

Safety issues in cell-based intervention trials

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We report on the deliberations of an interdisciplinary group of experts in science, law, and philosophy who convened to discuss novel ethical and policy challenges in stem cell research. In this report we discuss the ethical and policy implications of safety concerns in the transition from basic laboratory research to clinical applications of cell-based therapies derived from stem cells. Although many features of this transition from lab to clinic are common to other therapies, three aspects of stem cell biology pose unique challenges. First, tension regarding the use of human embryos may complicate the scientific development of safe and effective cell lines. Second, because human stem cells were not developed in the laboratory until 1998, few safety questions relating to human applications have been addressed in animal research. Third, preclinical and clinical testing of biologic agents, particularly those as inherently complex as mammalian cells, present formidable challenges, such as the need to develop suitable standardized assays and the difficulty of selecting appropriate patient populations for early phase trials. We recommend that scientists, policy makers, and the public discuss these issues responsibly, and further, that a national advisory committee to oversee human trials of cell therapies be established. (*Fertil Steril*® 2003;80:1077–85. ©2003 by American Society for Reproductive Medicine.)

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The potential of stem cells and related cell-based therapies to treat disease and injury in humans has generated great excitement in the scientific community, as well as among patients and their advocates. At the same time, ethical and political debates about the use of human embryos in medical research have captured the public eye. There is considerable disagreement about the importance of embryonic sources of stem cells to the advancement of cell science, and there is no agreement as to whether the anticipated benefits justify either the destruction of existing embryos or the creation of embryos for purposes of research.

This debate, however energetic, does not exhaust the moral questions that need to be considered in policy-making about stem cell research. We consider it essential to engage in serious discussions about the next generation of ethical and policy issues in stem cell research on an ongoing basis while research advances, rather than to react to scientific developments after they occur. As an interdisciplinary group with expertise in science, medicine, law, and ethics, we considered the following question: does the transition in stem cell research from the laboratory to first human trials and, ultimately, to human therapies raise

any particular ethical and policy issues that are either unique to the stem cell context or of heightened concern? We answered this question by focusing first on considerations of safety in early human trials, and second on considerations of justice. In this paper, we address the different safety considerations that emerge in the transition to research involving human subjects.

Like every new area of biomedical science, stem cell research is under considerable pressure from patient groups to develop human therapies as rapidly as possible. At the same time, however, there are moral obligations to refrain from moving to first human trials until the risks involved are properly characterized in laboratory and animal studies. In stem cell research, this tension is particularly acute. There are three ways in which stem cell research poses unique problems when compared with other new therapeutic strategies. First, controversies about the use of early human embryos in research have made it politically difficult, at least in the United States, to fund research that establishes new embryonic stem cell lines. Therefore, the selection of stem cell sources for preclinical and clinical testing is limited, at least in part, by policies established to protect human embryos from destruction. We shall describe how this results in tension between the goal of protecting human subjects and patients from risks and the goal of protecting early human embryonic life. Second, safety information about stem cells is very incomplete, partly because human stem cells have only been developed in the laboratory since 1998. Although research on human stem cells is based on 20 years of animal stem cell experiments, investigators working on nonhuman stem cells have not generally addressed the safety issues that come into play in human clinical applications. Third, the extensive regulatory structure and preclinical and clinical research methods used to evaluate new medical treatments are most sophisticated in the area of drug development. As exemplified by the field of human gene transfer, new biological agents, especially those that are inherently complex like cell-based therapies, pose especially difficult problems in risk evaluation, selection of appropriate assays for clinical outcomes, and selection of study populations.

SELECTION OF STEM CELL LINES AS SOURCES FOR EXPERIMENTAL INTERVENTIONS

The Food and Drug Administration (FDA), the institutional review boards (IRBs), and other bodies involved in the oversight of stem cell research with human subjects will need to address three types of safety concerns relevant to the selection of stem cell sources for human use: risk of infectious disease, transfer of genetic disorders, and quality control of stem cell lines and their derivatives.

Infectious Disease

The risk of infectious disease transmission from donor to recipient is common to all types of tissue and organ trans-

plant. Thus, for the most part, the risks associated with the transmission of infections in stem cell research can be characterized based on experiences with other types of tissue donation. Screening is essential to protect recipients from infectious agents, and emerging infectious diseases that escape detection pose a problem. With one notable exception, stem cells do not pose any greater risk of infection than any other tissue or organ transplant. This exception is a consequence of current U.S. policy, which restricts federal funding of embryonic stem cell research to a fixed number of stem cell lines approved in August of 2001. All of these lines were derived using mouse feeder layers; thus, any potential therapy developed from these lines carries the possibility of cross-species transfer of infectious agents. The nature or extent of this risk is not known. To date, the FDA has stated that the use of such stem cells would fall under the regulatory framework for xenotransplantation (1). Rules for xenotransplantation include specific testing and sampling requirements to assess the risks of infectious disease, as well as specific requirements that potential subjects and patients be informed about these risks and about the need to abstain from blood or tissue donation after receiving a xenotransplant (2).

There is currently considerable work under way around the world to identify reliable ways to grow human stem cell lines without mouse feeder layers (3–6). Although cell lines originally derived using mouse feeders can now be cultured and expanded with human feeders, there are no cell lines approved for federally funded research that avoided mouse contamination during the process of derivation. A central question for the ethics of stem cell research is whether human trials should await the development of new cell lines that are derived *de novo* without mouse feeders, thus providing maximum protection for the first human subjects. The policy implications of this conundrum are significant.

Depending on how the risks of cross-species infection are viewed, obligations to minimize risks to human subjects conflict with obligations to protect embryonic human life (7, 8). For those who believe that it is ethically acceptable to destroy embryos to advance biomedical science, there is no justification for exposing human subjects to the risk of cross-species infections, no matter how small. By contrast, those who place a high value on protecting embryonic human life are not likely to view a theoretical risk of cross-species infection as sufficient to justify the creation of new embryonic stem cell lines.

To minimize the risk of harm to human subjects, we recommend that only mouse-free stem cell sources be permitted in human testing. Although the risk of transmission of infectious disease across species barriers is likely quite small, a technical alternative appears to be at hand in which this risk is eliminated entirely. Thus, unlike other future uses of products carrying xenotransplantation risks, in which the potential benefits of the technology may outweigh the risks,

in this case, there are no medical benefits from using stem cells lines derived with mouse feeder layers to offset the risks, however small, to human subjects and to the population at large. Although we are not all of one mind with regard to the moral status of human embryos, we all agree that in this instance the imperative to protect human subjects and ultimately to produce safe human therapies justifies the destruction of human embryos that will be necessary to produce new mouse-free stem cell lines. Put more strongly, we believe that it would be unethical to expose human subjects to stem cell lines that have been derived with mouse feeder layers.

If our view about the ethics of human stem cell research prevails, first human trials will not be eligible for federal funding under current policy limits. The implications of such an outcome are worrisome. If the private sector is the only source of financial support in the United States, not only for the development of new (mouse-free) embryonic stem cell lines but also for human trials, the pace of this research is likely to be significantly slowed. Moreover, from the perspective of the protection of human subjects, the absence of federal funding means a reduced role for federal oversight of the ethics of human stem cell research.

Quality Control

Additional concerns that are relevant to stem cell sources relate to their propagation and storage in laboratories. Good manufacturing practices and quality control procedures need to be established for stem cell lines that will potentially be used for future cell therapies. The FDA Biological Response Modifiers Advisory Committee (BRMAC) has identified the need for traceability of manufacturing processes, from the original donor to the final cell preparation used in human trials. Experience with other cell-based therapies provides some precedent for these quality control standards. However, embryonic stem cell sources pose some unique problems with regard to cell passage, expansion, and preservation. Unlike hematopoietic stem cell transplants, for example, which are harvested and expanded once for an individual patient, potentially immortal stem cell lines can be passaged in the laboratory for many generations. Appropriate standards and assays will need to be developed to test stability of the lines, and good manufacturing procedures must be established for every step of the development of specific derivatives of these cell lines to be used in human trials. It is unclear at this writing if the stem cell lines currently approved for federal funding will meet these standards. If they do not, then it will be unethical to use these lines in human subjects. And, once again under current policy, human stem cell research will not be eligible for federal funding (9).

Genetic Risks

Transfer of genetic disorders is a safety concern that is difficult to evaluate. It seems reasonable to assume that many genetic disorders will not have an effect on a specific cell

lineage derived from stem cells, particularly if the altered gene product is not essential for that cell type or for its function. However, in the absence of animal and human trials, it is not clear how specific cells will behave after transplant. It seems prudent to screen donor tissue for genetic disorders that would be directly relevant to the proposed use of the stem cells, such as type I diabetes for islet transplant.

It remains to be determined how much genetic screening should be done on a routine basis or whether donor tissue should be excluded if a genetic mutation unrelated to the disease being treated is present. Given that hundreds of tests for genetic mutations of clinical significance are now available, a key question is what, if any, genetic mutations stem cell lines should be tested for, in addition to those believed to be directly relevant to the intended therapeutic function of the cells.

Concerns about the transmission of genetic risks directly affect the suitability of existing approved lines for use with human subjects. Determining whether any of these lines should be used in the first human trials requires resolving not only concerns about cross-species infection and quality control, but also about genetic risks. In the absence of information about the potential for genetic risk in currently approved stem cell lines, there is the possibility of yet another conflict between a focus on safety and commitments to protect embryonic human life. Decisions need to be made as to which genetic tests should be performed on the approved lines, and the results of these tests should be made public. Looking to the future, insofar as embryos may serve as sources of stem cell lines, a priority on safety suggests that family medical histories should be collected from couples who are willing to donate leftover embryos in infertility clinics.

PRECLINICAL TESTING

There are a number of risks associated with cell-based therapies that can only be fully assessed using in vivo testing. At least four types of risks will need to be evaluated with preclinical animal testing, and in the future, in early phase human trials. First, cells might “misdifferentiate” in vivo, that is, differentiate into undesirable cell types or fail to express the properties of the fully differentiated cells that are needed for therapeutic purposes. The effects of misdifferentiation are extremely difficult to predict. Second, cells could mistarget and not reach the appropriate site or migrate to other tissues or organs, potentially causing dangerous side effects. Third, transplanted cells also have the potential to form tumors as a result of inadequate regulation of cell division. Active cell division is an inherent property of stem cells, but not of most fully differentiated cell types. Fourth, transplanted cells could also be subject to immune system rejection, and in some cases, may contain immune system cells that could result in graft versus host disease (GVHD).

Mistargeting and Misdifferentiation

The prospect that stem cells may miss their targets or differentiate improperly poses both short-term and long-term risks to potential human subjects. These risks are largely unknown. There is currently a paucity of data from animal experiments, and, in some cases, a lack of good animal models for testing. It is still unclear whether homogeneous cell populations can be generated, and if not, whether heterogeneity will cause problems *in vivo* after transplant. It is also not known whether cells can “de-differentiate” after selection and transplantation, forming tissue types other than that which was intended. Even when cells differentiate properly, there is no guarantee that they will migrate to appropriate organs or compartments in the recipient, and it is not known what consequences might ensue from migration to unintended sites. Furthermore, proper differentiation and migration does not ensure cell-specific function within a given environment. Although laboratory work is under way on most of these issues, much more animal testing is required before the processes of differentiation and migration can be better understood.

One potential strategy for dealing with cells that have gone awry after transplantation is the use of suicide genes to render cell lines susceptible to specific drugs, which could then be administered to the transplant recipient to ablate the transplanted cells. This strategy has been studied in oncology treatments, where retroviral vectors carrying specific drug sensitivity genes—for example, the herpes virus thymidine kinase gene (HSV-tk)—are used to target tumor cells *in vivo* (10). The HSV-tk gene renders cells susceptible to ganciclovir, a prodrug that is modified by the tk enzyme and becomes toxic to dividing cells. Although the approach is promising, in clinical trials there are technical obstacles, such as resistance to drugs in tumor cells, low prodrug concentrations within tumors, mutations in the TK gene, and low percentage of tumors in S-phase, which is required for the TK strategy to work (11). Laboratory work with mouse ES cells transduced with HSV-tk and *Escherichia coli* gpt genes showed that these cell lines were susceptible to ablation with ganciclovir and thioxanthene, respectively, at concentrations that were well tolerated by nontransduced cells (12). In the same study, mesenchymal stem cells and hematopoietic precursors were somewhat less sensitive to ablation; it is also not clear if fully differentiated cells will behave similarly *in vivo*. Recently, a private company obtained patent rights for a suicide gene strategy for use in stem cells (13). Thus, the suicide gene strategy is promising in the development of human cell therapies, but more investigation is needed.

There are challenges in selecting appropriate animal models for testing. In cases where embryonic stem cells are available from the same animal species used for testing, data on differentiation and targeting will accurately reflect cell-cell signaling pathways, hormone and cytokine effects, and other *in vivo* processes that involve species-specific mole-

cules. However, the use of animal stem cells in animal experiments will not answer questions about specific human stem cell lines or their derivatives. Given that the processes of culturing and differentiating cells are idiosyncratic and successful methods vary from one species to the next, the extent to which it is reasonable to extrapolate from test results with mouse cell lines to human cell lines is unclear. FDA requirements mandate that human cell-based therapies be tested in a least two animal models before testing in human trials. Although this is appropriate for determining the behavior of specific human cell lines before human use, again, it may be problematic to interpret the data from these experiments, given that cell-cell signaling, targeting, and response to other biochemical signals may all depend on species-specific signals.

One of the challenges in animal testing is determining what methods to use to monitor cell fate and performance. The FDA BRMAC has identified parameters that need to be assessed: cell migration and differentiation, cell phenotypes expressed, functional integration of cells, and postimplantation cell survival. There are as yet no standard assays for these outcomes. Some of the assessment techniques include histopathology, immunohistochemistry, biochemical data, electrophysiology, and behavioral studies. There will most likely be an evolutionary process and dialogue between regulatory officials, stem cell researchers, and research sponsors about how to carry out appropriate preclinical tests to monitor postimplantation outcomes; at present this area of investigation is so new that no specific criteria for testing have been established.

Tumor Formation

The potential for tumor formation is difficult to characterize in animal experiments. In some cases tumor formation may only occur over long periods; small animals have short life spans. Experiments involving larger species are expensive and, for some, more ethically problematic. Moreover, it could take decades to establish with confidence the risk of tumorigenesis in, for example, nonhuman primates. The problem of long-term risks of new therapeutic modalities was illustrated dramatically in the case of leukemia recently reported in two French boys who had achieved immune reconstitution after gene transfer for X-linked severe combined immunodeficiency (SCID-X1) (14). The treatment, considered a landmark success in the field of human gene transfer, resulted in clinical improvement in 9 of 11 treated children; however, after 3 years of follow-up investigation, leukemia was detected in two patients. The disease was caused by integration of the retroviral vector upstream of an oncogene related to leukemia (15, 16).

It may be that risks of tumor formation are greater in cases where the transplanted cells are not fully differentiated. Yet for some therapeutic applications, it may be preferable to have final maturation of cells occur *in vivo*, rather than in laboratory culture. For example, stem cells treatments seem

promising for amyotrophic lateral sclerosis (ALS), a neurodegenerative disease marked by inevitable progression to complete paralysis and the lack of any effective treatment options. Recent work in a rat model of ALS demonstrates potential for neuronal cells derived from embryonic germ cells to alleviate symptoms (17). One obstacle to the use of cell-based therapies for ALS may be the need for new neuronal cells to migrate to appropriate locations in the spinal cord in order to re-energize areas damaged by progressive loss of motor neuron function (18). This, in principle, may be more difficult than the use of cell-based therapies to provide secretory molecules such as dopamine for Parkinson's disease or insulin for diabetes. It is possible that cells that are not fully differentiated may be more likely to migrate and form new synaptic connections—but these incompletely differentiated cells may carry more risks of mistargeting or tumor formation. Also, recent experiments showing that transplanted adult-derived hematopoietic stem cells can fuse with liver cells in the mouse (19, 20) are also cause for concern about tumorigenesis. Fusion events that result in tetraploidy may produce cells that are inherently unstable and prone to chromosome loss and uncontrolled growth, thus producing tumors in the recipient.

The need for collecting adequate data regarding the potential for tumor formation will affect the choice of animal models and the duration of follow-up in these animal studies. In some cases, immunosuppression might be used to allow more rapid formation and detection of tumors. Also, it may be possible to make some predictions about tumorigenicity of individual cell lines based on their degree of differentiation and homogeneity with respect to specific markers.

Insofar as tumor formation remains a concern for first human trials, suicide genes of the sort described to address problems with mistargeting and misdifferentiation could be used here as well, as a strategy for dealing with cells that have gone awry after transplantation. An open question in the ethics of first human trials is whether these trials should wait until a suicide gene strategy is available. Much turns here on the quality of the preclinical evidence that is likely to emerge in the near future about the risks of mistargeting, misdifferentiation, and tumor formation, and the feasibility of the suicide gene approach.

Immune Rejection

Immune rejection presents another type of risk to human subjects in trials in which the cell lines used are from donor, as opposed to autologous, sources. In organ transplantation, the significance of immune rejection varies depending on the type of organ transplanted. For example, the liver is less susceptible to rejection than the kidney. Even with HLA matching and immunosuppression, problems with immunological reactions are relatively common and can produce serious side effects or graft failure. In addition, immunosuppressive therapy itself carries significant risks. The development of a "universal" stem cell, that could be successfully

transplanted without HLA matching or immunosuppression, would negate concerns about immune rejection; however, the creation of such a cell remains technically out of reach at present and in the foreseeable future.

The importance of immune parameters in specific applications of cell-based interventions remains unclear. For example, the question of whether there are immunologic reactions to foreign tissue in the brain or how relevant this may be to cell-based therapies is not yet resolved. Animal experiments with neuronal cells derived from embryonic stem cells (21, 22) and human clinical trials with fetal neuronal tissue transplantation for Parkinson's disease show evidence of success (23). These experiments have provided proof of concept for the use of stem cell-derived neuronal cells for treatment of neurodegenerative disease, but some important questions remain. The fetal cell transplants for Parkinson's disease were carried out without immunosuppression (23) and in other studies fetal cell transplants survived for greater than a year after immunosuppression was stopped (24); however, autopsy studies of some patients who had received fetal cell transplants showed presence of immune and inflammatory markers (25). There are some data to suggest that immune activity in the brain is suppressed, as compared with other sites (26). For example, some investigators have described unique and specialized immune system regulation within the central nervous system (27), where typical immune activation markers may be expressed at low levels, but elevated in response to injury or inflammation. Additional animal studies may help clarify whether HLA matching or immunosuppressive therapies should be required in early human neural stem cell trials.

By contrast, in other applications of stem cell science such as cardiac repair, there is already ample evidence that immune rejection will be an important issue in achieving successful transplantation and thus that participants in early cardiac stem cell trials will likely require both HLA matching and immunosuppressive therapies.

HUMAN CLINICAL TRIALS

The first testing of new agents in humans is typically carried out in phase I clinical trials. Phase I studies are designed to test side effects and toxicity, and in some cases, to determine the maximum tolerable dose. Phase II studies collect further data on safety and on efficacy for the purpose of directing continuing research on the intervention, if warranted. Phase III research typically involves comparison of new agents to existing interventions, if any, in a larger population that is sufficient for robust statistical analysis. Phase IV research is postmarketing or surveillance research conducted after regulatory approval for the purpose of obtaining safety and efficacy information from larger population groups.

The three-phase clinical trial design is most well-developed in the area of drug testing, where there is a 40-year

history of rigorous systematic testing of new drugs (28). However, the three-phase design is also used in testing biologics like vaccines and human gene transfer agents. The division of clinical research into three phases that address, respectively, safety and toxicity, efficacy, and comparative efficacy is somewhat theoretical. In practice, efficacy data are frequently collected in Phase I research, and safety data are always collected in Phase III trials.

Who should participate in the first Phase I trials of potential stem cell therapies? Are there certain characteristics that would make it more ethically acceptable to have some people, and not others, bear the burdens of these first “human proof of concept” studies?

At one extreme, is a conventional practice in Phase I oncology research in which subjects are recruited from among cancer patients who have failed standard treatments. For these patient-subjects, there is generally little hope left for remission of disease. Phase I oncology research often involves measurement of pharmacokinetics as part of the assessment of the dosage effects, and in addition, trials of molecularly targeted agents may measure specific biochemical parameters relevant to the action of the drug, such as metabolite levels that indicate whether the specific drug effect has been achieved on a molecular level (29, 30). Phase I research for new chemotherapeutic agents is often conducted with a dose-escalation design, whereby the first patient-subjects receive low doses of the new agent, and dosage is progressively increased as new patients are enrolled (31). Typically, the initial dosage is too low to be clinically useful even in cases where the drug is subsequently determined to be effective. Thus, there is no prospect of medical benefit for the first patient-subjects. Even for later patient-subjects, the prospect of benefit is frequently remote. At the same time, participation in Phase I cancer trials can be burdensome and noxious, particularly as dosage is increased until toxicity becomes unacceptable to determine the maximum tolerable dose.

In contrast to oncology research, Phase I research on vaccines is normally carried out with healthy volunteers. Vaccines are usually designed as preventive strategies to be used in healthy individuals (with a few exceptions) and are expected to be relatively low risk interventions. The typical outcomes in Phase I research on vaccines are immunological parameters (32), side effects, and adverse events; side effects are expected to be minor and adverse events rare. Because many new vaccines fail to protect from infection, participants in Phase I vaccine trials frequently do not benefit medically from the experimental vaccine to which they are exposed. However, participation in such trials is usually not burdensome, and serious harms are relatively rare.

It is unlikely that Phase I stem cell trials will be conducted in healthy volunteers. From a technical standpoint, unlike for most vaccines, healthy people are not the intended target for stem cell interventions and thus they would not be the most

informative research subjects. Moreover, even if only new, quality controlled stem cell lines are used, and even if first human trials follow extensive preclinical testing, there will still be considerable uncertainty about both the magnitude and probability of the risks that the first human subjects will face. It is not even clear how to measure the safety and toxicity of stem cell grafts in human beings. The kinds of assays used in testing new pharmacologic agents, such as drug metabolites, blood chemistry, and liver enzyme levels, may not be entirely relevant. Many of the histological tests used in preclinical testing would largely be impossible in human patients, except in autopsy cases. Therefore, it may be difficult to assess location and function on a cellular level. Some imaging techniques may be available to observe changes in metabolic activity in specific tissue areas. In a study of neuronal cell transplant for stroke patients, for example, investigators monitored brain glucose metabolism in patients before and after cell transplant using positron emission tomography (PET) with ^{18}F fluorodeoxyglucose (FDG) (33). This technique demonstrated deficits in metabolic activity in the stroke area and corresponding improvements metabolic activity in some patients following transplant. However, it is impossible to determine which cells caused these metabolic changes or what their specific phenotypes and functions are.

With such uncertain risks of potentially serious consequence, the oncology model of recruiting first human subjects from among seriously ill patients for whom medicine has nothing to offer seems appropriate. Phase I stem cell trials may not follow a dose escalation model and thus, in theory, might offer some prospect of clinical benefit to even the first participants. However, because the risks are so unclear and because there is no way of assessing how likely it is that the first human experiments will work as planned, it would be ethically unacceptable to ask patients to forego treatments that are even partially effective to participate in the first proof-of-concept studies. This lesson was learned painfully in the aforementioned SCID gene intervention trial that resulted in two cases of leukemia out of 11 patient-subjects (34). As a result of these two adverse events, the trial was terminated, and the FDA suspended 27 other gene therapy protocols using retroviral vectors in order to review safety considerations (35). The trial has since been reinstated, but enrollment is now restricted to patients who do not have a matched bone marrow donor and thus are without meaningful alternative treatments.

Recruiting subjects from among patients who are facing life-threatening disease and have no viable treatment options is not without its ethical pitfalls, however. As many have noted in the context of Phase I cancer trials, a central concern is avoiding exploitation of patients desperately seeking reprieve from an incurable disease. Even if researchers are scrupulous in insisting that early trials are not expected to produce any clinical benefits to participants, patients may be

manipulated by their own emotions into believing that the experimental intervention is the miracle for which they have been praying. The emotional tensions are also significant for physician-investigators who must balance honesty with compassion and with their felt duty to support a hopeful, optimistic mindset among their seriously ill patients. Whether it is appropriate to allow or even encourage hope for medical benefit in settings where such benefit is only remotely possible is a controversial question. Some commentators believe that allowing patients to continue to hope is reasonable in that it may alleviate stress and promote well-being. Others are more concerned that such hopes are unrealistic and that a physician-investigator's role should be to encourage an understanding and acceptance of the very poor odds of clinical improvement.

Concerns about the validity of the consent that is obtained from potentially desperate patient-subjects have led some to argue in favor of enrolling the less seriously ill in early trials, at least in some cases. This tension played itself out in the Phase I trial of a viral vector carrying the ornithine transcarbamylase (OTC) gene in which an 18-year-old participant died (36, 37). While sick, often terminally ill OTC-deficient infants could have been enrolled into the trial, the consent process was thought to be problematic. First person consent was, of course, impossible. There was significant concern that shocked and desperate parents might have their judgment clouded by their newborns' tragic plight. As a consequence, a decision was made to enroll adult patients with a milder form of OTC deficiency whose disease was manageable with current treatments. In retrospect, it seems easy to conclude that it would have been ethically preferable to have elected to conduct this first human test of concept in dying newborns. Prospectively, the choice was far from obvious.

In the context of early human stem cell trials, enrolling the sickest patients may not always make the best scientific sense. In some cases, gravely ill patients may not be ideal for early trials for medical reasons. In a controlled study of fetal cell transplants for Parkinson's disease (23), patients younger than 60 had a positive response to the surgery, at least at 1-year follow-up, as compared with older patients, who experienced no benefit. At longer follow-up times, some of the benefit was lost for the younger patients, and some experienced dyskinesias, typically seen as a side effect of L-dopa treatment. Insofar as longer follow-up times may be necessary to characterize both safety and effectiveness, older or more seriously ill patients may not be the best technical choice for certain clinical trials. Also, in some cases, proof of concept, let alone clinical effectiveness, may be difficult to establish in patients with advanced disease. Because an essential condition of ethical human research is that the science is sound enough to adequately answer the research question, scientific objections to enrolling the seriously ill may also provide grounds for moral concern.

Questions about the need to characterize long-term risks will emerge in the transition from early human research to more widespread trials and, ultimately, to approved human therapies. In response to the emergence of leukemia in the children who received a genetic intervention for SCID-X1, researchers and regulators are considering whether longer follow-up periods and patient registries should be established for gene modification trials (38). It is not too soon to consider similar questions with regard to research involving stem cells. Model protocols designed to capture long-term risks should be designed now, with an eye toward collecting sufficient follow-up data relating to adverse events.

Developing medical treatments using new technologies or new paradigms is always ethically challenging (39). Determining the conditions under which it is reasonable to proceed with human experimentation is inherently difficult in the face of new and uncertain risks, especially when the clinical benefit of a new modality has yet to be demonstrated. In this regard, the field of stem cell science is no different from other new areas of medical research. The structure of the dynamic tension between the need to alleviate the suffering of severely ill patients and the need to proceed with caution into uncharted territory remains the same. The stakes seem particularly high in the case of stem cells, which provide a new and promising approach to especially intractable diseases. We have faced such high stakes before; however, unlike other advances in biomedical science, with stem cells, the ethics of the transition to the first human trials and ultimately to human therapies is made even more complicated by controversies about the moral standing of the human embryo.

CONCLUSION

We believe that it is unethical to expose human subjects to the stem cell lines that are currently approved for use in federally funded research. If our view about the ethics of human stem cell research prevails, first human trials will not be eligible for federal funding under current policy limits. This outcome is of deep concern. The pace of the transition from the laboratory to clinical research will likely be slowed, and the protections for human subjects potentially weakened.

In the area of laboratory and preclinical testing, a great deal of work still needs to be done to develop appropriate assays for cell differentiation, migration, and functional integration. Both same-species and inter-species testing should be carried out to determine the relevance of species-specific signaling and host environment and to select the most appropriate animal models for human disease. Follow-up times in animal experiments will have to take into account the problem of potential tumor formation. The question of how much animal testing should be required before human trials may ethically proceed will be difficult to resolve, given that cell-based therapy is a relatively new paradigm and appropriate assays and outcomes have yet to be defined.

In early human trials, there will be similar challenges in determining ways to measure the differentiation, migration, and function of cells after transplantation. Specific criteria will need to be developed for safety and toxicity testing in the first Phase I tests. These technical challenges will be accompanied by difficult choices in the selection of the first human subjects, given that the risks of new technologies are inherently difficult to predict. As is generally true in research involving human subjects, an analysis of the ethics of any particular proposed trial requires a detailed understanding of the relevant science and of the social and clinical context. We here propose only some broad guidance. Enrolling patients in first human trials who have clinically viable alternatives is presumptively unethical and should be avoided. In some cases, for example, where there is great uncertainty about risk or the biggest worry is about tumor formation or other longer term consequences, enrolling the more seriously ill patients may have some appeal. At the same time, however, special concern must be given to the needs and interests of seriously ill patients, including the potential for an unrealistic hope of benefit and difficulties in adequately grasping the extent of possible risks. Furthermore, the health status and progression of disease of individual patients are relevant to the ability to measure necessary biological outcomes. Gravely ill patients should not participate in trials if as a consequence the data that are obtained are of no or limited use.

Over the years, there have been many proposals for strategies to improve protections for human subjects in high-stakes, high-risk research. First human stem cell trials certainly fit this description. Serious consideration should be given to the establishment of patient advisory boards, consent monitors, patient advocates, and other procedures that will concentrate attention and energy on the interests of patient-subjects and their families as these first human trials go forward. Given the challenges facing the transition to human research, an open question is whether both the science and potential subjects would benefit from a national advisory committee on stem cell research, similar to the national Recombinant DNA Advisory Committee (RAC), which has oversight over federally funded human gene intervention research. We believe that the challenges of human research in stem cells can and must be addressed responsibly by scientists, policy makers, and the public to realize the full capacity of these new advances for alleviating human suffering.

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