



“As to diseases, make a habit of two things —  
to help, or at least *do no harm*.”

— Hippocrates, *The Epidemics* —

## TWO MAJOR STUDIES SHOW: HUMAN PLURIPOTENT STEM CELLS WITHOUT CLONING OR DESTROYING EMBRYO

*“On both ethical and practical grounds, direct programming  
is superior to cloning as a means of obtaining patient-specific pluripotent stem cells.”*

Two major scientific papers published this week in *Science* and *Cell* unveil a proven way to generate patient-matched human pluripotent stem cells without human cloning, and without the use of human embryos or human or animal eggs. Research groups in Wisconsin and Japan have generated “induced pluripotent stem” (iPS) cells with the properties of human embryonic stem cells by direct reprogramming of adult cells.

These are the studies which prompted Ian Wilmut, creator of Dolly the sheep and one of the world’s leading authorities on the cloning process Somatic Cell Nuclear Transfer (SCNT), to announce that he was abandoning SCNT to focus on reprogramming instead.

The following summary and analysis was produced by Maureen Condic, Ph.D., Associate Professor, Department of Neurobiology and Anatomy, University of Utah School of Medicine, and Markus Grompe, M.D., Director, Oregon Stem Cell Center and Professor, Department of Molecular and Medical Genetics, Oregon Health Sciences University (affiliations are for reference only).

### Summary of results and significance

Unlike embryonic stem cells, which are obtained by destroying live embryos, iPSCs are made directly from adult cells by adding a small number of factors to these cells in the laboratory. These factors remodel the mature cells and convert them into stem cells that are functionally identical to stem cells obtained from embryos. No human eggs are required and no human embryos are generated. Adult cells are obtained from a simple skin biopsy, 1/10<sup>th</sup> inch in diameter and about as painful as a blood draw. One study was able to produce an average of 10 pluripotent stem cell lines from a single skin biopsy. This approach can be used to generate stem cell lines from patients with specific genetic diseases to better study these conditions, and to provide patient-specific stem cells for possible stem cell therapies.

Direct reprogramming of human cells is one of the most significant scientific findings of the last quarter century; more significant than cloning Dolly the sheep. Indeed, the scientist who originally cloned Dolly, Professor Ian Wilmut, recently stated that direct reprogramming is “extremely exciting and astonishing”, a scientific approach he finds “100 times more interesting” than cloning—so much more interesting that he will abandon cloning research and pursue direct reprogramming instead.

The power of direct reprogramming is that, like cloning, it generates stem cells that are genetically identical to the patient who donated the adult cells. Reprogrammed cells would not be rejected by the patient’s immune system if used for medical therapies. Unlike human cloning, which has thus far not been accomplished and remains only a theoretical possibility, iPSCs have been generated by two independent laboratories, making patient-specific pluripotent stem cells a reality today. Also unlike cloning, no eggs are needed for the iPS procedure and no human embryos are produced or destroyed, thus resolving major ethical and practical difficulties associated with the cloning procedure. Thus, on both ethical and practical grounds, direct programming is superior to cloning as a means of obtaining patient-specific pluripotent stem cells.

### Possible concerns and responses:

*Concern:* The studies by Thomson and Yamanaka use viral vectors that integrate into the DNA of the cell, potentially causing dangerous mutations. This approach is unsafe for use in human patients.

*Response:* The NIH has established guidelines for clinical use of genetically modified cells (<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>), and dozens of clinical trials are currently underway using a wide range of viral vectors, including retrovirus. The study by Thomson’s group used lentivirus, one of the safest vectors for use in humans. In addition, it is technically possible to remove integrated viruses from the reprogrammed stem cells once they have achieved their mission.

Finally, both studies strongly indicate that reprogramming requires only transient expression of the manipulated factors, suggesting these factors could be supplied by other means that do not require viral integration into the cell’s DNA.

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*Concern:* The studies by Yamanaka have used c-Myc, a gene that is associated with cancer in humans. The requirement for this gene indicates the iPS approach cannot be used in human patients.

*Response:* The study by Thomson's group used different factors than those used by Yamanaka, and accomplished direct programming without using c-Myc. Yamanaka also indicates that direct reprogramming is possible using only three of the four factors he previously described, and can be accomplished without c-Myc, albeit at a lower efficiency. Reducing efficiency would not be a problem for clinical or scientific purposes, since very large numbers of cells can be obtained from one simple biopsy of tissue from a patient.

*Concern:* iPS cells are not a natural cell derived from embryos. While these cells are scientifically interesting, we need to continue studying pluripotent stem cells from embryos obtained by fertilization and from cloned human embryos to determine which source of cells is best for research and therapies. All lines of research must go forward.

*Response:* Neither iPSCs nor cloned stem cells result from a "natural" process, yet both are superior to stem cells from "spare" IVF embryos in the sense that they could provide patient-specific stem cells. iPSCs are also superior to cloned stem cells in several respects. Making stem cells from cloned human embryos is only a theoretical possibility that has not yet been accomplished, while human iPSCs have been independently produced by two different laboratories. Moreover, cloned animal embryos and the stem cells derived from them have abnormal patterns of gene expression.

Finally, because iPS does not involve either human eggs or human embryos, this approach is ethically uncompromised. In light of these considerations, iPSCs are clearly superior to stem cells obtained from cloned embryos, and major leaders in the field are abandoning cloning in favor of the iPS technique.

*Concern:* The efficiency of generating iPS cells is quite low, suggesting that reprogramming may require unknown alterations to the normal genome.

*Response:* The low efficiency of generating iPS cells is not an issue for either clinical or scientific applications, due to the very large number of cells that can be obtained from a small biopsy of tissue from a patient. Moreover, any unknown genetic alterations produced by the reprogramming procedure would not reduce the utility of these cells for studies of human genetic disease in the laboratory—the most immediate scientific application of patient-specific pluripotent stem cells—because multiple lines can easily be generated from a single patient. Finally, prior to use in human patients, iPS cells, like stem cells obtained from any source (including both natural and cloned embryos), would have to be thoroughly tested to characterize their genetic state and determine whether they are safe for clinical use. Importantly, due to the nature of the possible abnormalities introduced by direct reprogramming (principally genetic defects), there are multiple ways to ensure the safety of iPS cells prior to use in patients, while it would be difficult to determine with confidence whether cloned embryonic stem cells were safe for clinical use.

*Concern:* The reprogramming factors may be oncogenic (i.e., promote the formation or development of cancerous tumors) and cause the iPCs to be tumor prone even if expressed only transiently.

*Response:* The basic biochemistry of reprogramming is very likely to be similar regardless of whether the process occurs naturally, by the egg or by direct manipulation. If this turns out to be a problem, it will be a problem for cloning or any other procedure requiring reprogramming of mature cells.

*Concern:* How do you know that the reprogramming process will not accidentally generate a totipotent cell, and therefore rekindle concerns about making human embryos for research?

*Response:* The one-cell human embryo is uniquely able to generate all the cells of the human body and organize these cells into a coherent, integrated body plan; i.e. this cell is "totipotent". Human embryonic stem cells and iPS cells are "pluripotent"; i.e., able to make all cell types of the mature body but not to organize these cells into an integrated body plan.

Pluripotency reflects the state of the cell's DNA, and direct reprogramming works by inducing the DNA of a mature cell to assume a pluripotent state. In contrast, totipotency depends *both* on the state of the DNA and on the precise organization of critical factors (proteins and mRNA) in the oocyte. Oocytes must contain specific factors in specific amounts and at specific locations to be capable of helping to generate a totipotent embryo at fertilization or following nuclear transfer. The critical factors present in oocytes are produced over a long period

of time during the complex process of egg formation, or “oogenesis”. Oogenesis is a ‘team process’ involving multiple cells in the ovary, and produces a unique cell containing numerous factors necessary for the generation of a totipotent cell.

Unlike cloning, direct reprogramming *does not use oocytes (eggs) in any manner*. Moreover, reprogramming does not and cannot replicate the complex cell-to-cell interactions required to produce an oocyte. Merely altering the state of a cell’s DNA, as is done during reprogramming, could not “accidentally” generate a totipotent cell because the multiple non-DNA-associated factors required for totipotency are uniquely produced during oogenesis.

To produce a totipotent cell by reprogramming, one would have to *first* reprogram a cell to be an immature oocyte, reproduce in the laboratory all the cell-to-cell interactions required to bring that oocyte to a mature state and *then* reprogram the DNA of the cell a second time to transform the DNA from an oocyte-state to that of a totipotent zygote. While it may be theoretically possible for a team of scientists to perform such a feat, it certainly could not happen “accidentally”.

Finally, there are strong molecular differences between an iPSC and a totipotent embryo or zygote. Several of the factors used for reprogramming, notably Nanog and Sox2, are not expressed in the zygote, but are present only in the pluripotent inner cell mass, thus clearly distinguishing a totipotent embryo from a pluripotent stem cell. Because such ‘pluripotency-only’ factors are used for reprogramming, they categorically prevent the reprogrammed cell from ‘accidentally’ becoming a totipotent embryo.

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