Direct Reprogramming and Ethics in Stem Cell Research

W. Malcolm Byrnes

Abstract: The recent conversion of adult cells into so-called induced pluripotent stem (iPS) cells through direct reprogramming opens a new chapter in the study of disease and the development of regenerative medicine. It also provides a historic opportunity to turn away from the ethically problematic use of embryonic stem cells isolated through the destruction of human embryos. Moreover, because iPS cells are patient specific, they render therapeutic cloning unnecessary. To maximize therapeutic benefit, adult stem cell research will need to be pursued in parallel with studies using iPS cells. Among the four alternative methods presented by the President's Council on Bioethics, direct reprogramming is the most ethically acceptable. Nonetheless, iPS cells are tainted by their association with the human embryonic stem cell lines, derived in the past, which will be required for their validation. This concern is one that can be resolved. Human iPS cells will serve to stem the tide of human embryonic stem cell research, changing it and diverting stem cell research in a more ethical direction. *The National Catholic Bioethics Quarterly* 8.2 (Summer 2008): 277–290.

W. Malcolm Byrnes, Ph.D., is an assistant professor in the Department of Biochemistry and Molecular Biology at Howard University College of Medicine, Washington, D.C.; e-mail: wbyrnes@howard.edu. The views expressed here are solely those of the author; they do not reflect the official views of Howard University or any of its schools and colleges, including the College of Medicine. The author thanks Dr. David Prentice of the Family Research Council in Washington, D.C., for helpful comments on an earlier draft of this paper.

The successful conversion of ordinary skin cells into pluripotent stem cells represents a turning point in the ethical controversy that has dogged human embryonic stem cells since they were first isolated from in vitro fertilization (IVF)-derived embryos almost a decade ago. In November 2007, two research groups, one led by Shinya Yamanaka of Kyoto University in Japan,[1] and the other led by James Thomson of the University of Wisconsin at Madison,[2] reported the production of *induced pluripotent stem (iPS) cells* from adult skin cells. The iPS cells that were generated appear to possess all of the characteristics of embryonic stem cells: they have their typical cell morphology and proliferative ability; they express cell surface markers and have gene expression profiles characteristic of embryonic stem cells; they have similar levels of a telomere-lengthening enzyme known as telomerase; they likewise form tumors called teratomas when injected into mice; and they can differentiate into different cell types of the three germinal layers (endoderm, ecotoderm, and mesoderm).

These experiments by Yamanaka and Thomson prove that it is possible to derive patient-specific pluripotent cells without the ethically problematic use of human oocytes and without destruction of human embryos. These facts make the studies groundbreaking from both scientific and ethical perspectives. They open the door to a variety of scientific and medical applications that scientists had thought were possible only with embryonic stem cells. These include the investigation of how human tissues develop, the discovery and testing of new drugs, and the development of cell replacement therapies.[3] Nevertheless, the iPS cells produced through direct reprogramming will have to be rigorously compared to genuine embryonic stem cells in order to test their authenticity; that is, they will have to be validated. What ethical problems, if any, does this requirement carry with it?

How Direct Reprogramming Works

In the experiments reported, iPS cells were generated by introducing the genes for a set of four protein factors, known as transcription factors, into adult cells. Transcription factors control the expression of target genes in cells by becoming

[1] K. Takahashi et al., "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors," *Cell* 131.5 (November 30, 2007): 1–12. Dr. Yamanaka is also affiliated with the Gladstone Institute of Cardiovascular Disease in San Francisco.

[2] J. Yu et al., "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells," *Science* 318.5858 (December 21, 2007): 1917–1920.

[3] In December 2007, less than one month after the publication of the papers by Yamanaka and Thomson showing production of human iPS cells, a proof-of-concept paper for use of iPS cells in transplantation therapy was published. The paper showed that iPS cells could be used to successfully treat sickle cell anemia in a mouse model of the disease. This achievement by Rudolf Jaenisch's group at Massachusetts Institute of Technology shows how rapidly this area of biomedical research is likely to progress. See J. Hanna et al., "Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin," *Science* 318.5858 (December 21, 2007): 1920–1923. See also Rick Weiss, "Scientists Cure Mice of Sickle Cell Using Stem Cell Technique: New Approach Is from Skin, Not Embryos," *Washington Post* (December 7, 2007): A2.

attached to the DNA upstream of the genes, thereby turning them "on" or "off." The expression of different sets of genes is characteristic of different types of cells and tissues; thus, a characteristic gene expression profile—the presence or absence (and level) of expression of each of the genes in the genome—exists for each cell type and developmental stage. In the developing embryo, transcription factors themselves are expressed according to particular spatial and temporal patterns that depend on the stage of development. Thus, for instance, the stem cells in the blastocyst's inner cell mass—embryonic stem cells—will have a characteristic consensus gene expression profile.[4] Starting with thousands of genes whose expression is characteristic of embryonic stem cells, Yamanaka and Thomson independently sought to discover the smallest possible set of genes, and the transcription factors they encode, that could induce pluripotency and self-renewal in cells. (From a technological standpoint, only a small set could work, since all of the genes would have to be introduced together into the cells; thus, a large set would be too unwieldy.) Through selective elimination, from the initial list of thousands of genes, they obtained a list that included a few hundred, then a dozen or so, and finally only four. Interestingly, the four transcription factors discovered and used by the two groups were not all the same. Yamanaka used a set—*OCT3/4, SOX2, KLF4,* and *c-MYC*—that had worked in mouse experiments he had reported sixteen months earlier. In the earlier experiments, mouse embryonic stem cells were successfully converted to mouse iPS cells.[5] From that time onward, his goal was to see if the factors would likewise work in human cells.[6] Thomson, from the beginning using human (not mouse) embryonic

[4] In a range of species (human, monkey, mouse), researchers have found that embryonic stem cell lines originating from different embryos have somewhat different gene expression profiles, that is, not all of the same genes are expressed in each one. For example, in a study of three independently derived human embryonic stem cell lines, only 52 percent of the genes that were expressed in one line were also expressed in the other two. M. J. Abeyta et al., "Unique Gene Expression Signatures of Independently Derived Human Embryonic Stem Cell Lines," *Human Molecular Genetics* 13.6 (March 15, 2004): 601–608. Other studies have confirmed this result showing variability in gene expression profiles. One explanation for the variability could be that the chromatin inside the nucleus of embryonic stem cells possesses what is known as *hyperdynamic plasticity*, such that chromatin-associated proteins are able to bind to, and be released from, the chromatin very readily. In this way, the cell would remain uncommitted, poised to move down one differentiation pathway or another until the right signal appears. E. Meshorer et al., "Hyperdynamic Plasticity of Chromatin Proteins in Pluripotent Embryonic Stem Cells," *Developmental Cell* 10.1 (January 2006): 105–116. Nevertheless, in recent years and months, scientists have been able to identify a consensus set of genes that characterizes pluripotency and self-renewal in embryonic stem cells from different origins. Y. Sun et al., "Cross-Species Transcriptional Profiles Establish a Functional Portrait of Embryonic Stem Cells," *Genomics* 89.1 (January 2007): 22–35. Great strides have been made in this area. In some ways, the work by Yamanaka and Thomson represents a culmination of this effort.

[5] K. Takahashi and S. Yamanaka, "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors," *Cell* 126.4 (August 25, 2006): 663–676.

[6] As we now know, they do work. This result shows that the gene regulatory network that functions in human embryonic stem cells is very similar to the one that functions in mouse

stem cells, worked his way through a similar process of elimination. Like Yamanaka, he found that *OCT3/4* and *SOX2* were important, but instead of *KLF4* and *c-MYC*, he identified *NANOG* and *LIN28*.

Among the six factors selected by the teams led by Yamanaka and Thomson, *NANOG, OCT3/4,* and *SOX2* were known from a number of other studies to be master regulators of pluripotency and self-renewal in embryonic stem cells; therefore, their discovery came as no surprise.[7] The other three were less obvious. Yamanaka and coworkers speculate that *KLF4* and *c-MYC* may be involved in modifying chromatin structure in embryonic stem cells so that *OCT3/4* and *SOX2* can access their target genes. On the other hand, *LIN28* appears to be important for the proper translation of so-called messenger RNA molecules that bridge the gap between genes and the proteins they encode.[8] Interestingly, Thomson reports that *NANOG* was not essential for the initial appearance of the iPS cell clones, but it did appear to increase the survival rate of the reprogrammed cells once they appeared. This observation parallels others suggesting that *NANOG* is important for the *maintenance* of pluripotency and self-renewal, but not its *establishment*.

What these studies show is that somewhat different routes, each involving a different set of factors, are possible for obtaining human iPS cells. This demonstrates, as Rudolf Jaenisch observed, that "apparently there are various ways to get to Rome."[9] It shows that cellular reprogramming is a somewhat flexible process.

Some Practical Hurdles

Several practical issues will have to be resolved before iPS cells will be able to be used in cell replacement therapies. First, *c-MYC* is a known cancer gene. As a result, it will have to be eliminated from the "magic brew" used in the direct reprogramming procedure. This apparently will not pose a problem, however. In recently reported work, Yamanaka and coworkers showed that *c-MYC* is dispensable; iPS cells can form when only the other three factors are present.[10] Moreover, *c-MYC* was not one of the four factors that Thomson and his group identified and used. A second,

---

embryonic stem cells. However, Yamanaka also found that mouse cell culture conditions are not appropriate for the human cells, indicating that the external signaling pathways through which human and mouse embryonic stem cells respond to environmental signals are different.

[7] See, for example, G. Pan and J. A. Thomson, "Nanog and Transcriptional Networks in Embryonic Stem Cell Pluripotency," *Cell Research* 17.1 (January 2007): 42–49; and Q. Zhou et al., "A Gene Regulatory Network in Mouse Embryonic Stem Cells," *Proceedings of the National Academy of Sciences U.S.A.* 104.42 (October 16, 2007): 16438–16443.

[8] E. Balzer and E. G. Moss, "Localization of the Developmental Timing Regulator Lin28 to mRNP Complexes, P-Bodies and Stress Granules," *RNA Biology* 4.1 (January–March 2007): 16–25.

[9] Rick Weiss, "Advance May End Stem Cell Debate: Labs Create a Stand-In Without Eggs, Embryos," *Washington Post* (November 21, 2007): A1.

[10] M. Nakagawa et al., "Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts," *Nature Biotechnology* 26.1 (January 2008): 101–106.

more critical problem associated with the direct reprogramming procedure is the fact that retroviruses were used to introduce the genes for the four factors into the cells. In this delivery method, the genes first enter the cells and then enter the cells' nuclei. Upon entering the nuclei, they become inserted at random locations in chromosomes. If they happen to become inserted in the wrong places, they can activate endogenous cancer genes that are normally turned off. In this manner, cancer can arise upon transplantation of the cells into the body. This serious problem, however, might be solved either by introducing the protein factors directly (not as genes in retroviruses) or by developing drug-like molecules that can indirectly activate endogenous cellular pluripotency genes, which are present but normally silent in adult cells. Alternatively, use of other gene delivery methods, such as harmless viruses called adenoviruses, or fat-like liposome vectors, might be possible.

A third problem with the use of iPS cells, and indeed all types of pluripotent cells including embryonic stem cells, for transplantation therapy is that they can cause tumor formation when injected into the body. It is well-known that the characteristics of undifferentiated embryonic stem cells are very similar to those of cancer cells;[11] indeed, one of the tests for pluripotency is the ability to form a type of tumor known as a teratoma upon injection into the body. But theoretically, one would not expect this to be a problem because, prior to use in patients, the pluripotent cells are differentiated into heart or nerve or kidney cells. It is these *differentiated* cells that are transplanted into the patient, and the differentiation process should convert all of the pluripotent cells into a terminally differentiated form that is not tumorigenic. Unfortunately, this is often not the case for two reasons: First, chromosomal, genetic, or epigenetic abnormalities can arise in the cells during their propagation in culture; this can cause them to become cancerous once inside the body.[12] Second, even if no abnormalities have arisen, the process of differentiation into tissues in the laboratory dish is not always complete. Some undifferentiated or poorly differentiated (or de-differentiated) cells might remain, and these unstable cells can, once transplanted, cause tumor formation.[13] Therefore, preparations of differentiated pluripotent cells

[11] See L. M. Postovit et al., "The Commonality of Plasticity Underlying Multipotent Tumor Cells and Embryonic Stem Cells," *Journal of Cellular Biochemistry* 101.4 (July 1, 2007): 908–917; and P. W. Andrews et al., "Embryonic Stem (ES) Cells and Embryonal Carcinoma (EC) Cells: Opposite Sides of the Same Coin," *Biochemical Society Transactions* 33.6 (December 2005): 1526–1530.

[12] See J. S. Draper et al., "Recurrent Gain of Chromosomes 17q and 12 in Cultured Human Embryonic Stem Cells," *Nature Biotechnology* 22.1 (January 2004): 53–54; A. Maitra et al., "Genomic Alterations in Cultured Human Embryonic Stem Cells," *Nature Genetics* 37.10 (October 2005): 1099-1103; and Y. Shen et al., "Abnormal CpG Island Methylation Occurs During *In vitro* Differentiation of Human Embryonic Stem Cells," *Human Molecular Genetics* 15.17 (September 1, 2006): 2623–2635.

[13] See S. Chung et al., "Genetic Selection of sox1GFP-Expressing Neural Precursors Removes Residual Tumorigenic Pluripotent Stem Cells and Attenuates Tumor Formation after Transplantation," *Journal of Neurochemistry* 97.5 (June 2006): 1467–1480; and A. Brederlau et al., "Transplantation of Human Embryonic Stem Cell-Derived Cells to a Rat

will have to be carefully purified and shown to not cause cancer in transplantation studies in animals before they will be able to be used for therapy in humans. Though worthwhile, this refinement process will be painstaking and will take a while.

The tumorigenic potential of pluripotent stem cells such as iPS and embryonic stem cells that might limit their usefulness in the clinic is not shared by so-called adult (or somatic) stem cells, which can be obtained from tissues such as bone marrow, umbilical cord blood, and amniotic fluid. Adult stem cells, once isolated, are not cultured outside the body for long before they are used, so genetic abnormalities typically do not arise in them. Moreover, adult stem cells are multipotent, not pluripotent, so they do not cause tumor formation when propagated in culture or transplanted into patients.[14] Even stem cells from amniotic fluid, which might be more-than-multipotent, apparently do not cause tumor formation when maintained in culture for significant periods.[15] Finally, different types of adult stem cells (i.e., from bone marrow and cord blood) are being tested for therapeutic benefit in humans. So far, no tumor formation has been reported in these studies.[16] Thus, while it is worthwhile to pursue the long-range therapeutic potential of iPS cells, it is equally important to maximize the more immediate therapeutic benefit of adult stem cells.

Despite the limitations that could affect their clinical usefulness, iPS cells nevertheless can be used almost immediately for laboratory experiments to study human genetic disease progression and to test drugs. Moreover, because the methods of Yamanaka and Thomson are relatively straightforward to perform, they are likely to be taken up and used by many laboratories very soon. Indeed, even Ian Wilmut, the Scottish scientist who cloned Dolly the sheep in 1996, is apparently abandoning the cloning technique he pioneered to take up Yamanaka's direct reprogramming method.[17]

Model of Parkinson's Disease: Effect of In Vitro Differentiation on Graft Survival and Teratoma Formation," *Stem Cells* 24.6 (June 2006): 1433–1440.

[14] C. Foroni et al., "Resilience to Transformation and Inherent Genetic and Functional Stability of Adult Neural Stem Cells *Ex Vivo*," *Cancer Research* 67.8 (April 15, 2007): 3725–3733; and Y. S. Yoon et al., "Clonally Expanded Novel Multipotent Stem Cells from Human Bone Marrow Regenerate Myocardium after Myocardial Infarction," *Journal of Clinical Investigation* 115.2 (February 1, 2005): 326–338.

[15] See P. De Coppi et al., "Isolation of Amniotic Stem Cell Lines with Potential for Therapy," *Nature Biotechnology* 25.1 (January 2007): 100–106.

[16] For a list of studies showing the therapeutic benefit of adult stem cells, see Do No Harm: The Coalition of Americans for Research Ethics, "Peer-Reviewed References Showing Applications of Adult Stem Cells That Produce Therapeutic Benefit for Human Patients," www.stemcellresearch.org/facts/asc-refs.pdf.

[17] G. Vogel and C. Holden, "Field Leaps Forward with New Stem Cell Advances," *Science* 318.5854 (November 23, 2007): 1224–1225.

Is Direct Reprogramming the Best Alternative?

Direct adult cell reprogramming is one of four alternatives presented in 2005 by the President's Council on Bioethics.[18] These alternative methods attempt to circumvent the ethical problems associated with the embryo-destructive method now used. In addition to direct reprogramming, the alternatives include altered nuclear transfer, embryo biopsy, and retrieval of cells from dead embryos. As I will show, each of the other three alternatives, all of which have been shown to work experimentally in mice if not humans, has ethical problems that direct reprogramming does not have.

Altered nuclear transfer (known more specifically as ANT-Cdx2), first introduced by William Hurlbut in 2004, would involve the production, through genetic engineering and the cloning process, of an embryo-like entity that cannot implant and cannot develop beyond the blastocyst stage.[19] Critically, the ANT-derived entity would be normal from the time of the cloning event until the developmental stage at which the engineered genetic defect (a lack of *Cdx2* in this case) would take effect. Since it involves cloning, which is notoriously inefficient, hundreds of human oocytes would be required for the production of a single ANT-derived entity. For these and other reasons, some argued that the procedure was ethically problematic, saying that it would produce a human embryo, albeit a disabled one, solely for the purpose of obtaining embryonic stem cells.[20] Others argued that, like all forms of cloning, ANT would involve the wasteful misuse of human oocytes, whose purpose is for reproduction, not the development of medical technology.[21]

Embryo biopsy, which was pioneered by Robert Lanza of Advanced Cell Technology in Worcester, Massachusetts, involves the removal of a single cell, called a blastomere, from an eight-celled human embryo.[22] The single blastomere is then allowed to grow and multiply in culture, forming embryonic stem cells. A similar procedure, known as preimplanation genetic diagnosis (PGD), is now routinely used in fertility clinics to test the quality of IVF embryos. As with Lanza's procedure, a

---

[18] President's Council on Bioethics, *White Paper: Alternative Sources of Human Pluripotent Stem Cells* (Washington, D.C.: PCB, 2005).

[19] The ANT method has been shown to work in mice. See A. Meissner and R. Jaenisch, "Generation of Nuclear Transfer-Derived Pluripotent ES Cells from Cloned Cdx2-Deficient Blastocysts," *Nature* 439.7073 (January 12, 2006): 212–215.

[20] ANT and its derivative, ANT-OAR (altered nuclear transfer–oocyte assisted reprogramming), have been debated vigorously in Catholic bioethics circles. For an account of this debate, see W. Malcolm Byrnes, "Partial Trajectory: The Story of the Altered Nuclear Transfer–Oocyte Assisted Reprogramming (ANT–OAR) Proposal," *Linacre Quarterly* 74.1 (February 2007): 50–59.

[21] See, for example, Kimberly Zenarolla, "Our Fascination with Embryonic Stem Cells," National Pro-Life Action Center on Capitol Hill, www.nplac.org/columns/kz-fall06-fascination.html.

[22] See I. Klimanskaya et al., "Human Embryonic Stem Cell Lines Derived from Single Blastomeres," *Nature* 444.7118 (November 23, 2006): 481–485.

single blastomere is removed. Instead of being cultured and used to derive stem cells, however, the blastomere is subjected to a battery of tests that can be used to identify possible genetic defects, or even to determine the sex of the embryo. Despite its routine use in the fertility clinic, there is some evidence that PGD is not completely harmless. Studies have shown that embryos subjected to PGD, once transferred to the womb, are less likely to result in pregnancy and live birth.[23] Thus, there are concerns about the safety of the procedure. But even if the procedure were found to be perfectly safe, there would still be ethical problems associated with its use in embryo biopsy. The most significant of these is that the embryo is being treated as a means to an end. In other words, it is being used in a utilitarian manner for the benefit of others.[24] For this reason, many would agree that embryo biopsy is ethically unacceptable.

The last of the three alternatives is the retrieval of (viable) cells from embryos that have lost all integrated function, that is, that are dead. This method was introduced by Donald Landry and Howard Zucker of Columbia University in 2004.[25] A version of the procedure that uses whole "arrested" human embryos has been shown to work experimentally.[26] Scientist Maureen Condic of the University of Utah and ethicist Edward Furton of The National Catholic Bioethics Center in Philadelphia recently presented this method as the best of the four alternatives to the embryo destructive method now used to obtain embryonic stem cells.[27] Indeed, retrieval of cells from dead embryos appears to have clear advantages over ANT and embryo biopsy. The most important of these is that no living human embryos are destroyed or harmed in the process. A disadvantage of the method, however, is that it depends on the continued production of IVF embryos—some of which will be found to be dead—by the fertility industry. Currently in the United States, the IVF industry is largely unregulated and is wasteful, producing many more embryos than are actually transferred to the womb. As a result, hundreds of thousands of embryos are now stored in freezers across the country; it is these embryos that some scientists and legislators wish to use for the generation of embryonic stem cells. Given the status of the IVF industry today, questions about retrieving cells from dead embryos arise, such as, Who would monitor the decision regarding whether or not an embryo

[23] See S. Mastenbroeck et al., "*In Vitro* Fertilization with Preimplantation Genetic Screening," *New England Journal of Medicine* 357.1 (July 5, 2007): 9–17; and B. Goldman, "Reproductive Medicine: The First Cut," *Nature* 445.7127 (February 1, 2007): 479–480.

[24] Some might argue that the stem cells generated via embryo biopsy could be stored and used in the future for regenerative medical treatment of the embryo herself after she has become an adult. However, the probable risks of harm almost certainly will outweigh the possible future benefit in this case.

[25] D. W. Landry and H. A. Zucker, "Embryonic Death and the Creation of Human Embryonic Stem Cells," *Journal of Clinical Investigation* 114.9 (November 2004): 1184–1186.

[26] X. Zhang et al., "Derivation of Human Embryonic Stem Cells from Developing and Arrested Embryos," *Stem Cells* 24.12 (December 2006): 2669–2676.

[27] Maureen L. Condic and Edward J. Furton, "Harvesting Embryonic Stem Cells from Deceased Embryos," *National Catholic Bioethics Quarterly* 7.3 (Autumn 2007): 507–525.

is dead,[28] and how would the unbiased objectivity of this decision be ensured? Would the link between retrieving cells from dead embryos and the IVF industry on which it depends, and the potential for profit, lead to abuse? Condic and Furton have addressed these questions, but doubts nonetheless linger. The direct tie to the fertility industry is the chief drawback.

Direct reprogramming sidesteps many of the ethical problems associated with the other three alternatives. Most important, an adult cell is converted *directly* into a pluripotent cell in the direct reprogramming method. Embryos are simply not used and not produced. Thus, there is no possibility of creating embryos that might be human (ANT), harming and using embryos for utilitarian purposes (embryo biopsy), or being unsure if the embryos used are truly dead (retrieval of cells from dead embryos). With direct reprogramming, these issues are all avoided.

The other advantage that is unique to direct reprogramming is that it renders so-called therapeutic cloning entirely unnecessary. The supposed advantage of using stem cells produced via cloning (somatic cell nuclear transfer, or SCNT) is that they are genetically matched to the patient from whom the somatic cell nucleus was obtained. However, direct reprogramming can achieve this same result—patient-specific stem cells—without using oocytes or producing embryos. With direct reprogramming, a cell from the patient himself is reprogrammed. Thus, direct reprogramming eliminates the need for *both* human embryonic stem cells *and* human cloning.

Despite its clear ethical advantages, direct reprogramming does have some nagging residual ethical problems. These are discussed and evaluated below.

Residual Ethical Issues

The ethical problems associated with direct reprogramming originate in the fact that, although the iPS cells that are produced appear to be very similar to embryonic stem cells, it is not yet known if they are functionally equivalent to them. Indeed, iPS cells will have to be *validated*; they will have to be compared side by side with

[28] Note that a difficulty associated with this alternative is that embryos will have to be objectively determined to be dead. Landry and Zucker propose a "retrospective" procedure for doing this; it involves examining fertility clinic records on IVF embryos. D. W. Landry et al., "Hypocellularity and Absence of Compaction as Criteria for Embryonic Death," *Regenerative Medicine* 1.3 (May 2006): 367–371. Condic and Furton argue that a "prospective" method is also needed. "Harvesting Embryonic Stem Cells," 519–522. For this, they propose that arrested embryos be transferred to a woman's uterus for observation. If the embryo implants normally, then the embryo had been, in fact, alive. If it does not, then it had not been alive. This could constitute a method for determining when an arrested embryo is actually deceased. However, there is a serious problem with this method: What woman would agree to allow her uterus to be used as a laboratory for testing the viability of suspected dead embryos? Moreover, what if an arrested embryo survives, but is found to be disabled in some way? Would it not be better to avoid this situation? Although this method of testing may be feasible from an experimental point of view, it may be untenable from a social and emotional one. Thus, the issue of how to determine whether or not an arrested embryo is deceased remains unresolved.

embryonic stem cells to see how they measure up. Moreover, if the iPS cells do not match perfectly, additional refinements of the experimental procedure—a factor added here or replaced there—may have to be made. In order to make these refinements, embryonic stem cells will need to be understood on a molecular level very well. For this, they will have to be studied in depth.

It appears, then, that embracing direct reprogramming does not mean that embryonic stem cell research can be completely abandoned—at least in the near term. In the more long term, once the validation process is complete, embryonic stem cells will no longer be needed, since iPS cells can effectively replace them. Moreover, as biologist Marcus Grompe of the Oregon Stem Cell Center has suggested, the validation process would not require the "harvesting and production" of any embryonic stem cell lines beyond those that already exist.[29] In other words, no additional human embryos would need to be destroyed in order to validate iPS cells obtained through direct reprogramming.

Having said this, it is very possible that some scientists may never be satisfied with any cells other than those derived from embryos. They may maintain that true pluripotency and true self-renewal are possible only with cells formed within an actual embryo. This may prove to be true. On the other hand, iPS cells may be perfectly acceptable. Moreover, it is likely that *practical* considerations, including a lack of availability of IVF embryos for research, the fact that iPS cells are easy to produce in the laboratory, and the availability of federal funding, will drive acceptance of iPS cells even if they are not a perfect match to embryonic stem cells. Also, because of the variability among different embryonic stem cell lines, it may not be possible for scientists to come up with a single, all-inclusive definition of an "embryonic stem cell." Indeed, the different human IVF embryonic stem cell lines that Thomson and colleagues studied had somewhat different gene expression profiles.[30] The gene expression profiles of some of the iPS cell lines they produced were more similar to one particular embryonic stem cell profile, whereas others were more similar to others. This inherent variability among embryonic stem cell lines might lead scientists to accept a broader definition of embryonic-stem-cell-like pluripotency, one that encompasses iPS cells. If this happens, acceptance of iPS cells could come sooner rather than later.

---

[29] Nathan Burchfiel, "Stem Cell Studies 'End Debate' on Embryos, Conservatives Say," Cybercast News Service, November 21, 2007, http://www.cnsnews.com/ViewCulture.asp?Page=/Culture/archive/200711/CUL20071121a.html. The quotations from Marcus Grompe for this news article read as follows: "Grompe said he supported continued research involving embryonic stem cells 'basically to validate the new [adult-based] cells' but not research that would require harvesting or cloning new lines of embryonic stem cells. 'The question here is what is meant by continuing of embryonic stem cell research,' Grompe said. 'If it means that the existing lines that were available through the Bush policy remain valuable resources in terms of comparing the new cells to them and their properties, I would say that this is a correct statement. … If continuing ES research means continued harvest[ing] and production of new embryo-derived cell lines, I would disagree very strongly,' he added."

[30] Yu et al., "Induced Pluripotent Stem Cell Lines," 1917–1920.

Regardless of how many embryonic stem cell lines are used for validation or how long it takes, in the final analysis, it is still true that human embryos will have been destroyed to obtain these cell lines used for validation. Moreover, not just the validation, but the production of iPS cells in the first place used knowledge of human embryonic stem cell pluripotency gained, in part, through the destruction of human embryos.[31] Given these facts, will therapies developed using iPS cells be tainted by their association with human embryonic stem cells? Would persons who use such therapies be cooperating, in some way, with the destruction of human embryos? Would it be ethical for persons to benefit from such therapies?

These questions that apply to iPS cells are similar to ones that apply to vaccines that are produced using cell lines derived from aborted fetuses. First, in both cases, a medical treatment (a vaccine or a cell replacement therapy) is being used that somehow originated from the destruction of human life. Second, in both cases, the origin of the treatment is in a one-time event: either the one-time use of aborted fetuses to obtain cell lines (e.g., MRC-5 and WI-38) to grow weakened virus strains for vaccine production, or the one-time destruction of human embryos to validate iPS cells, which then effectively replace embryonic stem cells. In the latter case, if no additional embryonic stem cell lines are derived, then derivation of the existing lines could be considered a "one-time" event in the past, albeit an event that extended over a several-year period. Third, in both cases, the treatments developed (or to be developed) are of great medical benefit, although, admittedly, the benefits of cell replacement therapy are still a long way off while those of childhood immunization are clear today.

In separate analyses, Edward Furton and philosopher Daniel Maher carefully examined the ethical issues that pertain to the use of vaccines derived from cell lines arising from aborted fetuses.[32] Both came to the conclusion that it is not unethical to

[31] Nonetheless, while it is true that Thomson and his group used human embryonic stem cells to identify the four factors they used for direct reprogramming, it is also true that Shinya Yamanaka and his team *did not* use human embryonic stem cells to identify the (somewhat different) four factors they came up with. Yamanaka's team worked exclusively with mouse embryonic stem cells. The four factors that successfully reprogrammed adult mouse cells earlier were also successful, as it turned out, in reprogramming adult human cells. The procedure had to be optimized, but it nevertheless worked. Thus, in the end, human embryonic stem cells were not required to develop the procedure for direct reprogramming, although it does appear they will be required for validation of the procedure.

[32] See Edward Furton, "Vaccines Originating in Abortion," *Ethics & Medics* 24.3 (March 1999): 3–4; and Daniel P. Maher, "Vaccines, Abortion, and Moral Coherence," *National Catholic Bioethics Quarterly* 2.1 (Spring 2002): 51–67. It should be mentioned that the Autumn 2006 issue of the *National Catholic Bioethics Quarterly* (6.3), titled "Ethics in Cell Research," was dedicated to a discussion of the use of vaccines from cell lines derived from aborted fetuses. Included were articles by Rene Leiva, "A Brief History of Human Diploid Cell Strains," 443–451; Very Rev. Angel Rodríguez Luño, "Ethical Reflections on Vaccines Using Cells from Aborted Fetuses," 453–459; Alexander R. Pruss, "Complicity, Fetal Tissue, and Vaccines," 461–470; Alvin Wong, "The Ethics of HEK-293," 473–495; Timothy P. Collins, "Human Technology Manufacturing Platforms," 497–515; and the Pontifical Academy

use such vaccines. Furton asked "whether or not [the use of vaccines originating in abortion] involves the Catholic in immoral cooperation with the evil of abortion." His answer was in the negative, saying that "use of a vaccine in the present does not cause the one who is immunized to share in the immoral intention or action of those who carried out the abortion in the past."[33] Maher likewise reasoned that being vaccinated, or agreeing to have one's children vaccinated, does not involve cooperation with the initial act of abortion from which the tissue used to generate the cell line was obtained. Moreover, he reasoned that being vaccinated would not lead others to believe that abortion is morally acceptable. He wrote that "vaccine production and, hence, use is morally separable from abortion, even though current production in fact depends upon cell lines derived from aborted fetal tissue. Vaccine production and abortion are morally independent."[34]

As part of his argument that those who agree to vaccination are not unethically benefiting from a past abortion, Furton says: "As for receiving benefits from past immoralities, that is a common feature of our fallen world. . . . Acts of wrongdoing in the past regularly redound to the benefit of descendants who had no hand in the original crimes. It would be a high standard indeed if we were to require all benefits that we receive in the present to be completely free of every immorality of the past."[35]

Might not these same words be spoken about the future use of iPS cells for medical therapies? Again, once embryonic stem cells are used to successfully vali-

for Life, "Moral Reflections on Vaccines Prepared from Cells Derived from Aborted Human Fetuses," 541–550. The last article is a moral analysis carried out by the Pontifical Academy for Life and approved by the Congregation for the Doctrine of the Faith of the Catholic Church. As such, it represents the official voice of the Catholic Church on the issue. The Pontifical Academy writes: "In any case, there remains a moral duty to continue to fight and to employ every lawful means in order to make life difficult for the pharmaceutical industries which act unscrupulously and unethically. However, the burden of this important battle cannot and must not fall on innocent children and on the health situation of the population—especially with regard to pregnant women" (548). The paper by Wong discusses the use of fetal human embryonic kidney-293 (HEK-293) cells in biomedical research.

[33] See Furton, "Vaccines Originating in Abortion," 3.

[34] See Maher, "Vaccines, Abortion, and Moral Coherence," 59. A similar line of reasoning has been used to argue that it is ethically acceptable for scientists directly to use cell lines from aborted fetuses in their research, as long as alternative cell lines are not available and there is no connection between the research and the abortion, which has occurred in the past. See Amy Argetsinger and Avram Goldstein, "GU to Continue Controversial Research: Use of Aborted Fetal Cells Prompts Probe at Catholic University," *Washington Post*, January 30, 2004, B01. Notice that the connection to abortion is more direct in this case than in the case of vaccines because the cell lines *themselves*, not a product derived from the cell lines (a vaccine), are being used. In any case, this same kind of reasoning could be used to argue that iPS cells that are validated using embryonic stem cells could be used ethically in the laboratory for drug development and testing experiments, for example. See also Wong, "Ethics of HEK-293."

[35] See Furton, "Vaccines Originating in Abortion," 3.

date iPS cells, they will be no longer needed, and their association with iPS cells will lie in the past.

One difference between the use of embryonic stem cells for validation of iPS cells and the use of cell lines from aborted fetuses for vaccine production is that, in the former case, there is no *physical* connection between the ethically tainted cells used and the medical product derived from them, while in the latter case, there is. Cells used in the production of vaccines are the material descendants of an abortion committed many years ago. In contrast, the connection in the former case is through the *knowledge gained* from study of embryonic stem cells. It is more indirect and is of a different sort, involving as it does the order of knowledge rather than the order of nature itself. No materials directly connected with the past destruction of human life are used. If anything, then, iPS cells validated using embryonic stem cells are less ethically problematic than vaccines produced using cell lines derived from aborted fetuses.

Even still, one who values embryonic life might find himself not fully convinced. Is there not an element of sadness and resignation in accepting something (iPS cells) associated with an unjust act (destruction of an embryo), even if this act was committed in the past? One might experience this same kind of sadness and resignation when, for example, one walks into a magnificent European cathedral that is adorned with gold stolen from native Americans by conquistadors centuries ago, or benefits from an economy that was forged on the backs of African slaves in the pre-Civil War United States, or plays a piano with keys made of ivory obtained from elephants slaughtered decades ago. And yet, should not our response to past injustices be to vow not to allow them to occur again in the future? Is not such a response more constructive than focusing on past events (although, clearly, we should learn from the past)?

It is also good to acknowledge the contingency of the current situation. If Shinya Yamanaka had not relentlessly pursued direct reprogramming, iPS cells would not exist today.[36] And, if scientists do not embrace iPS cells in their research, they could very well turn again to using embryonic stem cells. But it appears that many scientists *will* choose iPS cells over embryonic stem cells. As James Thomson noted, "Over time, these [induced] cells will be used in more and more labs. And human embryo stem cell research will be abandoned by more and more labs."[37] It should be emphasized here that the reasons scientists are switching to iPS cells are

[36] The motivation and drive of Dr. Yamanaka to find a replacement for human embryonic stem cells in research was highlighted recently in a *New York Times* article. See Martin Fackler, "Risk Taking Is in His Genes," *New York Times*, December 11, 2007, D1. In the article, Yamanaka describes his reaction to seeing a human embryo through a microscope at a friend's fertility clinic: "When I saw the embryo, I suddenly realized there was such a small difference between it and my daughters. … I thought, we can't keep destroying embryos for our research. There must be another way."

[37] Colin Nickerson, "Breakthrough on Stem Cells: Reprogramming of Human Skin May Circumvent Ethics Controversy," *Boston Globe*, November 21, 2007, A1.

not because their opinion of the embryo has changed. Before iPS cells arrived, most scientists did not believe that human embryos had moral status as individuals. They still do not. Virtually no one, on either side of the embryonic stem cell debate, has changed his mind. Rather, the scientific landscape has changed—dramatically.

Amazingly, it is now possible to use an adult cell to produce a patient-specific pluripotent cell that is practically indistinguishable from an embryonic stem cell. It is as if the rules of engagement in the battle over this type of cell research have changed; combatants on both sides are left standing, arms down, looking around, thinking, "What happened?" But in all of this, the ethical landscape has remained virtually unchanged. Both sides feel exactly as they did before. It is good to emphasize this point, and to realize that scientists have switched to iPS cells for very practical reasons: iPS cells are easy to generate in the laboratory, and federal funding is available to perform research using them. An awareness of this reality will allow those opposed to the destruction of human life to not become complacent.

The Way Forward

The successful conversion of adult cells into pluripotent stem cells that can change into all of the cell types of the human body opens a new chapter in the study of disease and the development of regenerative medicine. It also provides a historic opportunity to turn away from embryonic stem cells isolated from human embryos and to embrace an ethically acceptable alternative: induced pluripotent stem cells. However, it is true that the validation of induced pluripotent stem cells is tainted by association with human embryonic stem cell lines that were developed in the past and will be required for validation. But this association is similar to the one between vaccines and the cell lines originating in abortion that were used for their production. This association can lead to sadness and a sense of resignation on the part of those who value life. Still, it is important to move forward. Human induced pluripotent stem cells will serve to stem the tide of human embryonic stem cell research, changing it, and diverting stem cell research in a more ethical direction.

One final point is worth making. Direct reprogramming and the production of induced pluripotent stem cells have created some breathing room in the debate over the ethical use of human biomedical technology. This reprieve now provides an opportunity for those engaged in the debate to take a step back, forge ties with persons of different political affiliations, and think deeply about ethical challenges that may lie in the future. It is an astounding discovery that pluripotency, that is, the ability of a cell to change into all of the cell types of the body, can be so easily induced. This and other recent discoveries in biology have given us a deeper understanding of the nature of life and organisms. In the current scientific reality in which groundbreaking discoveries are being made on a frequent basis, we need to ask, "Is the philosophical framework we now have adequate? Or do we need to devise a new, modified philosophical framework that incorporates a more integrated view of the organism?" I would argue for the latter. It is imperative that philosophers have the tools to handle the scientific and technological breakthroughs that loom on the horizon.