakthrough discovery underscores the importance of basic science and demonstrates how basic science can lead to applied science, clinical trials (for example, stem cell therapies), and entrepreneurship.

Thomson, J. A., Kalishman, J., Golos, T. G., Durning, M., Harris, C. P., Becker, R. A. and Hearn, J. P. (1995). Isolation of a primate embryonic stem cell line. *Proc. Natl. Acad. Sci. U. S. A.*

Toh, C. X. D., Chan, J. W., Chong, Z. S., Wang, H. F., Guo, H. C., Satapathy, S., Ma, D., Goh, G. Y. L., Khattar, E., Yang, L., et al. (2016). RNAi Reveals Phase-Specific Global Regulators of Human Somatic Cell Reprogramming. *Cell Rep.*

Tonge, P. D., Corso, A. J., Monetti, C., Hussein, S. M. I., Puri, M. C., Michael, I. P., Li, M., Lee, D. S., Mar, J. C., Cloonan, N., et al. (2014). Divergent reprogramming routes lead to alternative stem-cell states. *Nature.*

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White, J. and Dalton, S. (2005). Cell cycle control of embryonic stem cells. *Stem Cell Rev.*

Wu, J., Okamura, D., Li, M., Suzuki, K., Luo, C., Ma, L., He, Y., Li, Z., Benner, C., Tamura, I., et al. (2015). An alternative pluripotent state confers interspecies chimaeric competency. *Nature.*

Wunderlich, S., Kircher, M., Vieth, B., Haase, A., Merkert, S., Beier, J., Göhring, G., Glage, S., Schambach, A., Curnow, E. C., et al. (2014). Primate iPS cells as tools for evolutionary analyses. *Stem Cell Res.*

Ying, Q. L., Nichols, J., Chambers, I. and Smith, A. (2003). BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell.*

Ying, Q. L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature.*

Young, R. A. (2011). Control of the embryonic stem cell state. *Cell.*

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science.*

See annotation of Yu et al., 2007 with Takahashi et al., 2007.

MODULE 4: ADULT STEM CELLS AND REGENERATION

Barker, N., Van Es, J. H., Kuipers, J., Kujala, P., Van Den Born, M., Cozijnsen, M., Haegerbarth, A., Korving, J., Begthel, H., Peters, P. J., et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature.*

The small intestinal epithelium is a single layer of columnar epithelium, lining the mucosa. Within the epithelium, several differentiated cells take over special functions. The main cell types are the enterocytes that absorb nutrients, goblet cells that secrete mucus, Paneth cells that secrete antimicrobial peptides, and enteroendocrine cells that secrete hormones. The epithelium has a turnover of every 4-5 days. It was long thought that the stem cells responsible for this cell turnover reside deep in the bottom of the intestinal crypt, a repeating subunit of the intestinal epithelium. This paper identifies Lgr5, a target gene of the Wnt pathway, that specifically marks cells at the bottom of the crypt. The authors used a genetic tracing model based on the Cre-Lox system to demonstrate that these Lgr5-expressing cells can generate all cell lineages of the intestinal epithelium, defining them as stem cells. Contrary to the expectation that stem cells should be rare and quiescent, The Lgr5-expressing stem cells are abundant and frequently cycling. Subsequent studies showed that Lgr5 marks adult, tissue-resident stem cell populations in many other tissues. This paper also laid the foundation to isolate these stem cells and culture them in vitro as adult stem cell-derived organoids.

- Becker, T., Wullmann, M. F., Becker, C. G., Bernhardt, R. R. and Schachner, M. (1997).** Axonal regrowth after spinal cord transection in adult zebrafish. *J. Comp. Neurol.*
- Blanpain, C., Lowry, W. E., Geoghegan, A., Polak, L. and Fuchs, E. (2004).** Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell.*
- Blau, H. M., Chiu, C. P. and Webster, C. (1983).** Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell.*
- Brinster, R. L. and Avarbock, M. R. (1994).** Germline transmission of donor haplotype following spermatogonial transplantation. *Proc. Natl. Acad. Sci. U. S. A.*
- Buczacki, S. J. A., Zecchini, H. I., Nicholson, A. M., Russell, R., Vermeulen, L., Kemp, R. and Winton, D. J. (2013).** Intestinal label-retaining cells are secretory precursors expressing lgr5. *Nature.*
- Chan, C. K. F., Gulati, G. S., Sinha, R., Tompkins, J. V., Lopez, M., Carter, A. C., Ransom, R. C., Reinisch, A., Wearda, T., Murphy, M., et al. (2018).** Identification of the Human Skeletal Stem Cell. *Cell.*
- Davis, R. L., Weintraub, H. and Lassar, A. B. (1987).** Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell.*
- Dor, Y., Brown, J., Martinez, O. I. and Melton, D. A. (2004).** Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation. *Nature.*

Tissues in the body have various degrees of turnover. The intestinal epithelium, for example, has a relatively rapid turnover, while in other tissues, such as the liver, turnover is much slower. During this process of homeostasis, cells can be replaced by either a stem cell population or cell division from pre-existing cells. Dor et al. describe a pulse-chase system to study this process. They use a tamoxifen-inducible form of Cre recombinase (CreER) to label cells at a given time point (the “pulse”). In this paper, the insulin promoter drives the expression of CreER to label the beta cells of the pancreas. This mouse line is crossed to a Cre reporter line, in this case, with a ubiquitous promoter driving a loxP - strong transcriptional stop - loxP - human placental alkaline phosphatase construct. Based on the amount of tamoxifen they add, a certain percentage of the beta cells are labeled. This percentage is noted. They then examine the degree

of labeling efficiency after a period of time (the “chase”). If the labeling efficiency is lower, that would suggest that labeled cells die off and are replaced by an unlabeled stem cell population. If the labeling efficiency remains the same, then as labeled cells die off, they are replaced by pre-existing beta cells (some labeled, some unlabeled) by cell division. This strategy can be repeated for any cell type of interest. There are a few caveats: there must have been cellular turnover during the time of the chase, the promoter used to drive CreER must be specific to the differentiated cell population (and thus not expressed in a potential stem cell), and cells have an equal likelihood of dividing. Knowing how cells normally are replaced due to routine “wear and tear” provides important clues for efforts at manipulating the system in cases of repair or disease.

Gerber, T., Murawala, P., Knapp, D., Masselink, W., Schuez, M., Hermann, S., Gac-Santel, M., Nowoshilow, S., Kageyama, J., Khattak, S., et al. (2018). Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration. *Science*.

Godwin, J. W., Pinto, A. R. and Rosenthal, N. A. (2013). Macrophages are required for adult salamander limb regeneration. *Proc. Natl. Acad. Sci. U. S. A.*

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Gurley, K. A., Rink, J. C. and Alvarado, A. S. (2008). β -catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science*.

Johnson, S. L. and Weston, J. A. (1995). Temperature-sensitive mutations that cause stage-specific defects in zebrafish fin regeneration. *Genetics*.

Kempermann, G., Gage, F. H., Aigner, L., Song, H., Curtis, M. A., Thuret, S., Kuhn, H. G., Jessberger, S., Frankland, P. W., Cameron, H. A., et al. (2018). Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell*.

Kizil, C., Kaslin, J., Kroehne, V. and Brand, M. (2012). Adult neurogenesis and brain regeneration in zebrafish. *Dev. Neurobiol.*

Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H. H. and Tanaka, E. M. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature*.

Lehoczky, J. A., Robert, B. and Tabin, C. J. (2011). Mouse digit tip regeneration is mediated by fate-restricted progenitor cells. *Proc. Natl. Acad. Sci. U. S. A.*

Lucila Scimone, M., Cote, L. E. and Reddien, P. W. (2017). Orthogonal muscle fibres have different instructive roles in planarian regeneration. *Nature*.

Mescher, A. L. (1976). Effects on adult newt limb regeneration of partial and complete skin flaps over the amputation surface. *J. Exp. Zool.*

Morgan, T. H. (1904). An analysis of the phenomena of organic “polarity.” *Science*.

Moss, F. P. and Leblond, C. P. (1971). Satellite cells as the source of nuclei in muscles of growing rats. *Anat. Rec.*

- Nacu, E., Gromberg, E., Oliveira, C. R., Drechsel, D. and Tanaka, E. M. (2016). FGF8 and SHH substitute for anterior-posterior tissue interactions to induce limb regeneration. *Nature*.
- Petersen, C. P. and Reddien, P. W. (2008). Smed- β catenin-1 is required for anteroposterior blastema polarity in planarian regeneration. *Science*.
- Porrello, E. R., Mahmoud, A. I., Simpson, E., Hill, J. A., Richardson, J. A., Olson, E. N. and Sadek, H. A. (2011). Transient regenerative potential of the neonatal mouse heart. *Science*.
- Poss, K. D., Wilson, L. G. and Keating, M. T. (2002). Heart regeneration in zebrafish. *Science*.
- Sandoval-Guzmán, T., Wang, H., Khattak, S., Schuez, M., Roensch, K., Nacu, E., Tazaki, A., Joven, A., Tanaka, E. M. and Simon, A. (2014). Fundamental differences in dedifferentiation and stem cell recruitment during skeletal muscle regeneration in two salamander species. *Cell Stem Cell*.
- Singh, S. P., Holdway, J. E. and Poss, K. D. (2012). Regeneration of Amputated Zebrafish Fin Rays from De Novo Osteoblasts. *Dev. Cell*.
- Sugiura, T., Wang, H., Barsacchi, R., Simon, A. and Tanaka, E. M. (2016). MARCKS-like protein is an initiating molecule in axolotl appendage regeneration. *Nature*.
- Takeo, M., Chou, W. C., Sun, Q., Lee, W., Rabbani, P., Loomis, C., Mark Taketo, M. and Ito, M. (2013). Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature*.
- Tata, P. R., Mou, H., Pardo-Saganta, A., Zhao, R., Prabhu, M., Law, B. M., Vinarsky, V., Cho, J. L., Breton, S., Sahay, A., et al. (2013). Dedifferentiation of committed epithelial cells into stem cells in vivo. *Nature*.
- Toda, T. and Gage, F. H. (2018). Review: adult neurogenesis contributes to hippocampal plasticity. *Cell Tissue Res*.
- Van Es, J. H., Sato, T., Van De Wetering, M., Lyubimova, A., Yee Nee, A. N., Gregorieff, A., Sasaki, N., Zeinstra, L., Van Den Born, M., Korving, J., et al. (2012). Dll1 + secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol*.
- Wagner, D. E., Wang, I. E. and Reddien, P. W. (2011). Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science*.
- Yanger, K., Knigin, D., Zong, Y., Maggs, L., Gu, G., Akiyama, H., Pikarsky, E. and Stanger, B. Z. (2014). Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. *Cell Stem Cell*.
- Zeng, A., Li, H., Guo, L., Gao, X., McKinney, S., Wang, Y., Yu, Z., Park, J., Semerad, C., Ross, E., et al. (2018). Prospectively Isolated Tetraspanin + Neoblasts Are Adult Pluripotent Stem Cells Underlying Planaria Regeneration. *Cell*.
- Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to β -cells. *Nature*.

MODULE 5: DIRECTED DIFFERENTIATION AND TRANSDIFFERENTIATION

Cohen, D. E. and Melton, D. (2011). Turning straw into gold: Directing cell fate for regenerative medicine. *Nat. Rev. Genet.*

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K. and Sasai, Y. (2008). Self-Organized Formation of Polarized Cortical Tissues from ESCs and Its Active Manipulation by Extrinsic Signals. *Cell Stem Cell.*

The ability for stem cells to self-organize in three dimensions and produce specialized progeny is a remarkable demonstration of genetically-encoded information. This paper is the first demonstration that human pluripotent stem cells can form brain-like structures, such as the human neocortex, with appropriate guidance using ectopic/extrinsic factors to drive fate commitment. These structures, which were not named at the time of publication, are now referred to as brain or cerebral organoids. These organoids are used today to study mammalian brain development and disease. The same techniques used to produce these brain organoids have since been applied to generate organoids from other tissues.

Höpfl, G., Gassmann, M. and Desbaillets, I. (2004). Differentiating embryonic stem cells into embryoid bodies. *Methods Mol. Biol.*

Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., Eliazer, S., Young, H., Richardson, M., Smart, N. G., Cunningham, J., et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol.*

Mascetti, V. L. and Pedersen, R. A. (2016). Human-Mouse Chimerism Validates Human Stem Cell Pluripotency. *Cell Stem Cell.*

Thomas, K. R. and Capecchi, M. R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell.*

Wichterle, H., Lieberam, I., Porter, J. A. and Jessell, T. M. (2002). Directed differentiation of embryonic stem cells into motor neurons. *Cell.*

Because of their ability to differentiate into any cell type found in the embryo or adult body, pluripotent stem cells promise to be transformative tools in medical research and clinical applications. A critical experimental step was determining how to make these stem cells exit pluripotency and differentiate into specific cell types for cellular studies and cell replacement therapies. Before this study by Wichterle et al., it was unclear how differentiating pluripotent stem cells would respond to signaling cues. This work demonstrated three critical aspects of pluripotent stem cell differentiation. First, it was determined that like differentiating cells in the embryo, differentiating pluripotent stem cells are patterned by responding to signaling molecules in a concentration-dependent manner. Second, these stem cells acquire terminal motor neuron fate progressively, further recapitulating embryonic development. Third, embryonic stem cell-derived motor neurons integrate into developing circuits when transplanted into a developing spinal cord, demonstrating that in vitro produced motor neurons are functional. These three principles that were uncovered in this landmark paper guide the vast majority of embryonic stem cell differentiation protocols.

MODULE 6: LEVERAGING TOOLS TO STUDY STEM CELL BIOLOGY

Arias-Fuenzalida, J., Jarazo, J., Qing, X., Walter, J., Gomez-Giro, G., Nickels, S. L., Zaehres, H., Schöler, H. R. and Schwamborn, J. C. (2017). FACS-Assisted CRISPR-Cas9 Genome Editing Facilitates Parkinson's Disease Modeling. *Stem Cell Reports*.

Chapman, K. M., Medrano, G. A., Jaichander, P., Chaudhary, J., Waits, A. E., Nobrega, M. A., Hotaling, J. M., Ober, C. and Hamra, K. F. (2015). Targeted germline modifications in rats using CRISPR/Cas9 and spermatogonial stem cells. *Cell Rep*.

Deuse, T., Hu, X., Gravina, A., Wang, D., Tediashvili, G., De, C., Thayer, W. O., Wahl, A., Garcia, J. V., Reichen-spurner, H., et al. (2019). Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat. Biotechnol*.

Knott, G. J. and Doudna, J. A. (2018). CRISPR-Cas guides the future of genetic engineering. *Science*.

Kobayashi, T., Yamaguchi, T., Hamanaka, S., Kato-Itoh, M., Yamazaki, Y., Ibata, M., Sato, H., Lee, Y. S., Usui, J. ichi, Knisely, A. S., et al. (2010). Generation of Rat Pancreas in Mouse by Interspecific Blastocyst Injection of Pluripotent Stem Cells. *Cell*.

Kretzschmar, K. and Clevers, H. (2016). Organoids: Modeling Development and the Stem Cell Niche in a Dish. *Dev. Cell*.

Kumar, P., Tan, Y. and Cahan, P. (2017). Understanding development and stem cells using single cell-based analyses of gene expression. *Dev*.

Masaki, H. and Nakauchi, H. (2017). Interspecies chimeras for human stem cell research. *Dev*.

Matsunari, H., Watanabe, M., Hasegawa, K., Uchikura, A., Nakano, K., Umeyama, K., Masaki, H., Hamanaka, S., Yamaguchi, T., Nagaya, M., et al. (2020). Compensation of Disabled Organogeneses in Genetically Modified Pig Fetuses by Blastocyst Complementation. *Stem Cell Reports*.

Rompolas, P., Deschene, E. R., Zito, G., Gonzalez, D. G., Saotome, I., Haberman, A. M. and Greco, V. (2012). Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. *Nature*.

Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., Van Es, J. H., Abo, A., Kujala, P., Peters, P. J., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*.

Tissue-resident adult stem cells constantly renew the tissues they are responsible for. For example, the Lgr5-positive small intestinal stem cells that reside in the intestinal epithelium constantly replenish the intestinal epithelial layer. But despite their enormous regenerative capacity, adult stem cells were long refractory to being grown in culture. In this paper, Sato et al. identified culture conditions to grow murine Lgr5-positive intestinal stem cells in a dish. When grown in a 3-dimensional matrix supplemented with epidermal growth factor (EGF), the Wnt-agonist R-spondin, and the BMP-inhibitor Noggin, Lgr5+ stem cells not only proliferate but produce daughter cells that differentiate into the cell types of the intestinal epithelium. Moreover, the Lgr5+ stem cells and differentiated cells self-organize into domains representing

either the intestinal crypt or the intestinal villus. Because of this resemblance to endogenous structures, the cultures are termed “organoids”. These organoids can self-renew long term. After this initial discovery, the culture conditions were adapted and applied to adult stem cells of many other organs. These cultures allow the study of cellular plasticity, differentiation capacity, and the stem cell niche.

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N. J., Livak, K. J., Mikkelsen, T. S. and Rinn, J. L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.*

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be coaxed to differentiate towards any lineage, thus providing a powerful model system to study the pathways and mechanisms underlying the differentiation of various cell types. This differentiation process is highly asynchronous, however, and results in cells at various stages along a differentiation trajectory at any given time point. Thus, accurately studying the molecular basis of cell differentiation with traditional bulk cell methods is challenging as the high variability in transcriptional programs is drowned out by averaging gene expression values across a heterogeneous population. Single cell RNAseq allows researchers to specifically study the transcriptomes of individual cells in a heterogeneous population. In this work, a computational method called Monocle was developed and combined with single-cell transcriptomics to interrogate the early and late gene regulatory programs governing the differentiation of primary human myoblasts. Monocle enabled the precise temporal resolution of gene expression dynamics by ordering single-cell expression profiles in ‘pseudotime’, a quantitative measure of progress through a biological process. This combined approach facilitated the identification of rare and transient cell states during myoblast differentiation and novel regulators of this process, demonstrating its power as a tool for studying cell differentiation.

Yin, X., Farin, H. F., Van Es, J. H., Clevers, H., Langer, R. and Karp, J. M. (2014). Niche-independent high-purity cultures of Lgr5 + intestinal stem cells and their progeny. *Nat. Methods.*

Zhang, Y., Schmid, B., Nikolaisen, N. K., Rasmussen, M. A., Aldana, B. I., Agger, M., Calloe, K., Stummann, T. C., Larsen, H. M., Nielsen, T. T., et al. (2017). Patient iPSC-Derived Neurons for Disease Modeling of Frontotemporal Dementia with Mutation in CHMP2B. *Stem Cell Reports.*

Induced pluripotent stem cells (iPSCs) made it possible to study patient-specific mutations during differentiation and mature cell function. However, these patient-specific iPSCs lack a critical genetic control. Because they derive from individual patients, the iPSCs genotype carries the patient’s genetic mutation plus the rest of their unique genome. Healthy iPSC controls were typically derived from family members without the disease-causing mutation. Thus, the patient and control iPSCs have two types of genetic differences: the presence or absence of the disease mutation (what we want to study), and background genetic differences across people (noise). To overcome this issue, researchers used genome editing to correct the disease-causing mutation in patient-derived iPSCs. As a result, researchers have two identical iPSC lines whose only difference is whether they carry the disease-causing mutation. In this work, researchers apply that strategy to iPSCs derived from Frontotemporal dementia (FTD) patients who carry a mutation in the gene charged multivesicular body protein 2B (CHMP2B), which is located on chromosome 3 (FTD3). Through genome editing, they generated an isogenic control (genetically identical) but with a “corrected” CHMP2B gene. Afterward, the investigators differentiated both iPSC lines into neurons. They could, therefore specifically study how CHMP2B mutations affect neuronal physiology. The study found that CHMP2B

mutations cause several neuronal defects, including mitochondrial dysfunction and transcriptional dysregulation in neurons. Creating a genetically corrected control was necessary to determine the genetic cause for these defects.

Zhao, T., Zhang, Z. N., Westenskow, P. D., Todorova, D., Hu, Z., Lin, T., Rong, Z., Kim, J., He, J., Wang, M., et al. (2015). Humanized Mice Reveal Differential Immunogenicity of Cells Derived from Autologous Induced Pluripotent Stem Cells. *Cell Stem Cell*.

Cellular reprogramming was a tremendous breakthrough that introduced the possibility that patient-specific human induced pluripotent stem cells (hiPSCs) could be a renewable source of autologous cells for transplantation. Theoretically, these host-specific cells should not be rejected by the immune system when transplanted. Several studies in mice, however, have shown that the reality is more complex. Interestingly, in some cases certain types of autologous cells can still trigger an immune reaction. This study used the humanized mouse model system, in which the immune compartment of irradiated mice is reconstituted with human cells. This model was used to test the immunogenicity of human-iPSC-derived smooth muscle cells (SMC) as compared to human-iPSC-derived retinal pigment epithelial (RPE) cells in the context of a human immune system. The authors found that hiPSC-SMCs elicited a T cell response due to abnormal expression of immunogenic antigens, while hiPSC-RPEs did not express such antigens. hiPSC-RPEs were immune tolerated when transplanted into both the eyes and skeletal muscle. Thus, this approach revealed differential tolerance of distinct hiPSC-derived cell types, demonstrating the feasibility of developing hiPSC-RPEs for clinical use and identifying strategies to improve the tolerance of hiPSC-SMCs.

MODULE 7: CLINICAL APPLICATIONS OF STEM CELL BIOLOGY

Aydin, B. and Mazzone, E. O. (2019). Cell reprogramming: The many roads to success. *Annu. Rev. Cell Dev. Biol.*

Barker, R. A., Parmar, M., Studer, L. and Takahashi, J. (2017). Human Trials of Stem Cell-Derived Dopamine Neurons for Parkinson's Disease: Dawn of a New Era. *Cell Stem Cell*.

Cossu, G., Birchall, M., Brown, T., De Coppi, P., Culme-Seymour, E., Gibbon, S., Hitchcock, J., Mason, C., Montgomery, J., Morris, S., et al. (2018). Lancet Commission: Stem cells and regenerative medicine. *Lancet*.

Cugola, F. R., Fernandes, I. R., Russo, F. B., Freitas, B. C., Dias, J. L. M., Guimarães, K. P., Benazzato, C., Almeida, N., Pignatari, G. C., Romero, S., et al. (2016). The Brazilian Zika virus strain causes birth defects in experimental models. *Nature*.

Dekkers, J. F., Wiegerinck, C. L., De Jonge, H. R., Bronsveld, I., Janssens, H. M., De Winter-De Groot, K. M., Brandsma, A. M., De Jong, N. W. M., Bijvelds, M. J. C., Scholte, B. J., et al. (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.*

Dimos, J. T., Rodolfa, K. T., Niakan, K. K., Weisenthal, L. M., Mitsumoto, H., Chung, W., Croft, G. F., Saphier, G., Leibel, R., Golland, R., et al. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects motor neurons. Until recently, there were limited human models to study how or by what mechanism motor neurons degenerate during ALS. In 2006, pluripotency reprogramming of adult tissues rocked the stem cell world. These advances laid the groundwork for this landmark paper in 2008 in which the protocols to differentiate pluripotent stem cells into motor neurons were established. Importantly, this work enabled the generation of motor neurons from patients with ALS so that researchers could examine the genetic and cellular underpinnings of this neurodegeneration. This concept was then repeated for an uncountable number of diseases and genetic disorders.

Fowler, J. L., Ang, L. T. and Loh, K. M. (2020). A critical look: Challenges in differentiating human pluripotent stem cells into desired cell types and organoids. *Wiley Interdiscip. Rev. Dev. Biol.*

ISSCR (2016). Guidelines for Stem Cell Research and Clinical Translation. In *International Society for Stem Cell Research*.

Kikuchi, T., Morizane, A., Doi, D., Magotani, H., Onoe, H., Hayashi, T., Mizuma, H., Takara, S., Takahashi, R., Inoue, H., et al. (2017). Human iPSC cell-derived dopaminergic neurons function in a primate Parkinson's disease model. *Nature*.

Death of dopaminergic neurons in the brain drives Parkinson's disease progression. Thus, replacing dying dopaminergic neurons could be a potential cure. By replacing damaged, diseased, or dead cells, cell replacement therapies promise to transform medical care. To be effective, pluripotent-derived cells must graft onto and survive in the host. This paper demonstrates how human induced pluripotent stem cell-derived human dopaminergic neurons have the ability to successfully graft, survive, and potentially improve Parkinson's disease-like symptoms in primates. Thus, this pre-clinical study establishes the feasibility of the approach, an essential and necessary step for clinical trials in humans with Parkinson's.

Kiskinis, E., Kralj, J. M., Zou, P., Weinstein, E. N., Zhang, H., Tsiaras, K., Wiskow, O., Ortega, J. A., Egan, K. and Cohen, A. E. (2018). All-Optical Electrophysiology for High-Throughput Functional Characterization of a Human iPSC-Derived Motor Neuron Model of ALS. *Stem Cell Reports*.

Li, X., Zuo, X., Jing, J., Ma, Y., Wang, J., Liu, D., Zhu, J., Du, X., Xiong, L., Du, Y., et al. (2015). Small-Molecule-Driven Direct Reprogramming of Mouse Fibroblasts into Functional Neurons. *Cell Stem Cell*.

Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., Amenduni, M., Szekely, A., Palejev, D., Wilson, M., et al. (2015). FOXP1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders. *Cell*.

Pellettieri, J. and Alvarado, A. S. (2007). Cell turnover and adult tissue homeostasis: From humans to planarians. *Annu. Rev. Genet.*

Qian, L., Huang, Y., Spencer, C. I., Foley, A., Vedantham, V., Liu, L., Conway, S. J., Fu, J. D. and Srivastava, D. (2012). In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature*.

Cardiac muscle cell (cardiomyocyte) loss is a driver and consequence of many forms of heart disease, the leading cause of death worldwide. The limited regenerative capacity of cardiomyocytes has precluded the development of effective strategies to treat this condition. Direct cardiac reprogramming employed over-expression of cardiac lineage-restricted transcription factors to convert cardiac fibroblasts to new cardio-

myocytes, offering a promising strategy to overcome the poor recovery of endogenous cardiomyocytes. This study demonstrated that cardiac reprogramming could be accomplished in vivo and contribute to cardiomyocyte replenishment and recovery of heart function after an acute heart attack, setting the stage for clinical translation to combat heart failure.

Rama, P., Matuska, S., Paganoni, G., Spinelli, A., De Luca, M. and Pellegrini, G. (2010). Limbal stem-cell therapy and long-term corneal regeneration. *N. Engl. J. Med.*

Rivetti Di Val Cervo, P., Romanov, R. A., Spigolon, G., Masini, D., Martín-Montañez, E., Toledo, E. M., La Manno, G., Feyder, M., Pifl, C., Ng, Y. H., et al. (2017). Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. *Nat. Biotechnol.*

Shi, Y., Inoue, H., Wu, J. C. and Yamanaka, S. (2017). Induced pluripotent stem cell technology: A decade of progress. *Nat. Rev. Drug Discov.*

Srivastava, D. and DeWitt, N. (2016). In Vivo Cellular Reprogramming: The Next Generation. *Cell.*

Sugimoto, S. and Sato, T. (2017). Establishment of 3D intestinal organoid cultures from intestinal stem cells. In *Methods in Molecular Biology.*

Velasco, S., Kedaigle, A. J., Simmons, S. K., Nash, A., Rocha, M., Quadrato, G., Paulsen, B., Nguyen, L., Adiconis, X., Regev, A., et al. (2019). Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature.*

Vlachogiannis, G., Hedayat, S., Vatsiou, A., Jamin, Y., Fernández-Mateos, J., Khan, K., Lampis, A., Eason, K., Huntingford, I., Burke, R., et al. (2018). Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science.*

Zhou, T., Tan, L., Cederquist, G. Y., Fan, Y., Hartley, B. J., Mukherjee, S., Tomishima, M., Brennand, K. J., Zhang, Q., Schwartz, R. E., et al. (2017). High-Content Screening in hPSC-Neural Progenitors Identifies Drug Candidates that Inhibit Zika Virus Infection in Fetal-like Organoids and Adult Brain. *Cell Stem Cell.*

Environmental factors can impact human development. One example is damage to the nervous systems when viruses infect the developing embryo. Human brain organoids were used to demonstrate the causal link between the circulating Brazilian Zika strain and congenital disabilities. While the mouse model showed that the virus could cross the placenta, brain organoids were instrumental in demonstrating how the recently mutated Brazilian virus could dramatically impact the proliferative zone of the brain, causing cell death and reducing populations of cortical neuron subtypes. The slow developmental trajectory of the mouse and human brain enabled researchers to more accurately observe viral infection progression. This model was further utilized to screen drugs that could prevent infection or block viral replication in human brain cells.

Bone marrow transplant, Mayo Clinic. <https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/about/pac-20384854?p=1>

EuroStemCell Getting involved: clinical trials and patient registries. <https://www.eurostemcell.org/getting-involved-clinical-trials-and-patient-registries>

A database of privately and publicly funded clinical studies conducted around the world: <https://clinicaltrials.gov/>

MODULE 8: ETHICAL ISSUES IN STEM CELL RESEARCH

Bracewell-Milnes, T., Saso, S., Abdalla, H. and Thum, M. Y. (2018). A systematic review investigating psychosocial aspects of egg sharing in the United Kingdom and their potential effects on egg donation numbers. *Hum. Fertil.*

Chan, S. (2017). Current and emerging global themes in the bioethics of regenerative medicine: The tangled web of stem cell translation. *Regen. Med.*

Fox, D. and Cohen G. (2019). The Law and Ethics of Trump Administration Restrictions on Fetal Tissue Research. *Health Affairs.*

Fu, W., Smith, C., Turner, L., Fojtik, J., Pacyna, J. E. and Master, Z. (2019). Characteristics and Scope of Training of Clinicians Participating in the US Direct-to-Consumer Marketplace for Unproven Stem Cell Interventions. *JAMA - J. Am. Med. Assoc.*

Haimes, E., Skene, L., Ballantyne, A. J., Caulfield, T., Goldstein, L. S., Hyun, I., Kimmelman, J., Robert, J. S., Roxland, B. E., Scott, C. T., et al. (2013). Position statement on the provision and procurement of human eggs for stem cell research. *Cell Stem Cell.*

Hawke, B., Przybylo, A. R., Paciulli, D., Caulfield, T., Zarzeczny, A. and Master, Z. (2019). How to Peddle Hope: An Analysis of YouTube Patient Testimonials of Unproven Stem Cell Treatments. *Stem Cell Reports.*

Human genome editing: Science, ethics, and governance (2017).

This National Academies of Sciences, Engineering, and Medicine report provides an overview of research covering scientific advancements and ethical perspectives on gene modification, irrespective of the molecular tools used to modify genetic material. The report outlines several ethical principles that form the foundation to develop a moral framework for governing gene editing technologies, including the promotion of well-being, transparency, due care, responsible science, respect for persons, fairness, and transnational cooperation. The two major debates that have been discussed in the ethics of gene modification are the somatic versus germline distinction and the therapy versus enhancement distinction, both of which have also played a major role in establishing policies on gene modification. The report also discusses public engagement practices and provides a summary of recommendations to move forward on gene editing practices.

Goldstein, A. (2020). Trump restrictions on fetal tissue research unsettle key studies and scientists. *Washington Post.*

Hogle, L. (2017). Risk vs. Access: new standards for evidence for evaluating regenerative medicines. *EuroStemCell.*

Isasi, R., Kleiderman, E. and Knoppers, B. M. (2016). Editing policy to fit the genome? *Science.*

ISSCR (2016). Guidelines for Stem Cell Research and Clinical Translation. In *International Society for Stem Cell Research.*

The ISSCR Guidelines for Stem Cell Research and Clinical Translation represent guidance to scientists, clinicians, ethicists, policymakers, review boards, and others about the lab-based embryo and stem cell research, the clinical translation and research involving humans, and best practices of communicating science to the public. The ISSCR Guidelines also discuss governance processes for different types of research. For the module on ethics, the ISSCR Guidelines serve to illustrate the difference between unproven stem cell interventions, clinical innovation, and clinical stem cell research. This module also provides a background on the marketing of unproven stem cell interventions and provides guidance on best practices for clinical stem cell research. Another relevant chapter of the ISSCR Guidelines covers the public communication of stem cell research findings. The report also explains the risks of exaggerating the benefits of marketing unproven stem cell interventions to the public.

Juengst, E. T. (2017). Crowdsourcing the Moral Limits of Human Gene Editing? *Hastings Cent. Rep.*

Marcon, A. R., Murdoch, B. and Caulfield, T. (2017). Fake news portrayals of stem cells and stem cell research. *Regen. Med.*

Master, Z. (2018). Ethics of issues and stem cell research: The unresolved issues. In *Encyclopedia of Biomedical Engineering*, .

Meagher, K. M., Allyse, M. A., Master, Z. and Sharp, R. R. (2020). Reexamining the Ethics of Human Germline Editing in the Wake of Scandal. *Mayo Clin. Proc.*

Sipp, D., Caulfield, T., Kaye, J., Barfoot, J., Blackburn, C., Chan, S., De Luca, M., Kent, A., McCabe, C., Munsie, M., et al. (2017). Marketing of unproven stem cell-based interventions: A call to action. *Sci. Transl. Med.*

Tanner, C., Petersen, A. and Munsie, M. (2017). “No one here’s helping me, what do you do?”: Addressing patient need for support and advice about stem cell treatments. *Regen. Med.*

Turner, L. and Knoepfler, P. (2016). Selling Stem Cells in the USA: Assessing the Direct-to-Consumer Industry. *Cell Stem Cell.*

This paper is one of a few that show a major shift in the operation of businesses selling unproven stem cell interventions directly to the public. Previously, the industry was believed to operate in countries which may not have had adequate safeguards or enforcement standards. This paper along with a few others were the first to demonstrate the increase in businesses selling unproven stem cell interventions in countries with robust regulatory safeguards and enforcement of biologics. The authors undertook a content analysis approach to systematically evaluate the websites of businesses found in the United States, showing that 351 businesses operated over 570 clinics marketing unproven stem cell interventions on the internet. The researchers highlighted that California, Florida, and Texas were three states with the highest number of these clinics. The authors also outlined the sources of stem cells and the clinical areas and conditions for which unproven stem cell interventions were marketed as treatments. Specifically, the study showed that more patients were seeking unproven stem cell interventions for orthopedic and sports related conditions and injuries, as opposed to using stem cells for life-threatening or severely debilitating diseases. In addition, the majority of cells were derived from adipose tissue, bone marrow, and amniotic fluid, whereas in the past, a wider range of stem cell sources were used.

Turner, L. (2017). U.S. Businesses using ClinicalTrials.gov to market unproven and unlicensed stem cell interventions. *EuroStemCell*.

International Society for Stem Cell Research. Sample Informed Consent: Egg donation for stem cell research: provided directly and solely for stem cell research. <https://www.isscr.org/docs/default-source/all-isscr-guidelines/hesc-guidelines/cfeggsresearch.doc>

International Society for Stem Cell Research (2006). Guidelines for the Conduct of Human Embryonic Stem Cell Research. Chapter 11. https://www.isscr.org/docs/default-source/all-isscr-guidelines/hesc-guidelines/iss-crhescguidelines2006.pdf?sfvrsn=91f5f996_0

International Society for Stem Cell Research (2017). Human fetal tissue: a critical resource for biomedical research. <https://www.isscr.org/docs/default-source/policy-documents/fetal-tissue-research-re-source-feb-2017-revfinal.pdf?sfvrsn=2>

International Society for Stem Cell Research (2018). Stem cell-based clinical trials: practical advice for physicians and ethics/institutional review boards. https://www.isscr.org/docs/default-source/clinical-resources/iss-cr-stem-cell-based-clinical-trials-practical-advice_final_23jan2018.pdf?sfvrsn=2

International Society for Stem Cell Research (2019). Informed consent standard for stem cell-based interventions offered outside of formal clinical trials. <https://www.isscr.org/docs/default-source/policy-documents/isscr-informed-consent-standards-for-stem-cell-based-interventions.pdf>

AAAS' Center for Public Engagement with Science and Technology. AAAS Communication Toolkit. <https://www.aaas.org/resources/communication-toolkit>

- Communication Fundamentals: <https://www.aaas.org/resources/communication-toolkit/communication-fundamentals>
- Communicating Science Online: <https://www.aaas.org/programs/center-public-engagement-science-and-technology/communicating-science-online>
- Working with Journalists: <https://www.aaas.org/resources/communication-toolkit/working-journalists>
- In-Person Engagement: <https://www.aaas.org/resources/communication-toolkit/person-engagement>



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