Stem cells for tissue repair

Research on therapy using somatic and embryonic stem cells

Health Council of the Netherlands

aanbiedingsbrief Engels ongetekende versie

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to:

the Minister of Health, Welfare and Sport

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The Health Council, established in 1902, has the task of 'informing (the government and Parliament) concerning the scientific situation with respect to topics in the realm of public health' (art. 21 Health Law).

The Health Council receives most requests for advice from the administrators of Health, Welfare and Sport, Public Housing, Town and Country Planning and the Environment, Social Affairs & Employment, and Agriculture, Nature and Fisheries. The Council can also issue recommendations of its own volition. This generally involves identifying developments or trends that might be or become significant in terms of government policy.

The Health Council's recommendations are almost always prepared by multidisciplinary committees of individually appointed experts. Although foreigners serve on some committees, the members are generally Dutch.

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Inhoud

Glossary

Adult stem cell

a stem cell derived from the tissues or organs of an organism after birth (in contrast to embryonic or foetal stem cells).

Blastocyst

an embryo just prior to implantation in the wall of the uterus; a blastocyst consists of an outer layer of cells that are needed for implantation, and an inner cell mass from which all somatic and germ cells develop (see also 'pre-implantation embryo')

Cell nuclear transfer

the transfer of a cell nucleus to an ovum (or another cell) from which the nucleus has been removed.

Cell line

a given type of cell continuously cultured in the laboratory is referred to as a cell line (or cell culture)

Cloning

creating an organism that is genetically identical to another organism, or a cell that is genetically identical to another cell provided that the so-called mother and daughter cells are subsequently separated (see also reproductive and therapeutic cloning).

Differentiation

the development of an unspecialized cell into a cell with a given function, such as a liver or muscle cell.

Embryonic stem cell

a stem cell that is derived from a blastocyst. These cells are derived from the inner cell mass of a pre-implantation embryo; they are pluripotent.

Fertilized ovum

an ovum that has fused with a sperm.

Foetal stem cell

a stem cell derived from foetal tissue (in biological terms 'embryo' covers all stages of development up to eight weeks of pregnancy, from then on the term 'foetus' is used). A distinction is drawn between the foetal germ cells, from which the gametes develop, and the remaining foetal stem cells, which are the foetal somatic cells.

Germ cells

ova and sperm, and their precursors.

HLA system

the most important substances and component substances that identify material to the immune system as either self or non-self (human leukocyte antigens)

Implantation

the embedding of a blastocyst in the wall of the uterus.

In vitro and in vivo

outside and inside the body; *in vitro* (literally, in glass) generally means in the laboratory.

Multipotent

a cell's potential to develop in various directions (see also pluripotent and totipotent).

Pluripotent

a cell's potential to differentiate into virtually any type of cell (ectodermal, mesodermal and endodermal cells; all embryonic cells except those of the trophoblast; see also multipotent and totipotent).

Pre-implantation embryo

an embryo in the stage prior to its implantation in the wall of the uterus; the term pre-implantation embryo is generally used with regard to embryos *in vitro* (see also blastocyst).

Precursor cell

a cell that is capable of developing into a single specialized cell type.

Proliferation

the multiplication of cells by division.

Reproductive cloning

cloning with the aim of creating an organism.

Somatic stem cells

adult and foetal stem cells, with the exception of the foetal germ cells.

Spare embryo

an embryo, created by means of *in vitro* fertilization, which is not returned to the womb of the woman who donated the original ovum.

Stem cell

a cell which retains the potential to multiply for a long period of time and which is capable of developing into more than one type of differentiated cell.

Therapeutic cloning

cloning with the aim of obtaining stem cells for the treatment of diseases.

Totipotent

the potential to develop into any type of cell; the fertilized ovum and the cells of the early embryo (at the stage of the first few divisions) are totipotent (see also multipotent and pluripotent).

Executive summary

Damaged cells in tissue can sometimes be replaced by cells from a donor. Striking examples are cell transplants used in animal models for studying diabetes and in clinical trials with Parkinson patients. This research has given rise to the hope that it will be possible to repair human organs and tissue using donor cells. The analogy is with the current use of bone marrow transplants for bone marrow failure.

Not only could cell transplantation open up new possibilities of a cure for patients whose therapeutic options are currently inadequate, it could also be possible to help patients who currently have to wait for an organ donor. These prospects, however uncertain they may be, have led to an intensification of research into cell transplantation for therapeutic purposes. Until recently, this research was mainly carried out using cells or tissue fragments from embryonic or foetal organs. Although successes were achieved here, the limited survival of the transplanted cells and the lack of human embryonic or foetal tissue means that it is difficult to test this cell therapy in large groups of patients. That is why an intensive search is going on for other donor cells.

Research, mainly in mice, has identified two new potential types of donor cell:

- *Embryonic stem cells*. These cells are obtained from pre-implantation embryos. In principle, they can be reproduced endlessly and they are pluripotent. In other words, they can mature into almost any type of cell. To a certain extent, this process of maturation (differentiation) can be directed *in vitro*. Researchers have

succeeded in obtaining a large number of various types of cell, including neurons, cardiac cells and epidermal cells.

- *Somatic stem cells*. Bone marrow, for example, usually contains cells of this kind, which can differentiate into any type of cell found in the blood. These cells are called 'multipotent' cells: in the right circumstances, they can develop into many cell types but not all. Recent research has led to the conclusion that, in adults, somatic stem cells are not only present in bone marrow but also in other tissue. In addition, indications have been found that these somatic stem cells can form more types of cells than was thought previously. For example, there are reports of somatic stem cells from bone marrow developing into cardiac cells or neurons in certain experimental conditions. Here and there, this has given rise to the expectation that somatic stem cells could, in the foreseeable future, replace embryonic stem cells as a source of cells for transplantation purposes. The Health Council committee which drew up this advisory report -- referred to hereinafter as the committee -- believes that this is over-optimistic. Research into somatic stem cells suffers from limitations in availability. There are as yet no simple procedures for obtaining these cells in pure form. They are located in very small numbers in the tissues of adult animals/people, and their reproductive capacity would appear to be less than that of embryonic stem cells. Given the current state of the art, it cannot be predicted whether these problems can be solved. The extent to which this research is still in the early stages is shown by the fact that doubts have recently arisen about the identity and the nature of these stem cells. In time, somatic stem cells could, however, have the advantage that they could possibly be taken from the patients themselves, thereby getting around the problem of rejection.

Research into possible cell therapy in humans is - with the exception of bone marrow transplants - still in the experimental stage. Research into the suitability of embryonic stem cells for cell therapy has only just begun. It is estimated that, world-wide, approximately seventy human embryonic stem cell lines have been isolated, some of which are the property of commercial companies. It is not certain in all cases whether the cell lines are entirely normal (for example, free of chromosomal abnormalities). Other limitations are that the donors are not a representative cross-section of the global population and that the possibility is not excluded that the cell lines may be contaminated with viruses and prion protein. So more cell lines are needed, particularly cell lines which have been isolated using public funds and which are freely available for research. Research into stem cell therapy is now concentrating primarily on improvements in the culture conditions and on the conditions for differentiation to specific cell types.

A problem with stem cell therapy is the rejection of foreign cells, a problem which can be compared to the rejection of a donor kidney or heart. If the range of available stem cell lines is adequate, it will perhaps be possible to obtain a good match between the donor cells and the patient, as is the case at present with organ donation. It will perhaps prove possible to make specific modifications in the genetic properties of the donor cells so that the donor cell will not be seen as foreign by the immune system of the patient. Another possibility currently under examination is the replacement of the nucleus in a human embryonic stem cell by a nucleus from the patient, so that the donor cells take over the properties of the cells of the patient. It is not yet possible to replace the nucleus of embryonic stem cells while preserving their function. That is why intensive research is being conducted into two alternatives:

- Replacement of the nucleus of an unfertilised human egg cell with the nucleus taken from a somatic cell from the target patient. The unfortunate term 'therapeutic cloning' is used for this nuclear substitution technique. After nuclear substitution, the embryonic development of the egg cell is initiated and embryonic stem cells are harvested from the pre-implantation embryo produced in the test tube. In addition to ethical problems, this procedure involves numerous problems in the practical and cellular biology fields. For each patient-specific cell line, human egg cells are needed and, for the time being, these cannot be obtained without burdening the donor. Furthermore, animal trials have shown that embryos obtained from nuclear substitution are often abnormal because the normal nucleus of a fertilised egg cell is different from the new nucleus taken from a somatic cell. The extent to which this complication could also limit the use of such stem cells for transplantation purposes cannot yet be estimated given the current scientific knowledge.
- The procedure above, but using animal rather than human egg cells. This solves the problem of the availability of donor egg cells but involves another complication. DNA from the original mammal is still to be found in the mitochondria in stem cells that are obtained from an egg cell containing a human nucleus. This could lead to problems with the production of mitochondria in specialised tissue. It is not clear whether this complication will, in practice, make the use of cells of this kind impossible in humans for transplantation purposes.

The isolation of human embryonic stem cells involves the sacrifice of human embryos and this has resulted in heated international discussions. In the Netherlands, research on human embryos has been discussed for the past 20 years. The Embryo Bill which was approved by the *Tweede en Eerste Kamer der Staten-Generaal* (Parliament) makes it possible to conduct research using human embryos, on condition that the required approval has been obtained, that the research can reasonably be expected to result in

new insights in the area of medical science and that those insights cannot be obtained using other methods.

The committee is of the opinion that research using embryonic stem cells could indeed result in important new insights and therapeutic possibilities. None of the existing alternatives holds out the advance prospect of the development of a wide range of therapies. The prospects for the use of somatic stem cells are uncertain. Xenotransplantation is, in principle, banned in the Netherlands and it remains to be seen whether the risks it involves can be eliminated.

The proposed Embryo Bill makes it possible to isolate new embryonic stem cell lines from existing embryos left over after IVF (section 8). It is not permitted to create embryos specially for this purpose (section 24a), but this ban is not irreversible (section 33(2)). If the ban is lifted, it will be possible to replace the nucleus in human (or animal) egg cells with a donor nucleus (therapeutic cloning), with the aim of developing a pre-embryo suitable for the isolation of stem cells. The committee does not believe these trials are urgent, but does believe that they will be important when it becomes clear that cell therapy with embryonic stem cells is effective and no feasible alternatives have been developed.

Embryos resulting from nuclear substitution can, in theory, also be returned to the womb in order to induce pregnancy (reproductive cloning). This is forbidden by the Embryo Bill. At present, it would also be completely improper given the considerable risk of children being born with severe congenital deformities. In this respect, there is a consensus in scientific circles. However, the committee does not see this as a reason to impose unnecessary restrictions on nuclear substitution in human egg cells ('therapeutic cloning').

Research on human embryos is covered by the proposed Embryo Bill. The harvesting of somatic stem cells is covered by the Foetal Tissue Act, the Organ Donation Act or the Medical Research (Human Subjects) Act. The experimental use of cell therapy in people is included in the remit of the existing medical-ethical committees. The committee does not see any reason to introduce supplementary regulation. The committee does advise placing nuclear substitution experiments in which animal egg cells are used under the supervision of the central committee which monitors experiments with human embryos. The embryos originating from egg cells modified in this way can be considered to be human embryos. On the other hand, the committee does not consider it to be necessary, in accordance with the proposed Embryo Bill, for cellular biology research on human embryonic stem cells to be placed under central supervision. These cells do not have the potential to become viable embryos. They are human cells, like epidermal cells or tumour cells, for which no supervision is in place and for which no supervision is necessary.

This advisory report places the emphasis on the practical question of what can be expected from stem cells for transplantation purposes. In addition, the committee also devotes some attention to the possibilities of using stem cell research to determine how a pluripotent stem cell differentiates into specialised cells such as neurons or cardiac cells. With stem cells, this differentiation process can be followed step-by-step *in vitro*. With new techniques for determining the activity of all human genes in a limited number of cells, it can be expected that, in the years to come, a cornucopia of information will be acquired about the central issues in human biology: what is the difference between a neuron and a cardiac cell, how is this difference established within our bodies and how can we influence it? In the long term, this insight into the programming of our cells may result in increasing possibilities for reprogramming cells so that, ultimately, perhaps even cultured epidermal cells can be reprogrammed and embryonic stem cells will become redundant. Dutch research, with its strong position in genetics and cellular biology, is in a good position to make a major contribution to this new research field.

For the clinical application of stem cell therapy in humans, a good picture will have to be established of the risks associated with this new therapy. The committee therefore advocates meticulous preclinical research prior to the introduction of therapy with stem cells to the clinic.

The committee *concludes* that:

- research into stem cell therapy is still in the exploratory stage
- this research opens up the possibility of determining how undifferentiated stem cells develop into specialised tissue cells such as pancreas, brain and cardiac cells
- research into human somatic (including foetal) and embryonic stem cells is important for the possible development of new forms of cell therapy
- the creation of new embryonic stem cell lines may be of major importance since the availability of the current cell lines is limited, and those lines may also be contaminated and be one-sided in their genetic composition.
- research dealing with nuclear transplants into enucleated egg cells is (in the long term) important for research dealing with the possibility of preventing rejection
- the proposed Embryo Bill makes it possible in principle to isolate new embryonic stem cell lines from ‘spare’ embryos
- no broad international consensus has been reached about the acceptability of nuclear transplants into egg cells and the creation of new embryonic stem cell lines from embryos which have been specially generated for that purpose
- there are no urgent scientific reasons based on stem cell research for lifting, in the short term, the moratorium in the proposed Embryo Bill on nuclear transplants into egg cells

- the development of organs using stem cells is at best a prospect for the long term and stem cell therapy does not for the time being constitute an alternative for organ donation (or xenotransplantation).

The committee *recommends*:

- for the purposes of strengthening the scientific basis for cell therapy and stem cell therapy, the encouragement of research into the factors which influence cell division and differentiation
- permitting the isolation of stem cells from 'spare' embryos for the purposes of stem cell research
- no ban (statutory or non-statutory) in advance on research into the possibility of nuclear transplants and the creation of new embryonic stem cell lines
- the inclusion in the proposed Embryo Bill of research into transplants of human nuclei into animal egg cells
- research into the practicality of using somatic stem cells for transplantation purposes
- looking at the possibility of harvesting stem cells during autopsies
- encouraging animal trials into the effects of cell therapy and stem cell therapy.

Hoofdstuk 1

Introduction

1.1 The request for advice

On 17 January 2001, the President of the Health Council received a request from the Minister of Health, Welfare and Sport for advice concerning the use of cells, particularly stem cells, for transplantation purposes (letter ref. CSZ/ME-2145714, see appendix B). In the letter, the minister enquires about the scientific situation pertaining to the use of embryonic, foetal and adult cells, particularly stem cells, in culturing entire organs and tissues (of parts thereof), and cells (clusters) for transplantation purposes. The minister also asks for details of developments which are sufficiently promising, in the Council's view, to merit further stimulation. The Council is also asked to provide advice concerning the medical-ethical aspects that are, or could be, associated with the use of the various types of cells.

In addition to this request for advice, on 26 March the Minister of Health, Welfare and Sport requested advice concerning the use of haematopoietic stem cells (letter ref. GMV/L-2163886). In addition to the scientific situation with regard to haematopoietic stem cells, that request also contained various practical questions concerning the use of such cells. The Health Council's response to this second request for advice is contained in a separate recommendation.

1.2 Previous advice

The Health Council has previously made reference to the major potential applications that appear to be taking shape as research into embryonic stem cells progresses (GR97). The recommendation of that report was that such developments should be borne in mind when drawing up sections of laws relating to embryo research. In that context, the Council noted that research into established cultures of embryonic cell lines should not be automatically labelled as embryo research. It is also recommended that research which could have major implications for public health should not be automatically excluded by a restrictive recitation of the law (GR97).

In the United Kingdom, a report on stem cell research was drawn up by a Chief Medical Officer's expert group. The experts provide a summary of the origins of stem cells and discuss their possible uses. The report states that a great deal of research is still required in order to develop therapeutic applications. The experts recommend that research be permitted, not solely for the purposes of investigating fertility problems and hereditary or congenital disorders, but also for the study of diseases. The use of cloning for the purpose of creating human beings must remain illegal (Cmo00). Similar views have been published by the Royal Society of London (RSc00). It is anticipated that it will be possible to develop therapies for a variety of disorders. Furthermore, the Royal Society recommends that a feasibility study be conducted into the use of deep-freeze cell banks for various types of stem cells (RSc00). In the United Kingdom, the relevant legislation dates from 1990 (Human Fertilisation and Embryology Act 1990). Subject to certain conditions, it is legal to create and use embryos for research. Research was initially restricted to problems associated with infertility, miscarriages, contraception and congenital or hereditary defects. In 2001, the field of research was broadened to include embryonic development and serious diseases (Human Fertilisation and Embryology (Research Purposes) Regulations 2001).

The question of what therapeutic options might be feasible, using the various types of stem cells, has been extensively debated elsewhere in the scientific literature. In the United States, a comprehensive summary has been published by a National Institutes of Health committee. That committee concluded that there is enormous scope for research into possible therapies. Furthermore, given the differences between embryonic and somatic stem cells, both cell types would have to be investigated (NIH01). A National Academy of Sciences committee concurred with this view. They consider that research into human stem cells is vital to the development of therapies, and consider it essential that new stem cell lines be established (NAS02). A European Science Foundation committee also concluded that it is of the utmost importance that research be conducted into various types of stem cells (ESF01). That conclusion is in keeping with the

recommendations of the above-mentioned working group in the United Kingdom (Cmo00). In addition to questions concerning the multiplication of cells and their growth to form certain tissues, the European Committee takes the view that problems of immunological rejection (of possible transplants) should also be the subject of research (ESF01).

In several countries, there have been wide-ranging debates on whether the use of embryos for research into therapies should be permitted, and if so, subject to what conditions (Cmo00, ESF01, Gre01, Eve02). In the Netherlands, this issue has been addressed by the 'Act governing activities involving human gametes and embryos' (Embryo Bill, Wet01b).

In the United Kingdom, some individuals are opposed to any and all uses of embryos, others have no objections, while an intermediate position exists, which is referred to as the ethical middle ground. This position is that embryo research is permissible, provided that an important interest is being served, such as a possible therapy for a serious disease (Cmo00). The above-mentioned European Committee too has considered the ethical aspects of stem cell research. It recommends that embryo research be permitted, and that clear, appropriate legislation be drawn up (ESF01). In the United States, the National Bioethics Advisory Commission considers certain types of embryo research to be acceptable. It has recommended that research of this kind be made eligible for financial support from the federal government. Those types of research involve stem cells derived from foetal tissue and embryos that are no longer required for fertility treatments (NBA99). While the committee in question has included the creation of new cell lines (foetal or embryonic) in its recommendations, it did not address the issue of the creation of new embryonic stem cell lines for research purposes. The government of the United States has decided to restrict its financial support to research using pre-existing cell lines (Wer02b). Beside objections to the use of embryos, the decision-making process was greatly influenced by the fear that entire human beings would be cloned (Ann02).

In Germany, the creation of new cell lines is prohibited. Nor is it permitted to import such cells, unless it can be demonstrated that there is no other way of carrying out certain lines of research (Der01). The Protocol on the Prohibition of Cloning Human Beings was drawn up under the Council of Europe's treaty dealing with human rights and biomedicine (Ceu01).

In the Netherlands, the bill proposed to ratify that treaty has yet to be processed.

In view of the differences of opinion concerning the acceptability of creating embryos purely for research purposes, the European Commission has decided not to subsidize such research (including cell nuclear transfer) within the context of the Sixth Framework Programme. Research on stem cells from spare embryos, however, is eligible for financial subsidies.

1.3 Structure of this advisory document

The present recommendation focuses on research into therapy using embryonic and somatic stem cells, with the exception of blood-forming stem cells (from bone marrow, blood, and placental blood) which will be the subject of a separate recommendation. Section 2 contains a discussion of the origins of the various types of stem cells. Section 3 contains a brief summary of the biological characteristics of the various types of stem cells. That summary also deals with the gaps in scientific knowledge. The potential for developing clinical applications is the subject of section 4. There is also a discussion of current knowledge of cell therapy in the broader sense, and of the various diseases for which stem-cell therapy is being considered. The problem of rejection responses will also be addressed in this section. Section 5 deals with the ethical and legal aspects of research and therapy using cells, especially embryonic stem cells. When weighing up the objections to embryo research and the opportunities that it offers, the question was addressed whether the means are appropriate to the ends, and whether there are other options. Also included are the legal statutes governing the use of embryos for the purpose of stem cell therapy. Section 6 contains the Committee's standpoints concerning the acceptability of the research, and whether or not it is permissible.

Hoofdstuk **2**

The origin of stem cells

Stem cells are classified into two groups, embryonic and somatic stem cells.

Embryonic stem cells can be obtained, directly from embryos or from the foetal germ line, or by procedures such as cell nuclear transfer.

Somatic stem cells can be obtained from foetal organs and tissues, placental blood or organs and tissues from adults and children.

In this section, we will discuss stem cells from embryos (embryonic stem cells) and stem cells from adult and foetal tissues (somatic stem cells). Procedures such as cell nuclear transfer are dealt with in section 4.

2.1 Embryonic stem cells

Following fertilization, the embryo that has resulted from the fusion of egg and sperm passes down the fallopian tube to the uterus. The number of cells increases as a result of cell division, but these cells are still undifferentiated. In the first stage, the cells are totipotent, and identical twins can develop if the cell mass splits in two. Differentiation starts after about five days. The outer layer separates from the innermost layer, and starts developing into the placenta. A hollow ball of cells then develops, this is known as the blastocyst.

The next step is implantation, which involves the blastocyst becoming attached to the wall of the uterus. The cells of the innermost layer are pluripotent. They are capable of developing into various types of cells (more than two hundred types are known), which form the basis for the various tissues and organs.

In the case of *in vitro* fertilization (IVF), the sperm and ovum fuse outside the body. Next, one or more fertilized ova are transferred to the uterus. Occasionally, more than two ova are fertilized. These can be frozen, thereby retaining their vitality, and used if required for a future IVF treatment. If the patient does not wish to undergo any further treatment, however, this leaves a number of fertilized ova that are surplus to requirement. These are referred to as spare embryos. In the laboratory, stem cells can be obtained from spare embryos. In by far the majority of cases, IVF is used in connection with infertility problems. However, it is also used in the context of pre-implantation diagnosis. The literature contains references to various embryonic stem cells. Some researchers take the view that these cell lines should be used to carry out the experiments needed to further our understanding of stem cell development (thereby supplementing experiments on non-human stem cells). The National Institutes of Health in the US has identified the following stem cell lines as suitable for this purpose (Hol01):

BresaGen Inc., Athens, Georgia, US: 4; CyThera Inc., San Diego, California, US: 9; Karolinska Institute, Stockholm, Sweden: 5; Monash University, Melbourne, Australia: 6; National Center for Biological Sciences, Bangalore, India: 3; Reliance Life Sciences, Mumbai, India: 7; Technion-Israel Institute of Technology, Haifa, Israel: 4; University of California, San Francisco, California, US: 2; Gothenburg University, Gothenburg, Sweden: 19; Wisconsin Alumni Research Foundation, Madison, US: 5.

The list contains 64 cell lines, which were to have been set up before 9 August 2001. However, experts have made a number of comments in this regard. For example, it is not entirely clear whether the cell lines listed have been sufficiently verified (for the characteristics of embryonic stem cells). Another important issue is that not all of these cell lines can be made available to others, for the purposes of research (Hol01). If the research into stem cells ultimately results in therapies, many more cell lines will probably be required. The existing cell lines have only limited genetic variation, which could be an obstacle to their wider use. Contamination of these cell lines with viruses and prions must be excluded.

Embryonic cancer stem cells derived from testis tumours are sometimes listed separately from other types of stem cells. While these cells can give rise to any type of tissue, the change in their genetic composition brought about by the formation of the tumour means that they are probably less well suited for therapeutic purposes. Stem cells with embryonic characteristics can also be obtained from germ-line tissue taken from aborted foetuses. It is from these embryonic germ cells that the gametes normally develop. Research has shown that germ cells have the ability to differentiate into various cell types, although they are more limited in this respect than embryonic stem cells (Sha01). It should be noted that these research results have yet to be confirmed by

other researchers. The stability of these cells' genetic material is still open to question (Ste99).

2.2 Somatic stem cells

Embryonic stem cells are distinguished from somatic stem cells. The latter are derived from the tissues or organs of foetuses, children or adults. The most well-known somatic stem cells are the blood-forming (or haematopoietic) stem cells that are derived from bone marrow or blood. These cells are used in clinical practice. If stimulated with certain cytokines, the cells in the bone marrow can be induced to migrate to the circulatory system. Giving blood is far less distressing for patients than having samples of bone barrow (or other tissues) removed. Placental blood is a special source of such cells. Somatic stem cells are found in many more types of tissue than was originally thought. Some examples are neural stem cells from brain tissue and stem cells in skin and adipose tissue (Sla00, Rie01, Zuk01). It had been assumed that adults were not capable of developing any new neurons, but some parts of the brain have been shown to contain stem cells that are capable of doing just this (Pal01). Samples of adipose tissue that have been extracted during liposuction have been found to contain stem cells that might be able to develop in any of several directions (Zuk01). In each of these cases, the number of cells involved is very small. There is also some debate about the identification of the cell types involved (see 3.2.2).

Somatic stem cells can be obtained by means of invasive interventions, such as that used in connection with the donation of bone marrow. It has also been claimed that stem cells can be isolated following autopsy, from *post mortem* brain tissue for example (Pal01).

Hoofdstuk 3

Characteristics of stem cells

In addition to retaining the ability to reproduce for a long time, stem cells are capable of developing into a variety of cell types (Wei00a). Multiplication occurs by means of cell division (proliferation), a process that takes place on an enormous scale during the body's embryonic and foetal stages of growth, and in some organs and tissues of the adult body (*in vivo*). Following proliferation, some of the stem cells commence the process of developing into a certain cell type, such as a liver cell or a muscle cell for example. This process of development is referred to as differentiation. It proceeds through a number of stages. Some researchers draw a distinction between the committed stem cells (progenitor cells) and the precursor cells that are formed at a later stage. Committed stem cells are only able to develop into a limited number of cell types. The precursor cells can only give rise to a single type of cell, for example heart muscle cells originate from cardiomyoblasts. The detailed distinctions between stem cells, committed stem cells and precursor cells are as yet unclear (Bla01). The following sections deal with aspects of stem cell biology that are of relevance to the committee's recommendation.

3.1 Capacity for division

Stem cells differ markedly in their capacity for division. This is closely related to the species involved, for example there are considerable differences between the stem cells of mice and humans, and between individual types of stem cells. While all types of stem cells are able to divide *in vivo*, even if not at the same speed, this has yet to be

imitated in the laboratory (*in vitro*). Under certain conditions, embryonic stem cells can be induced to divide relatively rapidly in the laboratory. This is less true of somatic stem cells. One way in which embryonic stem differ from other cell types is that they never seem to exhaust their capacity for division. In many other cells, the chromosomes become shorter after each division. This places a limit on the potential number of cell divisions. The enzyme telomerase cambats chromosome shortening. It is active in embryonic stem cells, and therefore it is theoretically possible to obtain an extremely large number of embryonic stem cells. The successful continuous culturing of human embryonic stem cells generally involved a so-called feeder layer of certain types of cells (fibroblasts) derived from mice. The fibroblasts are treated to prevent them from dividing but to keep them alive, and to ensure that they secrete proteins that are required for the division of the embryonic stem cells and for the inhibition of their differentiation. The cultivation of a feeder layer is a labour intensive process, and it places a practical restriction on the number of embryonic stem cells available. The use of mouse cells or animal serum components necessitates the use of additional checks for possible harmful materials, such as viruses and prions. A method has recently been developed for cultivating these cells without the use of a feeder layer. Here, the substances produced by mouse cells are used differently (conditioned medium; Xu01). Like many other types of cells, embryonic stem cells can be biologically deep frozen (deep frozen under certain conditions, so that they can later be grown in culture once again)

Gaps are present in our knowledge about the cell divisions of stem cells. Not enough is known about which growth factors and other elements are needed for division. These gaps relate to embryonic stem cells and to other stem cells. A greater understanding of these factors would probably produce considerable improvements in the processes used to multiply stem cells. These processes are usually labour intensive, one reason being that embryonic stem cells are not cultured as separate cells (in suspension). It should also be noted that human embryonic stem cells divide more slowly than mouse embryonic stem cells, they are also more inclined to differentiate. Greater knowledge of the process of cell division is required, to achieve practical results in the form of therapies (Cmo00,Bot01,NAS02).

3.2 Differentiation

The differentiation of stem cells into specialized cell types is a highly complex process. Most of the research into that process has been carried out using the cells of experimental animals, especially mice. A number of different proteins are involved in differentiation (Sch00), including transcription factors (factors that activate a gene) and proteins that influence the cell's biological clock, and with it the rate of cell division.

Other factors that are important in terms of differentiation include the proteins that are produced by neighbouring cells and tissues. The published scientific literature contains references to the stem cells' niche (the micro-environment; Wat00, Spr01). An example of a niche is the hair root for stem cells in the epithelium.

3.2.1 *Embryonic stem cells*

Various types of cells have been generated *in vitro*, starting with embryonic stem cells (Eva81, NIH01). In this way, it is possible to create cells that not only resemble pancreas cells but which are also capable of producing insulin when administered with glucose (Lum01). The cells form three-dimensional structures that resemble the islets of Langerhans. If these structures are injected into mice, a vascular system develops around them (Lum01). There are other examples of the *in vitro* differentiation of mouse embryonic stem cells. These include the formation of tubular structures by cells that are found in the blood vessels (Yam00) and the creation of neural cells that are capable of producing dopamine (Gag00, Lee00a).

In 1998, a method was described for isolating and multiplying human embryonic stem cells (Tho98). These cells are also capable of developing in various directions *in vitro* (Odo01). Some of the cell types produced include heart muscle cells and bone cells (Doe00, Reu00, Keh01). Insulin-producing cells and neurons can also be cultivated from human embryonic stem cells (Ass01, Zha01). It is likely that, under the right conditions, embryonic stem cells are capable of differentiating into almost any type of cell. As has been pointed out with respect to cell division, our understanding of the factors governing this process also is still far from complete (And01, Bla01).

3.2.2 *Somatic stem cells*

Somatic stem cells (both foetal and adult) appear to be capable of differentiating in any of several different directions. Their ability to form a range of cell types is probably greater than was first assumed, in particular with regard to the adult forms. It had been known for some time that various types of blood cells were able to develop *in vitro* from bone marrow (both foetal and adult) and placental blood (Bro89, Bau92, Eri00). However, it is also possible for bone marrow cells to give rise to neural cells. The cells were cultured for this very purpose, in the presence of cells such as rat neurons. They were able to synthesize proteins specific to nerve cells, including human nerve cells (Bra00, Mez00, San00, Zha02). Conversely, stem cells isolated from mouse brain tissue are capable of developing into a variety of cell types (Cla00). Some examples include the production of blood cells from neural stem cells (Bjo99) and the development of muscle cells. After the latter had been transplanted into a mouse, they

were shown to have given rise to muscle tissue (Gal00). Stem cells isolated from samples of adipose tissue obtained during liposuction have the capacity to develop into precursors of bone cells, cartilage cells, adipose cells and muscle cells (Zuk01). Stem cells from bone marrow, including human marrow, are also capable of developing into precursor cells of this type (Min01, Rey01b). In addition, stem cells from peripheral blood and bone marrow are capable of differentiating into liver cells (Kor02, Sch02). It has been shown that stem cells isolated from human skin are capable of giving rise to neural cells (Tom01). Other transitions have also been achieved *in vitro*, and laboratory research is likely to bring even more options to light (Fu01, Jia02). However, various criticisms have been levelled against the above-mentioned studies. Under certain conditions, embryonic cells from mice appear to fuse with bone marrow cells or brain cells. Such fusions might resemble transitions to neural cells, for example (Ter02, Yin02). It is not clear how many previous observations of transitions have been prompted by this type of fusion. Objections have also been raised to the assumption that peripheral blood cells are capable of developing into liver cells. Liver tissue could easily contain residual blood cells, and the markers could have been affected by factors present in that tissue (Abk02). It is clear however that stem cells from adult bone marrow are capable of developing in various directions (Min01, Jia02).

Somatic stem cells are being found in more places in the body than was originally thought. The best known example are stem cells in the central nervous system (the hippocampus, the subventricular zone and the spinal cord), which can give rise to neurons or glia cells (Mck97, Tem01). In connection with these discoveries, it should be noted that the number of cells involved is generally quite small, and that the identification of these cells is also sometimes disputed.

In addition, stem cells have the interesting characteristic of being able to migrate to a damaged area of tissue. Under certain conditions, neural stem cells that had been transplanted into rat brains were found to migrate to the site of an induced brain lesion. This process appears to lead to functional recovery (Gra00).

In the case of somatic stem cells, very little is known regarding the identity of the factors that control differentiation. Co-cultures, or mixtures of cultivated cells, are often used. In one example, bone cells are mixed with stem cells from adipose tissue, to induce the latter to develop into the precursors of bone cells (Zuk01). It is not usually known which of the substances present in such co-cultures are really important. Furthermore, little is known about the factors that maintain stem cells *in vivo*, by inhibiting differentiation for example.

Hoofdstuk **4**

Stem cell therapy

Many researchers consider therapy using stem cells to be the most important objective of stem cell research (Lov01). In addition, such research can also be used to find out more about the differentiation of cells, embryonic development, the origins of cancer and the toxicity of substances to various types of cells *in vitro*. Research into the factors and conditions that may be important for embryonic development can be important in the context of infertility problems and for preventing disruptions to such development.

Some of the many genes whose function is presently unknown are probably involved in the growth of the embryo. Stem cell research can provide a better understanding of that growth, especially with regard to the initial phases. Thus the importance of stem cell research is much broader than the development of therapies.

Cell therapy is not restricted to the use of stem cells. The scientific literature contains descriptions of therapies with various types of cells, such as the use of fibroblasts to treat burns, or skeletal muscle cells for muscle diseases. This section provides a brief summary of other forms of cell therapy than stem cell therapy (4.1). Next, there is a summary of the potential applications of stem cell therapy (4.2).

As with other transplants, cell therapy can encounter problems of immune rejection (4.3). These problems could be tackled in various ways. On such approach is cell nuclear transfer, which is also referred to as therapeutic cloning. There is an explanation of the term ‘cloning’ in section 4.4, since misunderstandings sometime occur in debates about the desirability and potential of stem cell therapy. Many researchers expect stem cells to offer major therapeutic opportunities. They do not,

however, believe that the *in vitro* creation of organs from stem cells is feasible in the short term (4.5). Nevertheless, there are several reasons for expecting the demand for new stem cell lines to increase. This may well lead to a shortage of ova (4.6). Any responsible use of stem cell therapy will, in addition to basic research, also require experimental animal research (4.7).

4.1 Other forms of cell therapy

The possibility of using stem cells to develop new therapies has been extensively debated, both in the scientific literature and in the mass media. People sometimes lose sight of the fact that cell therapy, in the sense of treatment involving the introduction of functional cells, is not limited to stem cells alone. A great deal of research has been carried out into various types of cells, such as foetal neural cells for Parkinson's disease and myoblasts for muscle diseases. To better understand the potential of stem cell therapy, this section includes a brief discussion of the results obtained with other types of cell therapy.

Cell therapy can potentially be used for conditions such as diabetes, to compensate for a deficiency in insulin-producing cells in the pancreas. The conventional therapy for diabetes is generally unable to prevent the ultimate development of complications (Ser01). One alternative would be the transplantation either of an entire pancreas or of clustered pancreas cells. However, the transplantation of an entire pancreas involves major surgery (Sut01). Cell therapy is much less invasive, but requires a large number of donors (Ken01, Ser01).

In addition, cell therapy could be significant in the treatment of burns, wounds, broken bones, muscle diseases, myocardial infarcts, liver disorders and retinal degeneration. As part of the research into possible therapies, these various cell types are cultivated *in vitro* and transplanted into experimental animals. In some cases, this approach is also used in the treatment of patients. Fibroblasts and keratinocytes (skin cells) are used to improve wound healing, especially in the case of severe burns (Dor99, Rus00). Bone tissue and cartilage can be restored by means of cell therapy. This includes the transplantation of autologous cartilage cells (Job01). Myoblasts (the precursors of skeletal muscle) have been tested with a view to developing therapies for muscle diseases, especially Duchenne muscular dystrophy. While transplants of this kind have been shown to be possible, they have produced no clinically significant effect (Gus92, Men95). The muscle cells do not survive for very long, possibly due to immune responses (Hua92, Smy00).

Heart muscle cells (cardiomyocytes) and myoblasts have been investigated with a view to using cell therapy in the treatment of myocardial infarctions. After temporarily ligating the coronary arteries of experimental animals, attempts were made to limit the

resultant damage by the transplantation of cardiomyocytes and myoblasts (Tay98, Men01, Tha01). Some researchers consider the results to be unsatisfactory (Orl01b). Experimental animal research appears to indicate that greater improvements can be obtained by transplanting or mobilizing stem cells (see section 4.2). Other types of cells have also been investigated, such as the precursor cell of the endothelium (which lines the walls of blood vessel). In experimental animal studies, treatment with these cells led to improvements (Kaw01, Koc01b). Before this approach can be used in humans, research is needed into ways of multiplying heart cells (including heart muscle cells) and into the means of introducing them into the body (Tay01).

A great deal of research has been carried out into liver cells that have been cultured *in vitro*, with the aim of supporting liver function in patients with liver disorders. Recent research appears to show that the transplantation of liver cells can help to bridge the waiting period for a suitable liver donor), it can also provide metabolic support in the case of liver failure (while awaiting regeneration) and can help with certain metabolic disorders (instead of resorting to liver transplantation; Fox98, Str99).

Cell therapy in connection with Parkinson's disease has also been extensively investigated. This is a neurodegenerative disease in which a deficit of dopamine-producing cells develops in a specific area of the brain (the *substantia nigra*). Cell transplants have been investigated in rats and apes with experimental brain lesions, which were considered to be experimental animal models for this disease. The transplantation of foetal dopamine-producing cells was able to suppress Parkinsonian behaviour. Not only do the cells survive and grow in their host, but they continue to function for protracted periods of time. Research has also been carried out on patients with Parkinson's disease. Various researchers have reported that the transplantation of human foetal dopamine-producing cells has beneficial effects on the major symptoms of the disease, claiming that it has no adverse effects. It is estimated that about 300 operations of this type have been carried out throughout the world. The results obtained appear to be highly dependent on the type of techniques used (Bry01). However, placebo-controlled studies found that there was little beneficial effect, and that adverse effects did occur (Fre01). Various researchers have criticized both the technique and the evaluation method used in this study (Bru01, Dun01, Nik01). It should be noted that, were an adequate cell therapy to become available for Parkinson's disease, there would presumably be insufficient donor tissue available to meet the demand (Hag01). Sources other than human foetal donor tissue, such as tissue from pig foetuses, have been investigated for their suitability in this regard, so far without beneficial results (Sub01). Studies into the effects of cell transplantation have also been carried out in connection with Huntington's disease, another neurodegenerative disease. The transplantation of neural precursor cells from foetal tissue into the brains of patients

might produce some improvement. However, as yet, this therapy has not been used widely enough to enable firm conclusions to be drawn (Bac00).

4.2 Potential therapeutic uses of stem cells

The scientific literature lists an assortment of diseases for which the development of stem cell therapy seems to hold out considerable promise (Wei00b, Bia01). This includes neurodegenerative disorders such as Parkinson's disease, and metabolic diseases such as diabetes mellitus. However, the expectations of a future therapy are not always based on experimental research. One such example is Alzheimer's disease. The cause is still unknown and it is unclear whether specific types of cell therapy might be effective. On the other hand, a great deal of research has been carried out into the effects of cell therapy on Parkinson's disease, as reported in section 4.1. Stem cells represent a new source of cells that may well have a beneficial effect on this disorder (Oka96, Bru01, Reu01). In an experimental animal model, functional neurons were found to have developed following the transplantation of embryonic stem cells (Bjo02). Stem cell therapy has also been cited as a future option in the case of different disorders of the central nervous system, such as spinal cord transection, multiple sclerosis and cerebral infarctions (the most common form of stroke) (Par99).

In the case of spinal cord transections, the possibility of stem cell therapy is based on the notion that damaged tissues could be either partially or completely replaced by new neural cells, which could then serve as a bridge for the regrowth into the long nerve tracks. The transplantation of neural tissue has been extensively studied in experimental animals (Chr01). The introduction of embryonic stem cells leads to the formation of support cells (oligodendrocytes), which deposit a layer of myelin around the nerve fibres (Liu00). This observation meant that, aside from their possible use in the treatment of spinal cord transection, neural stem cells were investigated as a possible treatment for multiple sclerosis (by remyelinization). Further research could lead to clinical applications, but this will have to be preceded by research into transplantation methods and into the functioning of the newly grown neural pathways. Nor is the multiplication of neural stem cells sufficiently well understood (Mcd02). However, research has shown that human neural stem cells can be implanted into the brains of experimental animals (Fla98, Our01). Some of these stem cells develop into neurons and other types of brain cells, while others remain in the stem cell stage.

Cerebral infarctions are associated with damage to brain tissue, particularly as a result of blockage of the oxygen supply. It is not yet clear whether, and if so to what extent, cell therapy (including stem cell therapy) could produce improvements in situations of this kind. Neural cells cultured from testis cancer stem cells (which behave

in vitro like committed stem cells from brain tissue) have been transplanted into patients with infarcts in certain parts of the brain (the basal ganglia) (Kon00).

The suggested use of stem cell therapy is not restricted to neurological diseases, but is also considered for disorders of organs such as the liver, the heart and the pancreas. The transplantation of liver cells can be very useful in the case of certain liver disorders (see section 4.1). In some cases, this approach could be used instead of transplanting an entire liver (Str99). Partly due to the limited availability of liver cell donors (Lag01), research is being carried out into the possibility of using embryonic stem cells or somatic stem cells. The liver has been shown to contain stem cells which are capable of developing, via the so-called oval cells, into various types of liver cell. In addition, stem cells from bone marrow are capable of differentiating into liver cells (Pet99, the00). It is not yet clear which type of cell (embryonic stem cells, oval cells, bone marrow cells or differentiated liver cells) would make the best starting material for clinical applications (Ves01).

The option of stem-cell-based therapy for cardiac disorders has been investigated in experimental animals (Mum02). When heart muscle cells that had been cultured from embryonic stem cells were injected into mice, this resulted in the formation of new heart muscle tissue (Klu96). Human embryonic stem cells can differentiate *in vitro* into heart muscle cells, but the number of cells produced is quite low (Keh01). In some experiments with an experimental animal model (rats in which the coronary artery has been temporarily ligated) introduced adult stem cells were found to form connective tissue. Other studies recorded actual functional improvement (Tha01, Min02). In mice, it has been shown that stem cells were able to form heart muscle tissue and that there was also a recovery of function (Jac01, Koc01a). Instead of transplanting stem cells, attempts can be made to activate the individual's own stem cells. Using certain cytokines, stem cells in the bone marrow can be mobilized and induced to migrate to the circulatory system. Experiments with mice showed that this approach was able to stimulate the creation of heart muscle cells and blood vessel tissue. This led to a significant decline in experimental animal mortality (Orl01a, Orl01b).

Cell therapy could be very important in the treatment of diabetes, provided that sufficient pancreas cells were available (see section 4.1). Stem cells could be used for this purpose. Mouse embryo stem cells can differentiate into insulin-producing cells (Lum01). Transplantation of these cells was found to improve the condition of experimental animals with diabetes (artificially diabetic mice) (Sor00). For this reason, researchers anticipate that cell therapy (including stem cell therapy) will ultimately represent a major form of treatment for patients with type 1 diabetes mellitus (Ken01, Ser01).

There is a special category of diseases in which stem cells are already in wide-scale use, namely leukemias and lymphomas. In these disorders, bone marrow transplants

form part of a treatment in which chemotherapy and irradiation are used to destroy the patient's own bone marrow. Bone marrow transplantation is also used in the case of hereditary immunodeficiencies. Stem cells from blood (or, alternatively, placental blood) can be used instead of stem cells from bone marrow.

Stem cells from the embryonic retina of birds can divide *in vitro* and, under certain conditions, they can form cellular aggregations with some characteristics of the retina. The various cell types in the retina have been shown to join up correctly. Similar experiments have been carried out in mammals, so far without results (Lay01). In humans it has proved possible to heal, or partially heal, damaged corneal tissue using autologous stem cells (Hol99, Tsa00).

Stem cell therapy is also important in connection with the possibility of replacing or supplementing tissues (such as muscle and bone). As stated, attempts to treat hereditary muscle diseases by transplanting myoblasts have been unsuccessful. Researchers have suggested that better results could be achieved by using stem cells and a different method of introduction, namely via the circulatory system (Fer98, Par00). In the course of experiments with an experimental animal model for muscle diseases (mdx mouse), however, hardly any muscle tissue was obtained (Fer01). Transplants of bone marrow stem cells have been carried out in a small number of patients with hereditary bone abnormalities (osteogenesis imperfecta). Here it appeared that new bone was being laid down, and there was a reduction in the number of breakages (Hor99, Hor01). In the case of bone and cartilage diseases, as with the treatment of liver and heart disorders, the question is which type of cells can most appropriately be used for this purpose. Stem cells obtained from bone marrow can also differentiate to form endothelial cells, which may be important in promoting the healing of wounds (Rey02). The experiments referred to here offer favourable prospects for the development of therapies. A clinical trial is being carried out to investigate the possible therapeutic use of bone tissue obtained from stem cells.

4.3 Rejection responses in stem cell therapy

Following the transplantation of organs and cells derived from other individuals (heterologous transplantation), immune responses may occur that ultimately lead to rejection. In the case of stem cell therapy, there are various ways of countering this rejection. As with transplanted bone marrow supplied by a donor, for example, immunosuppressive drugs (drugs that suppress the activity of the immune system) can be administered. The magnitude of rejection is dependent on the differences between the patient's HLA system and that of the donor. For this reason, the differences should be as small as possible. In practice this would only be possible if stem cell lines were

available for a wide range of HLA types (estimates given in the literature are in the hundreds, some even run into the thousands). Partly to this end, a stem cell bank has been established in the United Kingdom. Rejection can also be reduced by the generation of immunotolerance. Previous administration of embryonic material, or haematopoietic cells from the stem cell donor, might cause the patient's immune system to become habituated to some extent. Habituation during the initial step would mean that smaller doses of immunosuppressive drugs would be required, or none at all. In addition, research is being carried out into the possibility of preventing an immune reaction by enclosing the cells to be transplanted in a capsule of inert material (Dov02).

The rejection problem would not occur if the patient's own cells are used (which is referred to as an autologous transplant). To this end, therapy based on somatic stem cells must be developed. As stated in section 3, the potential of these cells seems rather limited compared to that of embryonic cells. Another option is cell nuclear transfer (see 4.4). The HLA type of cells obtained in this way is the same as that of the cell-nucleus donor, which makes rejection responses unlikely. Cell nuclear transfer, also known as therapeutic cloning, is still dependent on the availability of ova. To date, attempts at cell nuclear transfer using human cells have yielded few results (Cib01, Cib02).

Another option, as yet theoretical, is the direct reprogramming of cells. This involves processing a patient's cells in such a way that they acquire the characteristics of embryonic stem cells. They can then be continuously cultured, after which they will differentiate into the cell type to be transplanted. One approach that researchers are using to examine the possibility of reprogramming involves fusing the cells with embryonic stem cells (theoretically, both ova and embryonic cancer stem cells would also be suitable; Dew77, Sur01). The resultant hybrid cell can, under certain conditions, develop in a variety of directions (Tad01).

This research may well lead to a situation in which a patient's cells could be reprogrammed to make them suitable for cell transplantation. Cells derived from the skin can develop *in vitro* into a given type of white blood cell, or at least into cells with the same characteristics as these blood cells (Hak02). It may become possible to manipulate body cells, using growth and differentiation factors, so that they could be continuously cultured to produce usable quantities of cells for therapeutic transplantation. Since these cells were obtained from the patient, such a transplantation would probably not give rise to a rejection response. If direct reprogramming proves possible, it would also neatly side-step the objections to the use of embryonic cells.

In theory, cell nuclear transfer and reprogramming are methods of avoiding a rejection response. However, much more research is still required into growth, differentiation and de-differentiation before these methods can be put into effect.

4.4 Cloning and cloning

The word ‘cloning’ can have a range of different meanings (Web03, NAS02, Mai02). If cells *in vitro* divide a number of times, and the daughter cells are cultured separately, these cells will be genetically identical clones. This would make it possible to culture embryonic stem cells and to continuously culture the daughter cells. This type of cloning produces cell lines *in vitro*. Culturing fragments of plant tissue to produce descendants is also described as cloning. A special use of the term ‘cloning’ is in relation to the possibility of cloning animals by means of cell nuclear transfer.

To this end, a cell is taken from an animal and the nucleus is removed (since this is where the animal’s hereditary characteristics are encoded). That nucleus is then introduced to an ovum from which the original nucleus has been removed. After cell nuclear transfer, and several cell divisions *in vitro*, the resultant embryo is introduced into the womb. This method has been used to clone sheep and other animals. It is described as reproductive cloning. The method’s low level of efficiency has meant that the use of reproductive cloning to increase livestock numbers is still not being widely used, which runs contrary to initial expectations. Animal cloning now primarily involves the introduction of genetically modified nuclei from somatic cells into ova, which then develop into transgenic embryos. Efficiency is less critical here since a single genetically modified animal can yield large quantities of a scarce product, for example a coagulation factor.

In addition, it has recently been proposed that human cloning should be carried out (Pis02). In most cases, however, the procedures currently used in animals do not produce a pregnancy. Where pregnancies do occur, there are a large number of abnormalities (Hum01). The most common form of abnormality is large offspring syndrome, while other problems such as abnormalities of the heart and lungs are also relatively common (Rid01). It would therefore be completely irresponsible to use this procedure on humans at the present time.

In the literature, cloning that is used to increase the numbers of animals or plants is referred to as reproductive cloning. Another form is described as ‘therapeutic cloning’. In this case, the intended product is not an organism but an embryonic stem cell that is capable of growing *in vitro* to form a clinically useful amount of cell material. To this end, the nucleus of a somatic cell taken from a patient (in this case, the intended recipient of the transplant) is inserted into an enucleated human ovum. Following cell nuclear transfer, the embryo is cultured *in vitro* to the blastocyst stage. At this point it consists of a ball of several hundred cells, from which embryonic stem cells can be isolated and continuously cultured. Therapeutic cloning is important in connection with possible rejection responses in the recipient of the cells. Transplanting embryonic stem

cells is classified as an allogenic transplant, which means that the tissue does not come from the patient (if it did, then the term autologous transplant would be used). In allogenic transplants, differences in genetic composition often lead to responses from the immune system. Cell nuclear transfer may be a way of avoiding such problems. Some researchers discourage the use of the term 'therapeutic cloning' since this could lead to confusion, in particular it could be confused with reproductive cloning. By preference, therefore, the term 'cell nuclear transfer' should be used (GR01, Vog02).

One particular form of cell transplantation is the creation of hybrid cell lines, by inserting a human nucleus into the enucleated ovum of another mammalian species. This method has been described as a means of compensating for the shortages of ova that might arise if stem cell therapy were to progress to the point of large-scale use. However, mitochondrial DNA also differs from one species of mammal to another. Thus, the mitochondrial proteins that were coded by the DNA in the enucleated ova do not combine well with the other mitochondrial proteins, and the resultant mitochondria function poorly (Bar98, Bar00). These differences are quite large compared to some slight changes in mitochondrial DNA which cause severe diseases (Leo00). It is unclear whether that problem can be circumvented by the co-transfer of mitochondria from a suitable donor. If, in order to generate cells that would be suitable for therapy, it were possible to use nuclei from the patient's body cells in combination with animal ova then both the rejection problem (4.3) and a possible shortage of ova could be avoided. Meanwhile it is still not certain that the development of stem cell therapies will actually lead to shortages of ova (see also sections 5.1 and 6).

4.5 The isolation of new embryonic stem cell lines

The desire to avoid rejection responses can lead to a demand for the number of existing stem cell lines to be increased. There are also other reasons for this:

- it is important to obtain embryonic stem cell lines which, unlike all current cell lines, have not been cultivated in contact with mouse cells; contact with animal cells and serum components involves an unknown risk of contamination with viruses and other infectious agents.
- the cell lines that are currently in use have only a limited amount of genetic variation; cell lines with a different genetic basis can have different characteristics
- the current cell lines are covered by patents; it is important not to be dependent on biotechnology companies
- it is important to create cell lines from embryos which, as a result of pre-implantation genetic diagnosis, were found to have an anomaly; in such cases, the research can focus on the anomaly in question.

It is therefore important to retain the option of isolating new embryonic stem cell lines from spare embryos. It is not yet clear whether sufficient spare embryos are available for a possible expansion of the number of embryonic stem cell lines.

It might eventually be possible to obtain ova by culturing of oocytes derived from ovarian tissue (following an operation or autopsy) (Cor01, Smi02). For the time being, however, no practical means towards this aim have been developed.

4.6 Stem cell therapy versus organ transplantation

The shortage of donors for organ transplants is fuelling a demand for alternatives, such as xenotransplants and stem cell therapy. The Health Council has pointed out the problems associated with rejection responses in cases of xenotransplantation, and the possibility of viral transmission and viral modification (GR98a). This form of transplantation is prohibited in the Netherlands. Nor can stem cell therapy provide a short-term solution to the shortage of donors. A great deal more research is required to facilitate the possible application of stem cells. This must focus on cell cultures, on the factors that are important for the development of various cell types and on the problems associated with scaling up production. Research is also required to determine whether stem cell therapies are safe. In addition, for the time being any clinical application of stem cells will involve cell therapy, in other words treatment with dissociated cells that are not organized into an organ. Dissociated cells may well be sufficient for some purposes but not for others, such as the replacement of a diseased kidney. Although research is being carried out into the feasibility of creating organs (or parts thereof) from stem cells, the creation of organs such as kidneys and hearts *in vitro* is unlikely to be possible in the foreseeable future.

4.7 Safety and experimental animal research

Various factors are important in terms of the clinical application of stem cell therapy. One is the immunological barrier, another is the outgrowth of transplanted cells, and a third is the purity of the materials used (Don01). Immunological rejection can lead to rejection responses. As stated in section 4.3, there are various means of combating these reactions (immunosuppressive drugs, cell nuclear transfer, habituation as a result of co-transplants and the use of a patient's own stem cells). As yet, too little is known concerning the pros and cons of the various methods. As with the use of a patient's own cells, cell nuclear transfer has the advantage that the HLA system of the transplanted cells is the same as that of the patient. According to sources such as the

National Academy of Sciences, research into that method should not simply be prohibited as a matter of course (NAS02).

Another risk of possible clinical application is that the cells will not simply grow into a replacement or supportive tissue, but that tumours will develop or inappropriately differentiated tissue (Bot01, Don01). An understanding of the process of differentiation is therefore essential. In addition, purification methods should be developed in order to avoid the transplant of unwanted cells. It must be possible to guarantee the purity of the transplant. This also means that the cultured material must not contain any viruses, mycoplasmas or prions.

Experimental animal research is important in order to check the efficacy and safety of stem cell therapy. Experimental animal models have been developed for various disorders (see also sections 4.1 and 4.2). The effects of stem cell transplants must be investigated, using these models. Both the extent to which disease symptoms are improved, as well as any adverse effects, can be recorded. Research of this kind does not reveal all types of risk. There may also be therapeutic options that cannot be investigated using experimental animals. Nevertheless, in many cases, such research is a pre-requisite for any well-grounded clinical application.

Hoofdstuk **5**

Ethics and Law

This section addresses the acceptability and permissibility of isolating and using various types of cells, particularly stem cells, in research that is aimed at the development of cell therapy.

5.1 Ethical aspects of cell therapy in general and stem cell therapy in particular

The ethical debate focuses on two types of cells. The first type is cell material obtained from aborted foetuses (and aborted embryos) and the second type is embryonic stem cells derived from the pre-implantation embryo.

5.1.1 Foetal cells, particularly stem cells

Cells derived from aborted foetuses can be classified either as differentiated foetal cells and tissues (which have been used for years in the context of small-scale clinical trials associated with foetal tissue transplantation) or as foetal stem cells (both somatic and germ cells).

There is no international consensus concerning the question of whether it is acceptable to transplant human material that has been obtained following clinical abortion. Until recently, the ethical debate was restricted to the use of differentiated foetal cells and tissues. There are those who consider such practices to be improper (Bop88). The main arguments are that such use makes all those involved accessories to

the preceding clinical abortion, and that this practice can lead to an increase in the number of abortions. The argument is that any women who are in doubt about whether to continue their pregnancy might be unduly influenced by the knowledge that foetal material can be used for a beneficial purpose. As a result, they might well decide to terminate their pregnancy. However, the assumption that the transplantation of foetal tissue will lead to an increase in the number of abortions is highly speculative. Furthermore, this risk can be greatly reduced by imposing conditions on the performance of abortions and on the donation of foetal tissue. According to the predominant view in ethics, which also forms the basis of the Foetal Tissue Act (see section 5.2), foetal tissue can properly be used for transplantation purposes provided that a number of guidelines are observed (GR84, Wer91, Boe99, Kem93, Wer02a). Conditions that are broadly supported include:

- the use of foetal material for transplantation purposes requires the permission of the pregnant woman in question
- permission can only be asked once the woman has decided to proceed with an abortion
- foetal tissue may only be removed from a dead foetus
- the donation of foetal tissue must always be free of charge
- the decision to proceed with a clinical abortion should not be prompted by the decision to donate foetal tissue; one special aspect of this condition is that the woman is not permitted to stipulate who will be the recipient of the foetal tissue (to avoid situations in which women become pregnant specifically with the aim of having a clinical abortion and donating foetal tissue).

Clinical trials have shown that it is difficult to obtain enough foetal tissue for standard or experimental treatments. This is most clearly illustrated in the transplantation of foetal neural tissue for the treatment of Parkinson's disease. Each treatment currently requires the neural tissue from 6-10 foetuses.

Partly because of the scarcity of foetal tissue, research is now being carried out into the possible use of foetal stem cells. The use of such cells does not give rise to any new ethical issues.

5.1.2 Embryonic stem cells

Research into human embryonic stem cells, and the associated ethical debate, are both very recent phenomena (Gue01, Mcl01). To some extent this is an extension of the debate about experimentation with embryos, which has been going on for about 20 years now (Lee00b).

The moral status of the pre-implantation embryo.

The isolation of stem cells from an embryo implies the destruction of that embryo. Can such instrumental use of embryos for research aimed at cell therapy be morally justified? A comparable issue had previously been raised in association with other forms of so-called non-therapeutic research on embryos, aimed at improving the various methods for medically assisted reproduction. All answers to this question are based on a view of the moral status of the pre-implantation embryo and of its right to protection.

Broadly speaking, there are three visions, which can be summarized as follows (GR98b):

Equal right to protection. According to this view the embryo is an individual, right from the outset. In such a case, the embryo has the same rights as a child or an adult. This means that, in view of the violation human dignity, embryos may never simply be used as a resource. One variant of this view is that, although the embryo is not yet an individual, on the basis of its status as a potential individual it should nevertheless be considered to be an individual and protected as such. This is the strict version of the so-called potentiality argument.

No right to protection. The diametrically opposite view is that the embryo is not an individual, thus it has no status whatsoever. Although the embryo has the potential to develop into a person, this potential brings no additional ethical weight to bear. From this point of view, the embryo is essentially the same as a reproductive cell. Its destruction is, in principle, morally neutral.

Relative right to protection. This vision, the middle position, states that since it is human in origin and has the potential to develop into a human individual, the embryo has an intrinsic value on the basis of which it deserves respect. However, this moral value is relative rather than absolute. This means that, under certain circumstances, the embryo's right to protection can be weighed up against other values and interests. One variant of this view is that the embryo's value is symbolic rather than intrinsic or independent. The imprudent, instrumental use of embryos is prohibited since this might otherwise undermine the protection of other, more developed forms of human life. This means that the embryo is entitled to a certain degree of protection, due to 'importance by association'.

The most widely-held standpoint in ethical circles supports the relative right to protection. This has been adopted by bodies such as the Health Council 'Review of the IVF Planning Decree' Committee (GR98b) and it forms the basis of the Embryo Bill (see section 5.2), This vision means that instrumental use can be morally justified under certain conditions. One condition relating to the procedure is that permission is required for embryo research, both from those who donated the embryos and from an

ethics committee, or national ethics committee. The material conditions, for which there is a ‘strong’ consensus are:

- the research serves an important purpose (the requirement of proportionality)
- there is no suitable alternative way of achieving this objective (the requirement of subsidiarity)
- no more embryos are used than is strictly necessary, from the scientific viewpoint, to find an adequate answer to the study question
- the study will be limited to a period of 14 days after fertilization (the 'two week limit').

One major point of discussion is whether such work should be restricted to the spare embryos from IVF treatment, or whether embryos can be created specifically for the purpose of research.

The acceptability of isolating stem cells from spare embryos.

The possibility of using embryonic stem cells for research into cell therapy makes the instrumental use of embryos a topic for continuing debate (Har02, Rob01). This is primarily concerned with putting into effect the requirements for proportionality and subsidiarity, and with the issue of whether it can be appropriate to create embryos purely for research purposes (Wer01a, Wer01b).

According to the principle of *proportionality*, embryos can only be used instrumentally if this serves an important purpose, such as a major health interest. Opinions differ, however, about how best to put this condition into effect. Thus, in some countries, it has been determined that embryo research should be limited to reproduction-related research. In 1995, the Minister of Health, Welfare and Sport announced that the government also opted for a restriction, namely infertility, artificial reproduction and hereditary or congenital disorders (Lower House of the Dutch Parliament, March 1995). These restrictions are increasingly under fire at international level, partly because of the therapeutic prospects associated with the use of embryonic stem cells (Per00, Wer02a). In the Netherlands, bodies such as the Health Council have recommended that sufficient scope be granted to research into the practicality of using embryonic stem cells for the purposes of transplantation (GR97). The Embryo Bill does indeed offer scope of this kind.

According to the principe of *subsidiarity*, embryo research can only be acceptable if there is no suitable alternative means of achieving the purpose of the investigation. Critics of the use of embryonic stem cells claim that there are a variety of alternatives, including the use of xenotransplants, foetal germ cells and somatic stem cells. On this basis, they advocate that a moratorium, or even an outright ban, be imposed on the

isolation of embryonic stem cells from pre-implantation embryos (see section 1.2). Others, however, take the view that a moratorium would be undesirable. They support the idea that parallel research should be conducted on the various types of cells, including human embryonic stem cells. This argument is partly inspired by the restrictions, drawbacks and health risks of the supposed alternatives to embryonic stem cells.

Xenotransplantation. This option is also the subject of some controversy. To start with, there is the risk of an infection capable of jumping the species barrier. Starting with the individual who received the transplant, and those closest to him, this could even pose a risk to public health (GR98a). According to many, this risk currently constitutes an ethical threshold to prudent clinical trials. Against this background, a ban is in place in the Netherlands on the clinical use of xenotransplantation. There are also objections related to the animal ethics. The 'strict' variant of the animal ethics takes the view that xenotransplantation is quite simply unacceptable. The 'moderate' variant holds that it is only acceptable if there are no suitable alternatives. This moderate view of the ethics of animal use therefore gives rise to the question of whether it is acceptable to breed animals to supply transplants while human spare embryos are available. It should be noted that the development of organs from stem cells does not appear to be possible for the time being, so in this respect stem cell therapy is not an alternative to xenotransplantation.

Foetal germ cells. From the moral standpoint the use of germ cells from an aborted foetus is preferable to the isolation of cells from living, pre-implantation embryos. However, it is unclear whether foetal germ cells are as useful.

Somatic stem cells. The international debate is focusing on the third possible alternative to embryonic stem cells, namely somatic stem cells. Recent research suggests that these cells have more potential than was previously thought. Some critics of the use of embryonic stem cells postulate that these cells are not needed, since it has now become clear that somatic stem cells have the same therapeutic potential. On the other hand, while most experts consider the research into somatic cells to be very promising, they do not feel that these cells have the same potential as embryonic stem cells (see 3.2.2). Somatic stem cells seem to have less developmental potential. Many experts believe that the results of research into somatic cells have been over-interpreted. They take the view that, these cells will have, at best, in the long term the same range of applications as embryonic stem cells (Cmo00, Vas01). Dissent concerning the issue of whether, given the existence of possible alternatives, research on human embryonic stem cells is ethically sound, derives partly from disagreement concerning the interpretation of the principal of subsidiarity (Wer01b).The restrictive view is that research using embryonic stem cells is only permitted if it can be proven that the use of somatic stem cells is either less effective or not a viable option at all.

The permissive view is that research on embryonic stem cells can continue while there is still some doubt about whether somatic stem cells are just as useful as embryonic stem cells. On the basis of a permissive interpretation, it can be concluded that the isolation of embryonic stem cells for the purpose of cell-therapy research is quite proper. If a moratorium were to be imposed on research using these cells, patients might be disadvantaged.

Some take the view that any type of research on embryonic stem cells is unacceptable, for reasons of principle. Others feel that while research on existing embryonic cells is acceptable, they want to call a (provisional) halt to any further isolation of cells from embryos. Besides objecting to the instrumental use of embryos, some assert that the number of embryonic cell lines currently available is sufficient for research. There is considerable doubt concerning the latter claim, however. There are major arguments in favour of creating new embryonic cell lines (see 4.5).

5.1.3 Cell nuclear transfer

Cell nuclear transfer ('therapeutic cloning') is much more controversial than research using cells derived from spare embryos. This approach confronts society with a controversial issue that had previously been raised in connection with the 'classic' debate on embryo research, which is can it be acceptable to create human pre-implantation embryos for instrumental use? Thus, if the answer to that question is affirmative, we must then ask whether it can be proper to create embryos for research into the development of autologous cell therapy.

The discussion about the acceptability of creating embryos for purposes other than pregnancy has been comprehensively summarized by the IVF Committee of the Health Council (GR98b). In view of the debate, that committee adopted the standpoint that the creation of embryos for research can be both acceptable and permissible. They do not dispute that there is a moral distinction between the use of spare embryos for purposes of scientific research on the one hand and the creation of embryos for research on the other. However, they do not feel that this is of critical importance. Justification of the use of spare embryos is, after all, based on the consideration that the pre-implantation embryo has only a relative moral value. The moral requirement that this value should be respected as much as possible can be superseded by the possibly greater weight of the interests involved in scientific research. If this involves research that cannot be carried out using spare embryos and which is, beyond any doubt, in the interests of human health then, in the view of the above-mentioned committee, the creation of embryos for the purposes of research can also be justified. For that matter, the committee would like to stress that the creation of embryos for research is not

acceptable when it would also be possible to carry out that research using spare embryos.

What then should we make of the use of cell nuclear transfer to create embryos in order to obtain embryonic stem cells for autologous cell therapy, or for research in this area? The potential benefit of this strategy is that the transplant will be genetically virtually identical to the patient, which means that there will be no problems with rejection.

Some critics dismiss cell nuclear transfer on the basis that it would inevitably lead to reproductive cloning. However, this objection is a matter of controversy. Some authors reject the premise that reproductive cloning is, in principle, improper. They recognize that, given the enormous health risks involved, the appropriate course of action would be to impose a moratorium. However, they ask whether, if the risks involved ultimately appear (or are shown) to be minimal, reproductive cloning could be appropriate under certain circumstances and subject to a number of conditions. A second, broader reply is that if we, as a society, determine that reproductive cloning is simply unacceptable and impermissible, then a ban on this application is sufficient (GR01).

The question of whether cell nuclear transfer satisfies the requirement of subsidiarity is answered by some in the affirmative (NAS02). Others are more cautious (Wer01a, Wer01b). Since the development of stem cell therapy still requires a great deal of research (see section 4), it is proposed that, for the time being, such research can and should be restricted to currently available spare embryos. At the same time, possible alternatives such as the above-mentioned use of somatic stem cells will have to be considered. As a result, instrumental use, or use for scientific research, could perhaps be restricted to such spare embryos or it might even be rendered unnecessary. Other options referred to in the literature are an embryonic stem cell bank, immunotolerance, direct reprogramming and the use of enucleated animal cells.

Provided that there were sufficient spare embryos available, embryonic stem cell banks would contain stem cells with a suitable HLA type for any patient. Many hundreds of different stem cell lines would be needed to create such a bank.

Immunotolerance could also be an alternative (for cell nuclear transfer, in order to avoid rejection problems). This involves an initial transplant of embryonic stem cells in order to generate tolerance for this foreign material, after which actual therapy could be started, using cells from the same spare embryo.

Furthermore, an alternative would be to transfer a cell nucleus from a patient into an enucleated animal ovum. Some take the view that this approach is not comparable to the creation of a human embryo. In addition, this form of cell nuclear transfer would obviate a possible shortage of ova. It is not yet clear whether or not cells created in this way would be functional. From the ethical viewpoint, there is the question of the

ontological and moral status of interspecies embryos created by this means. The 'special combinations' section of the explanation provided for the passage of the Embryo Bill through the Dutch Parliament makes no mention of the variant in question. This does not require a discussion of the possibility of creating novel organisms, since embryonic development would be terminated several days after cell nuclear transfer. It should be noted in passing that, in the case in point, any such research would be subject to the provisions of the Experiments on Animals Act (Wet 96a) and of the Animal Health and Welfare Act (Wet96b).

The literature also makes mention of the possibility of directly reprogramming a patient's cells (see section 4.3; Cmo00, Nuf00, Ald01, Sur01). This would involve culturing the cells in such a way that the programming of the cell nucleus for its specific cell function would be terminated. Using growth and differentiation factors, it would then be possible to produce large numbers of cells with new functions. A vast amount of research will be needed before this objective can be achieved. Possible, such research will require the creation of embryos (by means of cell nuclear transfer).

Clearly, research aimed at the development of cell therapy is exceptionally dynamic and varied. Furthermore, in theory at least, there are several alternatives to cell nuclear transfer. These might make it unnecessary to use embryos as a source of stem cells, or restrict such use to spare embryos. At present it is impossible to say whether these alternatives will indeed prove to have any practical value, whether they will be able to satisfy the need for cell material and if so, when this might be.

5.2 Legal aspects of research into therapies using somatic and embryonic stem cells

In legal terms, regulation of the use of human tissue samples for various purposes, including scientific research and therapeutic applications, is a field that is still in a phase of rapid development. Making human tissue samples available for transplantation purposes is subject to the provisions of the Organ Donation Act. The use of foetal tissue is regulated by the Foetal Tissue Act, which came into effect in November 2001. A proposal for legislation in the field of embryos and human gametes (Embryo Bill) has been approved by the Lower and Upper Houses of the Dutch Parliament. Other proposals still awaiting approval include a bill aimed at guaranteeing the safety and quality of human tissue samples (used for therapeutic purposes; proposed Bill on the safety and quality of human tissue samples) and legislation concerning the authority over further use of body material (in draft form).

This section sets out a brief outline of the developing legal framework and of the legal principles, arguments and considerations upon which it is founded. These have been included because of their relevance to the issue of the exact conditions under

which research into therapies using somatic and embryonic cells can be permitted. The objective is to test the extent to which lines of research considered by the committee to be relevant can be achieved within the legal framework (both existing and under development), and where necessary to provide recommendations for further modification of that framework.

The major legal principles which regulate the use of human tissue samples are:

- the principle that the individual from whom the tissue sample was obtained has the authority to determine how it is used,
- the principle of non-commerciality in relation to the donation of human tissue samples,
- the Dutch principle of '*doelbinding*' (similar to the American legal concept of 'conversion'), which means that the tissue sample may only be used for the purpose or purposes for which it was donated,
- the protection of the health interests both of those who donate the tissue sample and those who receive it,
- the special nature of certain types of tissue samples and the interests that are at stake, which can be a reason for imposing additional requirements,
- the principle that the use of human tissue samples for scientific research and/or therapeutic applications is permissible in principle, and that it should not be obstructed any more than is strictly required by the need to safeguard the above-mentioned interests.

In identifying the options within the current legal framework for using stem cells for scientific research into therapies, it is important to make distinctions on the basis of the source from which the stem cells were obtained. This can involve living stem cell donors (minors/adults, possibly legally incompetent), deceased stem cell donors, aborted foetuses or embryos (spare after IVF or specially cultured) as a source of stem cells.

5.2.1 *Living stem cell donors*

The scope of the Organ Donation Act is restricted to situations involving the donation of human tissue samples for the purpose of transplantation. The Act does regulate the use in scientific research of tissue samples that were originally donated for transplantation purposes, but this only applies in situations where such tissue samples have been found to be unsuitable for the purpose of transplantation. The removal of human tissue samples for the purposes of scientific research is governed by the Medical Research Involving Human Subjects Act (WMO). The principle of informed consent is applicable to such situations, while testing by the medical ethics committee can provide

protection for the participants against health risks associated with such donation in the interests of science. The WMO virtually eliminates any possibility of obtaining tissue samples specially for scientific research from minors and individuals who are legally incompetent. The Embryo Bill contains a special clause governing the donation of gametes where their collection involves the use of an invasive procedure on the individual or individuals involved. In such instances, those involved must be informed concerning the associated risks and objections. Furthermore, the institution's medical ethics committee must give its permission after assessing whether the interests served by making the tissue sample available are proportionate to the risks and objections associated with the procedure, taking into account the circumstances of the individual involved. The Embryo Bill also includes the principle of provision without remuneration, in relation to gametes (article 5, paragraph 2). As far as the donation of human tissue samples is concerned, the principle of non-commerciality has been fully established. However, with regard to subsequent phases, the possibility of commerciality (in the form of the patenting of research results, commercial use) cannot be excluded. It is essential to carry out a thorough analysis of any possible adverse effects that this may have on the availability of tissue samples. In addition, consideration should be given to possible adverse effects on the preparedness of donors to donate tissue 'without remuneration', but also to the obstruction of important research and applications - as a result of the presence of exclusive rights and associated financial thresholds.

5.2.2 *Post Mortem stem cell donation for scientific research*

The *post mortem* donation of a body and/or human tissue samples for the purposes of scientific research can take place by means of a written advance directive (codicil).

In this connection, it is important that the potential donor should be informed about, and is in agreement with, the nature of the research to be carried out using the body and/or human tissue samples in question. Take, for example, the Netherlands Brain Bank, which is specially concerned with the *post mortem* donation of brains and spinal cords in the interests of scientific research. Its codicil stipulates that the research in question will involve neurological and psychiatric diseases. On the basis of the principle of conversion, the tissue sample can only be used for such explicitly stated purposes. The legal acceptability of a more generic use (such as a use obtained for all types of scientific research) is still very much a matter for debate.

It is important to face the fact that, in order to obtain stem cells from tissue samples made available for that purpose, certain 'preservative procedures' must be carried out soon after death (or possibly even before), as is presently the case with organ donation. It is important that this information be given to potential donors, since their permission

can be important here too, in order to legitimize procedures of this kind. The early involvement of next of kin when the donor is drawing up an advance directive has the advantage that they are informed in good time and that they are prepared for the requisite procedure. In formal terms, the donor's advance directive is decisive, but in practice great importance will be attached to any objections lodged by the next of kin to the implementation of the advance directive.

5.2.3 Obtaining stem cells for scientific research from foetal tissue

In the current legal framework, the use of both foetuses (including aborted foetuses) and embryos as a source of stem cells for scientific research into therapies is not obstructed by any overriding fundamental objections. However, it is considered to be important that the use of these types of human tissue samples be subject to a number of further conditions.

The regulations and conditions governing the provision and use of foetal tissue are set out in the Foetal Tissue Act. The definition of foetal tissue used within the framework of the Act is important. It defines foetal tissue as the parts making up a human foetus that was born following a pregnancy of less than 24 weeks and which is no longer alive, or parts thereof. The Act limits the use that can be made of foetal tissue to medical purposes, medical and biological university education and medical and biological scientific research (article 2). In addition, the Act prohibits the use of cultured cells derived from foetal tissue for purposes other than medical purposes, medical and biological scientific research or medical and biomedical education (article 11).

On this basis, it can be concluded that there is no prohibition against the use of stem cells derived from foetal tissue for the purpose of scientific research into therapies. This is, however, subject to the condition that permission is obtained from the woman, who must be informed about the nature and purpose of the scientific research involved. The woman's husband, registered partner or other companion has a right of objection against the use of the foetal tissue in question. The ban on the storage and use of gametes and other parts of the human foetus for purposes of reproduction and for non-medical purposes (article 10) is no obstacle to the use of foetal tissue as a source of stem cells.

The principle of the donated tissue's non-commerciality is set out in article 9.

5.2.4 Obtaining stem cells for scientific research from embryos

On the basis of the theory of progressive legal protection, there are no overriding objections to the use of embryos for scientific research. However, such use is subject to

certain conditions, namely that the study satisfies high scientific standards, that the study does not exceed a period of two weeks and that the embryos will not be implanted once the study has finished. The normative basis for this permissive legal framework was derived from Leenen's theory of the progressive protection of embryos' legal rights, which is considered to be the dominant view of the law in this field (Lee00b). In that theory, the embryo's origin from human gametes and its potential to grow into a human being argue in favour of caution in all dealings involving the embryo. However, in the early phase (preceding implantation in the womb) the embryo has only a limited right to protection (see also section 5.1). It is that limited right to protection which opens the possibility of weighing this against the interests that might be involved in the use of the embryo for scientific and/or therapeutic purposes. Further requirements may lie in the sphere of the importance of those purposes and in the subsidiarity of the use of such a human tissue sample.

From the point of view of the right to protection, there is no good reason to distinguish between research using spare embryos and that using embryos that were specially cultured for the purpose. The manner in which an embryo came into being has no bearing on its right to protection. Accordingly, it is by no means self evident that there should be a ban on the production of embryos specially for scientific research. However, the conditions relating to an important interest and to subsidiarity must be met.

The Embryo Bill is largely a reflection of those views. That Bill presently includes a ban on the special creation of embryos for reasons other than reproduction. This ban can be lifted within a period of five years.

In the Embryo Bill, an embryo is defined as a cell or coherent mass of cells that is capable of growing into a human being. The term foetus is used to describe the embryo in the human body. The Bill contains a prohibition against allowing an embryo to develop outside the human body for more than fourteen days.

On the basis of the Embryo Bill, the use of spare embryos for scientific research (including obtaining stem cells from such embryos for the purposes of scientific research) will be allowed, subject to checking of the research protocol by the CCMO (The Central Committee on Research Involving Human Subjects). In addition, permission to use the embryo for this purpose must be obtained from the 'donors' of the gametes. The CCMO will evaluate the research protocol in terms of 'it is a reasonable assumption that the establishment of new insights in the field of medical science cannot be achieved by means of scientific forms or methods other than by research with the embryos in question or by research of a less radical nature'. In this way expression is given to the requirement for subsidiarity and proportionality.

On the basis of the Embryo Bill, it is currently prohibited to create embryos for purposes other than reproduction (ex article 24, part a), but this ban can be lifted within

5 years (ex article 33, paragraph 2). The ban on scientific research using embryos specially created for the purpose, that is still contained in the bill, can also be lifted within 5 years. However, there are still clauses restricting such research (in contrast to the less constrained uses of spare embryos) to the areas of infertility, artificial reproduction techniques, hereditary or congenital disorders or transplantation medicine. Furthermore, on the basis of the principle of subsidiarity, there is a requirement that the research may not make use of embryos specially created for the purpose. This type of scientific research is also subject to checking by the CCMO.

When the ban on the creation of embryos specially for research purposes is lifted, it is implicit that the creation of embryos by means of cell nuclear transfer for the purpose of obtaining stem cells for scientific research will also be possible. The provision in the Embryo Bill banning cloning (article 24, part f) relates only to so-called reproductive cloning (with a view to the birth of genetically identical human individuals).

The Embryo Bill also establishes the principle of 'non-remuneration' with regard to the provision of embryos (article 8 paragraph 2).

5.2.5 *Obtaining stem cells by the combined use of human and animal tissue samples*

The creation of human-animal hybrids is prohibited on the basis of prevailing legal doctrine. However, this does not apply to the use of methods that cannot lead to the production of such beings. In line with that, the Embryo Bill (article 25) contains a number of restrictions concerning the combined use of human and animal tissue samples, but these do not relate to the combination of human and animal gametes in order to create pre-implantation embryos that will be used as a source of stem cells for scientific research. One important issue is the status to be assigned to the embryos created by the transfer of human cell nuclei into enucleated animal ova. If these are to be considered as human (see section 6), then research on such embryos should be subject to checking by the CCMO. In addition, research into the creation of such embryos should be submitted to the Committee for Biotechnology in Animals. A favourable evaluation by an animal ethics committee will also be required for the collection of animal ova (Wet96a).

It is also important that a proposed amendment to the Special Medical Procedures Act incorporates a ban on xenotransplantation. That ban covers the introduction into or onto a person's body of living parts from an animal. This is not so much a rejection on principle as the introduction of a ban with a view to possible risks for test subjects, patients and those closest to them. It provides the option of lifting the ban for special techniques that eliminate unacceptable risks both to the patient and to public health. For the time being, the ban on xenotransplantation is no barrier to stem-cell research into possible therapies. However, it is a potential barrier to the application of the results

of such research to human subjects, if the source of the stem cells was a combination of human and animal cells.

One matter that is still unresolved involves the patenting of research results and/or the process of obtaining stem cells. Patented therapies could form a barrier to scientific research. This point also demands consideration due to the considerable controversy at international level concerning the patenting of biological material.

Hoofdstuk 6

The committee's standpoint

In recent years, a consensus has been achieved in international scientific circles with regard to the importance of stem cell research for the development of new forms of regenerative cell therapy (section 1.2). The committee endorses this view. Promising results have already been obtained using human cells (including cultured cells) from the tissues of adults and of aborted foetuses. This research does not clash with any overriding ethical objections and is legally permissible, provided that the applicable precautionary conditions are observed. There are considerable technical limitations, however. The number of cells that survive transplantation is very small and there is insufficient foetal tissue available (4.1). The committee takes the view that this research should be stimulated, but that at the same time a determined effort should be made to find new sources of human cells for transplantation purposes, given the promising prospects for the use of cell therapy in a variety of chronic diseases (section 4.2) and the lack of sufficient alternative prospective options for the treatment of these diseases.

The most important alternative is embryonic stem cells (2.1). It has been demonstrated in mice that there is no limit to the number of times that embryonic stem cells can divide (section 3.1) and, again in mice, that they can develop into any kind to tissue (3.2). The committee expects that this will also prove to be the case with human stem cells, which have been available since 1998. Throughout the world, well over sixty human stem cell lines have been isolated from human embryos (section 2.1). The committee feels that existing cell lines provide insufficient options for research into cell therapy (section 4.5) and that Dutch researchers should be able to contribute to the isolation of new cell lines from embryos left over from IVF procedures. Based on the

embryo's relative right to protection and the permissive interpretation of the principle of subsidiarity (section 5.1), the committee believes that, in association with previous recommendations made by the Health Council (section 1.2), the use of spare embryos for this important scientific research is both acceptable and permissible. It would be inconsistent to give the go-ahead to research with embryos in relation to reproductive technology while prohibiting the isolation of embryonic stem cells aimed at the development of cell therapy, under circumstances in which no equivalent alternative is available. The committee takes the view that the alternatives which have been presented for use in regenerative therapy using embryonic stem cells are less promising (sections 3.2.2 and 5.1). The extent to which somatic stem cells can be used for cell therapy is still unclear, nor can it yet be said with any certainty whether they will be able to replace embryonic stem cells in the long term. However, the committee considers it essential that research be carried out into somatic stem cells, and into other alternatives that may not rely so heavily on the use of embryos. In the long term it may well be possible to reprogram body cells into cells, including stem cells, that can be used for therapy. Research into embryonic stem cells can lead to fundamental insights into the programming (and reprogramming) of cells. Its strong position in international research into genetics and cell biology would enable the Netherlands to make a major contribution to this process of acquiring fundamental knowledge.

One important question is whether spare embryos will be able to provide a sufficient diversity of embryonic stem cells in all cases. In order to counteract rejection responses, a huge number of different cell lines (an embryonic stem cell bank) may well be required (section 4.3). Other factors that will contribute to the demand for more stem cell lines (sections 1.2, and 4.5) include the possible contamination of existing cell lines, the lack of genetic variation, the limitation imposed by patients and research into some hereditary diseases. It is not yet certain whether these can be obtained from spare embryos. The committee feels that the legal option of generating embryos specifically for scientific research should remain open (section 5) in the interests of acquiring important new knowledge (the principle of proportionality) that cannot be obtained by any other means (the principle of subsidiarity). The committee considers that, in ethical terms, the distinction between conducting research on spare embryos and creating embryos specifically for the purpose of research is comparatively small (section 5.1). As stated, an embryonic stem cell bank can be set up to counteract rejection responses. Attempts can also be made to obtain embryonic stem cells with all the genetic characteristics of the cells' intended recipient. To this end, the nuclei of somatic cells are already being inserted into enucleated ova (4.4).

Such cell nuclear transfer ('therapeutic cloning') is controversial because it involves the creation of an embryo for instrumental use (sections 4.4, and 5.1.3), and - in the Netherlands – is subject to a ban that can be lifted (the Embryo Bill). The committee

endorses the human pre-implantation embryo's relative right to protection (Lee00b). However, in the view of the committee, this right to protection cannot be used to make a convincing *a priori* objection to the creation of such an embryo by means of cell nuclear transfer. Nor is the committee convinced by the argument that 'therapeutic cloning' will lead to reproductive cloning, and that 'therapeutic cloning' should therefore be banned. Reproductive cloning is banned in the Netherlands. In addition, there is a scientific consensus that the reproductive cloning of humans at the present time is medically extremely irresponsible. It is still unclear whether an alternative means of preventing rejection responses can be developed, such as the induction of immunotolerance or the reprogramming of somatic cells (section 4.3). The committee takes the view that cell nuclear transfers in human ova are appropriate if research using spare embryos leads to usable forms of cell therapy, while the alternatives are either less usable or totally unusable. However, the committee sees no forceful reason to make cell nuclear transfer into enucleated ova legally possible at short (by lifting the moratorium contained in the Embryo Bill). The development of stem cell therapy still requires a great deal of preliminary research (sections 3.2.1 and 4.2). The committee feels that, for the time being, such research can and must be carried out using stem cells from spare embryos.

If research into stem cell therapy based on embryonic stem cells leads to applicable results, this may lead to a shortage of ova. The donation of ova is a radical procedure that is regulated by the Embryo Bill. It is important to remain alert to the possibility of potential donors being pressured into providing ova. Research should also be carried out to determine whether ovarian tissue could be used as a source of ova. Rather than transferring a human cell nucleus into an enucleated human cell, efforts are being made to use enucleated animal ova as acceptors instead (section 4.4). The advantage of this approach is that scarce human ova are not required for the cell nuclear transfer. The scientific and ethical debate about this new type of cell nuclear transfer is still in full swing. The committee does not share the view that the embryos created by this means would not be human embryos. After all, the DNA contained in this embryo is almost entirely human. For this reason, research on such embryos should be subject to the Embryo Bill and should satisfy the same conditions as cell nuclear transfers using human ova.

The committee feels that the current and planned legal framework is largely sufficient for the requisite regulation of Dutch stem cell research. The use of adult somatic cells is subject to the Organ Donation Act or the Medical Research Involving Human Subjects Act (section 5.2.1). The use of cells of foetal origin is subject to the Foetal Tissue Act (section 5.2.2), which also sets out the requirements concerning due care to be met by such use. On the basis of the Embryo Bill, the use of spare embryos for scientific research will be permitted, provided approval is obtained from a central

monitoring committee (5.2.3). The Embryo Bill also incorporates a ban that can be lifted, which covers the creation of embryos specially for purposes other than the achievement of a pregnancy (5.2.4). This also excludes cell nuclear transfer into enucleated ova (therapeutic cloning). While this ban does not currently form a barrier to research, it could well impede progress in the event of the rapid development of this field of research. The transfer of human cell nuclei into animal ova should be incorporated into the Embryo Bill. On the other hand, the committee does not feel it necessary that experiments with existing embryonic stem cell lines should be subject to supervision, because, on the basis of current scientific knowledge, it is not possible to create embryos from such cell lines. Since, as shown in this report, stem cell therapy is still in a very early stage, the committee feels that it is essential that sufficient preclinical research be carried out before stem cell therapies are introduced into clinical practice.

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A The request for advice

B The committee

Appendices

Bijlage **A**

The request for advice

On 17 January 2001, the President of the Health Council received a request from the Minister of Health, Welfare and Sport for advice concerning the use of cells, particularly stem cells, for transplantation purposes (letter ref. CSZ/ME-2145714). The following is an extract from the minister's letter:

In response to the General Consultation on xenotransplantation, held on 2 February 2000, the Lower House of the Dutch Parliament has adopted the motion by Van der Vlies cs (parliamentary papers II 1999/2000, 26 335, no. 12; enclosed) concerning the search for credible alternatives to xenotransplantation. If the associated problems can be solved, then xenotransplantation could even be an alternative to the transplantation of organs of human origin, thereby contributing to the shortening or even elimination of the waiting lists for organ transplants.

In my view, for the time being the only factors that can really contribute to reducing the current organ shortage are the incidence of organ failure on the one hand and promoting the availability of human organs on the other. These are therefore the only credible alternatives to xenotransplantation. Some recent literature has broadly indicated that the use of stem cells, for example, might eventually produce material that could be used for certain transplantation purposes. If this were also to contribute to a reduction in the shortage of organs, then it would constitute an alternative to xenotransplantation.

In view of the above, I would ask your Council to inform me of the scientific situation pertaining in areas such as the use of embryonic or foetal cells (including stem cells) or adult cells (including stem cells) in culturing entire or partial organs, tissues and cells (including cell clusters) for transplantation purposes in the widest sense of the term, i.e. both for organ replacement and for cell therapy. I would also be grateful if you would advise me of any developments that are sufficiently promising that the Council

considers additional stimulation to be essential. Finally, rather than just being informed about the medical/scientific aspects associated with the use of various cell types, I would also be grateful for your advice concerning the medical ethics issues that are (or could be) involved. I would ask that Dr GJ Olthof be included in the committee (or committees) to be established by you, as an observer representing the Ministry of Health, Welfare and Sport.

On the basis of the Health Council's 2001 working programme, I understand that the Council will not be able to produce the advisory report in question before the year 2002. However, perhaps unnecessarily, I would request that you provide me with interim reports concerning new developments with which it is appropriate that I should be conversant prior to the appearance of the final report.

The Minister of Health, Welfare and Sport,
sgd
dr E Borst-Eilers

Bijlage **B**

The committee

Bijlage

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- dr GJ Boer
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- dr PAFM Doevendans
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- prof. Dr G de Wert
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- dr GJ Olthof
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- dr PA Bolhuis
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