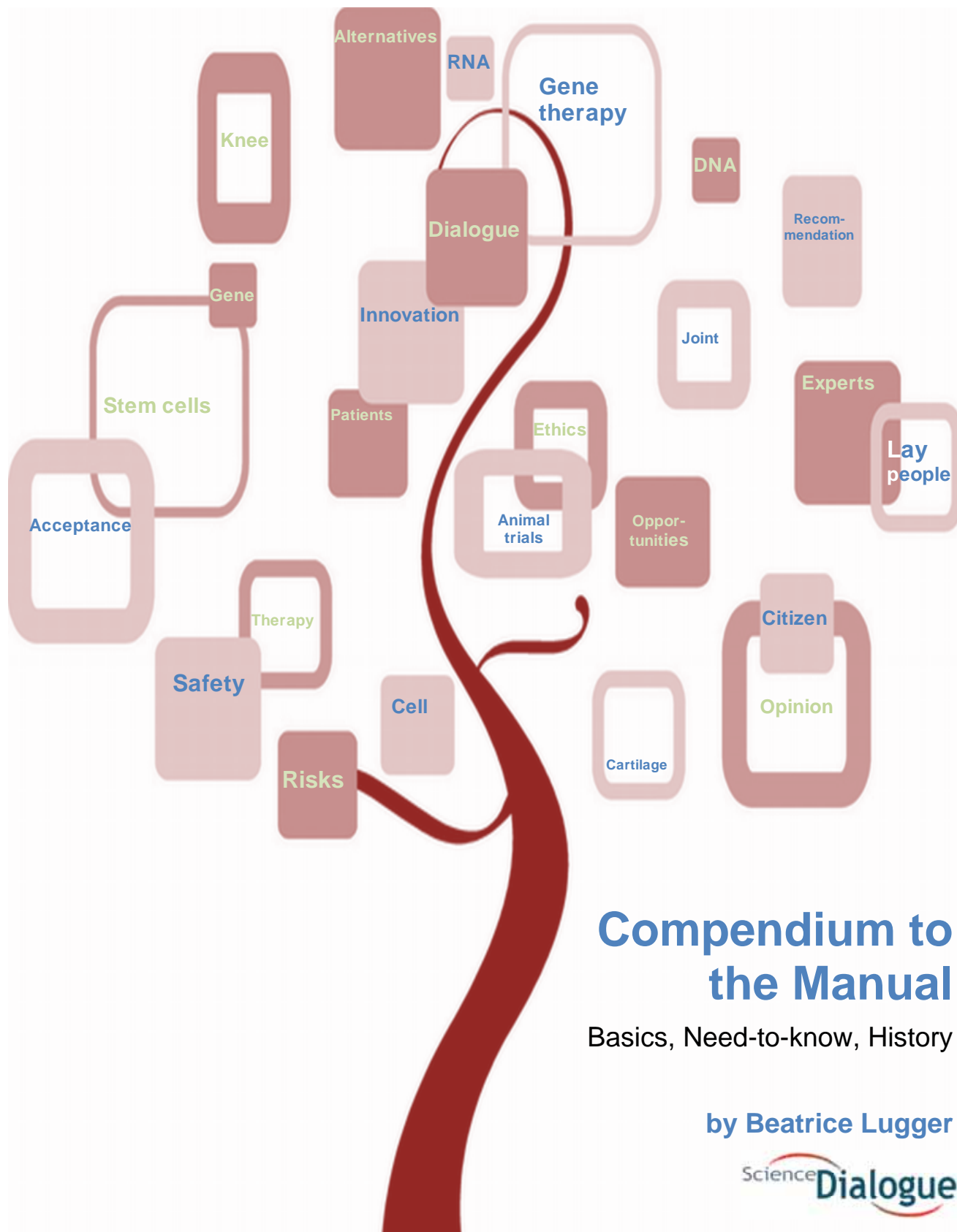


EU Project GAMBA

Basic research into regeneration
of cartilage and bone in osteoarthritis



Compendium to the Manual

Basics, Need-to-know, History

by Beatrice Luggner

Science **Dialogue**

How to read this compendium

This Compendium to the Manual for the EU Project GAMBA is meant to give you a deeper insight into the topics around the project. You will find more information about the symptoms and the treatment of **osteoarthritis** as well as contact points and selfhelp groups. There is background information about the **basic components of life, stem cells, gene therapy and nanomedicine**. What is what exactly, which innovations are already in use, and what is the current state of research? You will also find information on **legal and ethical aspects** of the GAMBA topics that are worth considering.

Last but not least the extensive **Glossary** with its → cross-references will be a valuable source of information.

This compendium was originally written for citizen and patient panels in Germany, which took place in May 2011 and January 2012. Therefore the numbers and statistics often refer to Germany, Irish numbers were added on a case by case basis, if available.

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1. Osteoarthritis

1.1 Symptoms of osteoarthritis patients¹

The complaints associated with osteoarthritis tend to increase over time, however this does not happen continually, but in stages. Especially after long periods of rest– e.g. in the morning, when getting up or after sitting for some time – there is pain and a feeling of stiffness in the joints (start-up pain). This usually improves after a few minutes of movement. Some patients say it feels as if the tendons were too short to stretch the joints completely.

With the progression of the disease the joints become more and more immobile and stiff. Every movement causes great pain. Additionally there is pressure pain in the affected joints. Finally the pain occurs even in rest – quite often even at night. Little by little more areas of the joint are affected and cartilage, bone, tendons and ligaments undergo changes. This leads to misalignments, the joint becomes unstable, it crunches occasionally and its function becomes increasingly restricted.

Typical complaints of osteoarthritis patients are pain, flare-ups of inflammation, swelling and deformation of the joints (see Manual p. 13) and finally the onset of rigidity.

1.2 Diagnosing osteoarthritis

Osteoarthritis is mainly defined by the condition of the patient. There are patients who are in very little pain even though a joint is almost destroyed. Also pain is not an indicator for osteoarthritis, but the joints affected by pain should be examined.

Usually the diagnosis begins with a thorough review of the patient's medical history in a conversation with the doctor. An important indicator for a potential disease is the so-called start-up pain. After phases of rest patients initially suffer from a lot of pain, but this decreases with further movement. Finally the doctor palpates the joint and examines it for swellings, changes in the mobility range and the stability of the ligaments.

When the detailed consultation and the physical examination give rise to the suspicion that osteoarthritis is present, the affected joints are usually x-rayed. The characteristic signs of osteoarthritis are usually clearly visible in an x-ray, especially a reduction of the joint fissure, mismatched joint surfaces, bone compression, the formation of osteophytes and newly formed cavities in the bone and a deformation of the joint.

The state of the cartilage can be analysed with the help of an MRI scan. Ultrasound pictures may show liquid-filled areas and can help to assess inflammations in the joint.

1.3 Common osteoarthritis therapies

Arthritis Ireland (arthritis.ie) is offering information and support for Irish arthritis patients. In Germany the Deutsche Arthrose Forum (German Osteoarthritis Forum), is a self-help forum with approx. 125,000 members. Their website has a constantly updated overview of around 230 osteoarthritis therapies. The members share their experiences in different groups with

¹ Deutsche Arthrose-Hilfe e.V.(undated) u.v.m.

topics such as nutrition and weight, stiffness, medication, allergic reactions to prostheses, sport, physiotherapy and wellness. The main concern of that forum is to curb unrealistic expectations and to monitor the chances of success of different therapies over a long time. Therapeutic success in osteoarthritis is usually measured subjectively, i.e. by questioning the patient. Under the title “Promise and Reality” authors of the forum wrote the following: “Osteoarthritis is curable at last - and similar headlines in the media repeatedly give the impression that there are therapies which can repair the destroyed cartilage and restore it to its healthy state. Unfortunately, to date this is not possible. If one believes statements like that the patient could easily get the impression that he has always made the wrong choices. If he had taken the right supplements, had decided on a treatment with certain therapy device or had followed the strict nutrition and lifestyle rules of some of the self-proclaimed “health missionaries” – he might not suffer from osteoarthritis or would have been healed a long time ago.”

Despite all diagnostic tools which help to track the progression of osteoarthritis, the decisive factors for the therapy are ultimately the severity of the pain and the associated restriction of mobility. At present, osteoarthritis cannot be cured as there is no way to reverse the wear and tear. Therefore it is very important to halt the degeneration of the joints as early as possible.

Depending on the requirements and the state of the patient, different therapy aims are pursued: pain relief, improvement of the quality of life and of mobility and, above all, delaying the progression of osteoarthritis.

1.3.1 Prevention and treatment without surgery and with simple measures

Weight loss is one of the easiest and most effective ways to prevent osteoarthritis or to alleviate the symptoms. Less weight means less burden on the joints, misloads and overloads have lesser consequences and pain can be reduced.

Orthopaedic devices such as splints, canes, forearm crutches, buffer heels or special orthopaedic shoes ensure a temporary protection, for example after injuries, or a long-term relief of joints.

Movement without load is also very helpful. This ensures a better nutrition of the joint cartilage and self healing processes, for example after injuries, are promoted. The progression of osteoarthritis can also be slowed down. Swimming or cycling in low gears is recommended.

Physiotherapy and physical therapy are essential elements of conservative treatment and for the prevention of osteoarthritis. Beside classical massages and thermal treatment (warm and cold) further physical therapies are available. However, the benefits of these have not yet been proven by either scientific insights or findings of traditional medicine. Examples of the therapies on offer are laser therapy which is supposed to stimulate the cell metabolism to produce new connective tissue; stimulating x-ray therapy which is supposed to change the metabolism in the inflamed tissue and electrotherapy, which is supposed to stimulate the nerves.

1.3.2 Drugs as important aids²

Osteoarthritis can be painful at times and causes inflammations and swellings. Therefore the therapy employs alleviating drugs: pain killers (e.g. Paracetamol), decongesting and anti-inflammatory cortisone-free drugs (e.g. Ibuprofen, Diclofenac, non-steroidal anti-rheumatic drugs (NSAR) and even some weak opoid analgesics such as Tramadol), corticosteroids and synovial fluid substitutes such as hyaluronic acid. Drug based therapies always carry the risk of side effects. This can have severe consequences for long-term patients such as osteoarthritis sufferers.

Thus long-term use of paracetamol can lead to kidney and liver damage. The interaction of non-steroidal anti-rheumatics (NSAR) with other drugs can cause gastro-intestinal complaints. Therefore patients on NSAR are usually also given antacids. "If pain killers are taken too often, they can cause pain themselves" (Albrecht 2011). Permanent use of active agents such as Diclofenac, Ibuprofen or Naproxen is also likely to double or even quadruple the risk of a heart attack or stroke (as before).

As a possible substitute for NSAR and cortisone, new drugs are used, the so-called COX-2 inhibitors. They slow down the function of a protein that plays an important role in the development of inflammation and pain. This is a more targeted intervention in the metabolism of inflammation. The incidence of ulcers and bleeding in the gastro-intestinal tract is much lower, but they may also have side effects such as effects on the stomach lining and the kidney function.

Drugs such as NSAR are also administered locally in the form of ointments, cremes or gels. However so far there is no sufficient evidence for their ability to improve circulation and their ability to reduce swelling.

In the case of an inflamed osteoarthritis the use of cortisone-based drugs can be advisable. The drug is injected directly into the affected joint and the duration of the treatment is limited. Cortisone is a hormone and has a strong anti-inflammatory effect. This reduces the reaction to inflammation, such as a chronic irritation of the joint. However cortisone may only be used locally, as otherwise too much of it reaches other organs through the blood stream and could cause severe side effects there.

1.3.3 Liquid prostheses

Hyaluronic acid is the main component of synovial fluid and a building block of the cartilage structure. It binds liquids and forms a kind of firm gel that helps to buffer impacts. As a so-called liquid prosthesis hyaluronic acid injected into the joint is supposed to remedy a deficiency, to alleviate pain and to improve mobility. It is called a prosthesis because the hyaluronic acid is taking over the function of the synovial fluid. Furthermore, it is supposed to improve the metabolism of the cartilage. At present hyaluronic acid is used for age-related wear as well as for joint damage caused by injuries. However, the effect is only temporary and doesn't show the same level of efficiency in all patients. A study shows an improvement and alleviation of pain for a period of no more than three weeks (Michael 2010). Undesirable

² Techniker Health Insurance 2002, www.arthrose.de.

side effects can be allergic reactions or even infections caused by inappropriate handling. A complementary further development of the pure liquid prosthesis is a therapy sold under the brand name Orthokine©. This injection also contains a strong antagonist of the cartilage de-generating substances, i.e. Anti-Interleukin-1 (Anti IL-1). These proteins are first isolated from the patient's blood and then enriched. The combination therapy with hyaluronic acid and Anti-IL-1 is supposed to protect the cartilage and to inhibit inflammations in the joint. Studies that claim to prove the efficacy of this therapy are controversial (Bajer 2005).

1.3.4 Food supplements

Food supplements are substances derived from food that are supposed to have a specific effect in concentrated form. So far, a scientific proof of effectiveness (as for drugs) is not required, pharmacies and drug stores sell a whole range of food supplements meant to treat osteo-arthritis– vitamins, gelatine, devil's claw or millet. A particular favourite is rose hip: "Standardised rose hip powder is a well investigated food supplement for patients with painful osteoarthritis" (Bielenberg 2007).

Presently there is intensive research into substances that are marketed as cartilage protectors. These include plant and animal ingredients such as chondroitin sulphate, glucosamine sulphate or methylsulfonylmethane. Some scientific studies found an alleviating and stabilising effect but others did not. A survey study on glucosamine showed that it did not achieve better results than a placebo (Rozendaal et al 2009). Chondroitin sulphate also did not fare better when compared with placebos (Jüni et al. 2007).

1.3.5 Surgical prevention and therapy

1.3.5.1 Surgical procedures to protect or heal the cartilage³

Cartilage damage could lead to osteoarthritis in the long term. Therefore many current surgery techniques aim to heal the cartilage at an early stage. There are three well established therapy principles:

a) Autologous stem cells

Blood and bone marrow contain so-called mesenchymal stem cells (see Stem Cells Chapter 3). These cells are able to differentiate into cartilage, bone, muscle, connective tissue or fat cells over several generations. Different methods are used to cause a targeted injury of the bone just below cartilage damage, to enable stem cells to reach the cartilage where they stimulate the natural repair processes and the production of new cartilage. To this end a bit of the bone is shaved off (abrasion arthroplasty), drilled (Pridie drilling) or a special instrument is used to punch small holes into the bone close to the joint (microfracturing). As these techniques only improve the function and pain symptoms for a limited time, they are mainly used to delay a necessary prosthetic replacement (Vogt 2007). The improvement achieved with microfracturing only lasts for 18 to 36 months (Groß 2010).

³ Vogt et al 2007, www.arthrose.de, Groß 2010.

b) Autologous cartilage cells

Due to the fact that cartilage cells divide at a very slow rate and there is only very limited regrowth of cartilage tissue, it is a good idea to take cartilage cells from a healthy joint (part) during an arthroscopy and then to grow these in the lab over several weeks, until they are inserted into the damaged area. This transplantation of the body's own cartilage cells (Autologous Chondrocyte Transplantation, ACT) is primarily used in the case of damaged knee cartilage and is supposed to prevent the development of osteoarthritis. This procedure which is only a few years old is already seen as established. The rate of success is dependent on how early cartilage damage is treated in this way.

New methods of cell cultivation and modern materials have led to an advanced variation, where the cartilage cells are grown in the lab on a prefabricated three-dimensional fabric. Then the entire structure is implanted into the damaged area. The carrier material, the so-called Matrix Associated Autologous Chondrocyte Transplantation (MACT), can consist of certain synthetic materials, hyaluronic acid or collagens (proteins that are also the main component of the connective tissue). Both ACT and MACT are only feasible up to an age of 55 years at the most. After that the body's own cartilage cells are usually not suitable any more (Groß 2010).

c) Bone and cartilage grafts

For an osteochondral transfer a cartilage-bone cylinder is taken from a healthy area and then inserted into a cylindrical hole made in the diseased area. In the donor area the body's healing processes usually ensure a regrowth. This technique is known as mosaicplasty or OATS (Osteochondral Autologous Transplantation).

1.3.5.2 Straightening of joints

Osteoarthritis is often the result of a misaligned joint. Knock-knees and bandy legs put strain on the knee and hip joints. If these misalignments are corrected osteoarthritis can be avoided or can delay the progression. For this so-called corrective osteotomy a wedge of bone is removed to correct the misalignment of the joint.

1.3.5.3 Cleaning of the joints

An arthroscopy makes it possible to smooth the cartilage, to remove cell and tissue fragments or to perform a joint lavage without surgery. This is supposed to alleviate the symptoms of patients, but can only delay a replacement of the affected joint. During the cartilage trimming (Chondroplasty or Shaving) superficial ragged, broken or instable joint cartilage is removed and straightened. The particles swim in the irrigation liquid which is suctioned off. The joint lavage can generally be used to eliminate irritating cartilage and bone fragments from the joint.

1.3.5.4 Joint replacement

An artificial joint replacement (endoprosthesis) is currently the final therapy of osteoarthritis and is routinely done (see Osteoarthritis, Manual Chapter 1). The implantation of artificial joints requires major surgery, and different surgery techniques used for this. In older patients

(60 and older) artificial joints for knee, hip or shoulder achieve good results. Patients are finally pain-free and can use the affected joint again. The personal effort of the patient post surgery, i.e. during the rehab phase, plays a significant role for the long-term success. In the case of ankle, hand or elbow joints a replacement is much more complicated. Therefore arthrodesis surgery (artificial ossification of the joint) is still commonly used in ankle osteoarthritis. Its main aim is pain relief.

1.3.6 Alternative medicine

Many clinics and doctors in Germany also offer complementary therapies. This is controversial and these therapies may not be funded by health insurance.

Some of the classical alternative therapies are listed here. Acupuncture, for example, is supposed to induce healing by inserting needles into specific points. In the case of electronic acupuncture, weak electronic pulses are administered through the needles.

Various motion techniques aim to achieve better posture, strengthening of the muscles; special motion sequences combined with breathing techniques are meant to bring relief. One of these is the Alexander technique, a kind of instruction for overcoming unconscious bad postural habits when sitting, standing, carrying or walking. The Feldenkrais method seeks to improve the movement repertoire, i.e. to replace painful movements with more comfortable ones. Slow movements in combination with breathing techniques are the basics of Qi Gong which is meant to balance the vegetative system of the body.

One of the manual therapies is osteopathy, which tries to find and alleviate limitations of the locomotor system; this is also extended to inner organs and the nervous system.

1.4 Self Help Groups

Arthritis Ireland

1 Clanwilliam Square
Grand Canal Quay
Dublin 2.

Tel: 01 661 8188

Fax: 01 661 8261

Email: info@arthritisireland.ie

Web: www.arthritisireland.ie

Chronic Pain Ireland

Carmichael Centre
North Brunswick Street
Dublin 7

Tel: +353 1 8047567

Fax: +353 1 8047567

Email: info@chronicpain.ie
Web : www.chronicpain.ie

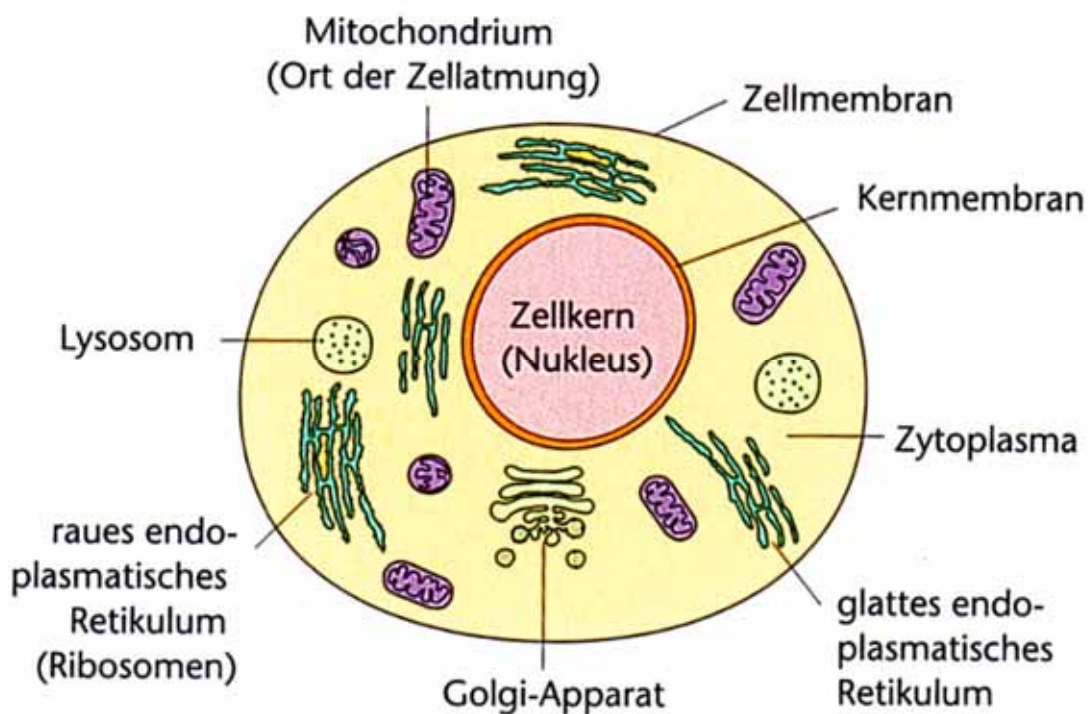
2. The basic building blocks of life⁴

All organisms are composed of cells. The most basic, like bacteria, consist of only one single cell. Plants and animals are composed of numerous cells. The human body contains approximately 100 trillion cells (100,000,000,000,000), which are constantly being renewed. These can be differentiated into about 200 cell types (skin, lung, bone, cartilage cells and so on).

2.1 Cells as protein factories

All cells in the human body have the same structure. They are enclosed by a membrane. Inside (cytoplasm) are different functional units such as energy factories (mitochondria), waste disposal (lysosomes) or packaging system (endoplasmatic reticulum) and, most important of all, the nucleus which contains the genes.

1: Cell structure



→ Explanation (clockwise): Mitochondrion (Cellular respiration), Cell membrane, Nuclear membran, Cytoplasm, smooth endoplasmatic reticulum, Golgi apparatus, Rough endoplasmatic reticulum (ribosomes), Lysome; Explanation (center): Nucleus.

Artwork: Dettmer et al © Elsevier

Cells are continually producing various proteins. These proteins are responsible for all life functions. They serve the communication between the cells, they are needed for repairs, control processes, regulate which genes are activated or deactivated. Last but not least they

⁴ GenSuisse undated, Kirchner & Mühlhäußer 2009, Hacker et al 2009

determine what type of cell the individual cell is – e.g. skin or muscle. The blueprint for the proteins can be found in the genome, which is identical in all body cells. This is called the “Dogma of life”: The genetic information contained in the nucleus (Deoxyribonucleic acid, DNA) is transcribed into RNA (Ribonucleic acid) and then the information is translated into the production of the proteins. Not all genetic information is continuously transcribed and translated into proteins. Which protein is produced and when depends on varied interactions. Furthermore the environment influences the proteins – they mature and change (see Epigenetics Chapter 2.2).

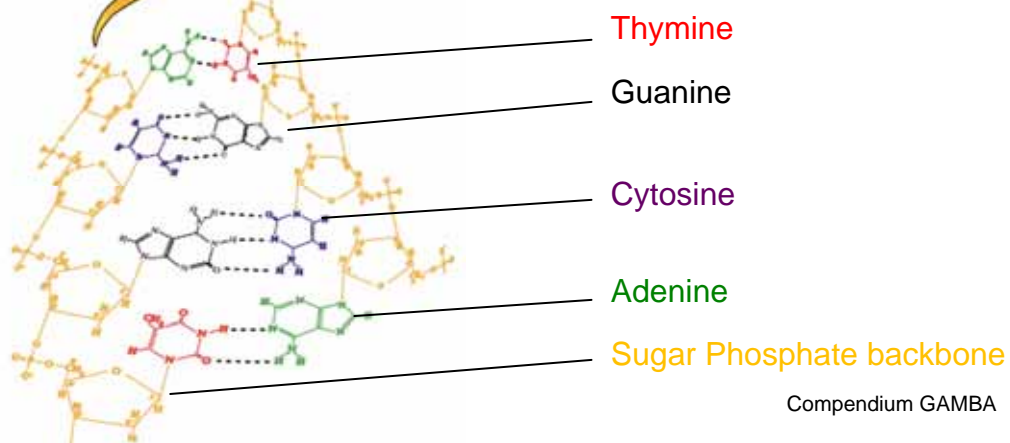
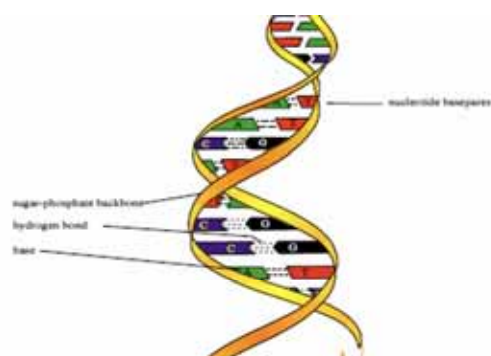
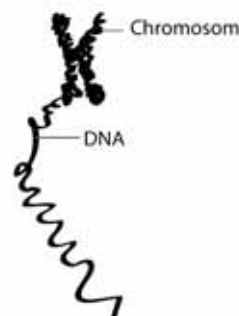
The human genome

The human genome is stored in 46 chromosomes (23 chromosome pairs), in which the DNA strands are tightly coiled. It is estimated that the number of genes contained is less than 30,000. These genes contain the code (blueprint) for the protein production. The length of a gene is determined by start and stop codes within the DNA strand. Most genes are blueprints for protein production. The areas between the genes control the activity. The small ribonucleic acids (RNAi) play an important role in this control.

Fig.2: DNA

Set of chromosomes

Chromosomensatz



Source: Vierstraete, modified

The DNA entwines like vines, in the shape of a double helix. It consists of two strands running in opposite directions; the outside is made up of sugar molecules and phosphates. The inside contains sequences of four different organic chemical molecules, so-called bases, which together with their counterpart on the opposite side form the steps of the helix. These four bases are Adenine (A), Cytosine (C), Guanine (G) and Thymine (T) and they write the genetic code. In contrast to our alphabet which has 26 different letters from A to Z, the gene alphabet only has four letters. These four components Adenine (A), Cytosine (C), Guanine (G) and Thymine (T) form two couples that match like lock and key.

Transcription – copying of DNA

As the proteins are not produced in the nucleus the information has to be exported from the nucleus first. For this transport the DNA sequence is transcribed letter by letter with the help of certain enzymes (polymerases) from the beginning to the end of gene into the so-called messengerRNA, (mRNA). The mRNA replaces the Thymine (T) with an Uracil (U). The mRNA is small enough to pass from the nucleus into the plasma.

The translation of gene sequences in protein chains

The cell plasma contains molecular protein factories (ribosomes), which follow the “building instructions” of the mRNA and produce the proteins by stringing together the corresponding amino acids. In the mRNA three consecutive bases (codon) represent a certain amino acid. These amino acids are freely available in the cell plasma where they are collected by carrier molecules (tRNA) and brought to the ribosomes.

The ribosome then reads the information on the mRNA step by step. For each codon a further amino acid is added to the growing protein filament. Once a protein filament is complete a stop codon is inserted and the ribosome detaches from the mRNA. The gene sequence has been translated in a protein.

All proteins in the human body are made up of different sequences of only 20 different amino acids. The protein chains can have different lengths (between 20 and 10,000 amino acids) and have specific functions.

2.2 Silent and active genes and epigenetics

For many decades the following rule was applied: One gene – one protein. 1990 saw the start of the Human Genome Project, which was meant to find out, letter by letter, what makes us humans what we are. In 2000 the sequence of three billion letters was hailed as the »Book of Life« by US President Bill Clinton. The results were published in February 2001 and the analysis of the data began.

Since then, scientists have been trying to ascertain in large-scale mass screenings whether certain areas of the human genome occur more often in patients with certain diseases. At or close to a conspicuous area, so they propose, there should be a gene that causes the disease in question. A bewildered public was told about genes causing homosexuality, violence, alcoholism, depression and much more (Kegel 2009).

Most of these reports can't be taken too seriously. There are actually around 7000 classic hereditary diseases, in which a certain gene defect has been proven to be the cause. But in most of these diseases the cause cannot be pin-pointed to "the one gene". What we call disease genes are in reality mathematical associations. The search for genes that are associated with cardiovascular diseases, so far has turned up around 100 such associations. These associations have a mathematical significance, but have hardly any practical use (Blech 2010).

The Human Genome Project also shook up the old dogma of life with its relationship between genes and proteins. When the scientists started the project they were expecting, based on the estimated amount of different proteins, to find more than 100,000 genes. In the end they only found less than 30,000 and even this number decreased further. Only 1.5 percent of all DNA strands actually write the text of the genes (Spork 2009). The remainder was labelled as junk DNA.

But how can these few genes possibly be the code for a multiple number of proteins? The answer to the question is the fact that proteins, once they have been produced by translating the mRNA, can still be modified. Tiny chemical reactions are sufficient and give the proteins a different form and consequently a different function too.

A further wealth of variety is due to a process that was already discovered in 1977. According to this the DNA sequences (exons) needed for a protein do not exist as connected base sequences, but are interrupted by long sequences that do not contain protein code (introns). During the transcription of the DNA, the exons as well as the introns are translated into mRNA. Only afterwards a special enzyme complex cuts off the RNA fragments needed for the production of proteins and joins them. This process is called splicing. Because there are distinct possibilities of variations of what is cut and joined, different proteins can be the result (alternative splicing) (Kegel 2009). Also introns may contain gene sequences and much more. Even the remainder of the DNA labelled as junk DNA seems to be full of control modules (Bahnsen 2008).

External influences may alter genes chemically or switch them on or off. These interactions of environmental influences and the genome are being researched by the fairly recent field of epigenetics which focuses on the information on genes. The epigenetic information is even passed on to the daughter cells. The body has a changeable memory (Blech 2010).

According to current knowledge the following three biochemical processes are important epigenetic switches:

1. **DNA Methylation:** The genome is based on chemistry: Methyl groups ($-\text{CH}_3$) attach to a specific component (the base cytosine) of the DNA strand. This makes it possible to switch off genes permanently. This process has been known for quite some time, but geneticists had assumed that methylation only plays a role in the embryonic development, when the methylated stem cells specialise into various body cells. However, methylations can also occur as a reaction to environmental influences and can change the cells permanently. This change is then passed on with every division.

2. **Histone code:** The DNA of a human body cell forms a strand with a length of two metres. To make the DNA fit into the nucleus it is wrapped around miniscule packaging proteins (histones). The tighter a certain DNA sequence is packed the more difficult it is to activate the

genes located in this sequence. The density of the packaging can be influenced by proteins attached to methyl groups (Blech 2010).

3. RNA interference: The genome does not only contain genes, but also codes for so-called micro-RNAs. With the help of enzymes they destroy their corresponding messenger RNAs, which carry the genetic information from the nucleus into the interior of the cell. This results in a reduced number of translations of a gene into a protein. Because these two RNAs affect each other we are talking about an interference (Spork 2009). The micro-RNAs control various development and disease processes (Bahnsen 2008).

In January 2011 the German Ministry of Education and Research announced that they are participating in the new "International Human Epigenome Consortium" (IHEC), an international network for co-ordinated epigenome research. The ministry gave the following reasons: "Epigenetic factors such as DNA methylation, histone modifications, structural changes of the chromatin or non-protein-coding RNAs (ncRNAs), play a significant role in the regulation of various gene activities. They influence (cell) aging processes, the reaction to environmental impact and the development and manifestation of diseases, such as cancer, diabetes, schizophrenia or rheumatism" (BMBF 2011).

This means the characteristics of living beings are not only determined by the mere "gene text", but also by biochemical systems. The latter can control the activity of single genes or gene groups and therefore play an important role, too.

2.3 Communication between cells

There are several options that allow cells to exchange information. Electrical signals which are usually transmitted quickly play an important role, as does the slower chemical information exchange.

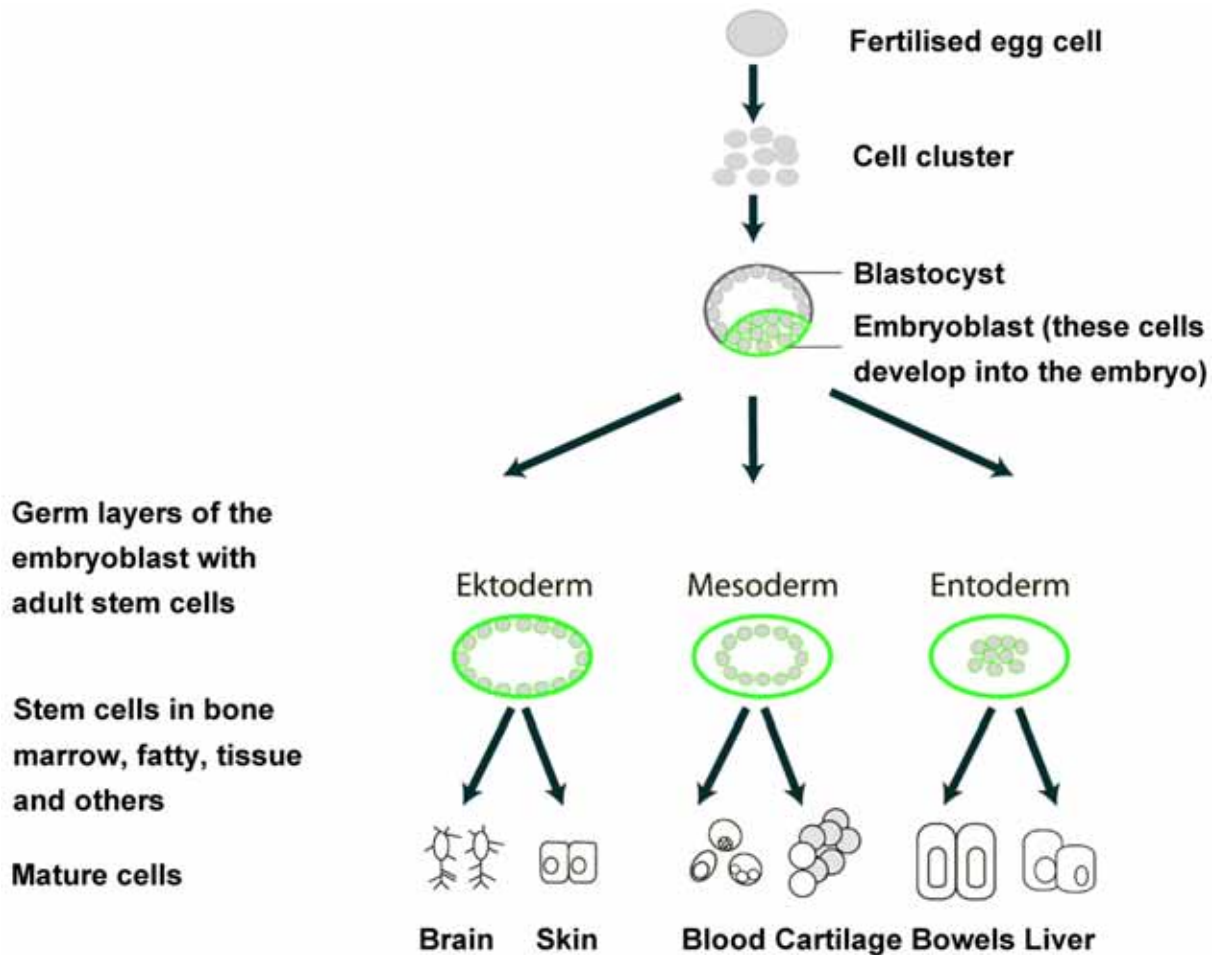
One group of informants are the so-called signal molecules. These are proteins, which include the interleukins (IL-10) and growth factors (TGF- β and BMP-2) used for GAMBA. They influence inflammatory reactions, as well as the duration and strength of the immune defence and regulate division, movement and growth of other cells.

To transmit a signal a cell produces signal molecules and releases them. The signal molecules then bind to a matching receptor on the target cell. There they can initiate a sequence of reactions, which reaches as far as the nucleus. By binding to the cell membrane and initiating several reactions within the cell, growth factor can influence whether certain genes are transcribed or not. For example TGF- β and BMP-2, which are used for the GAMBA project, can influence the differentiation of stem cells into specific cells – such as bone or cartilage cells.

3. Stem cells

For the first few days after fertilization, until the egg cell has divided several times and the blastula contains around 100 cells, all cells are the same. These cells are **embryonic stem cells (ES)**. They are not yet specialized and can develop into the more than 200 cell types in the human body (Pluripotency).

Fig. 3: Stem cells



Artwork: ScienceDialogue, adapted according to Hacker et al 2009

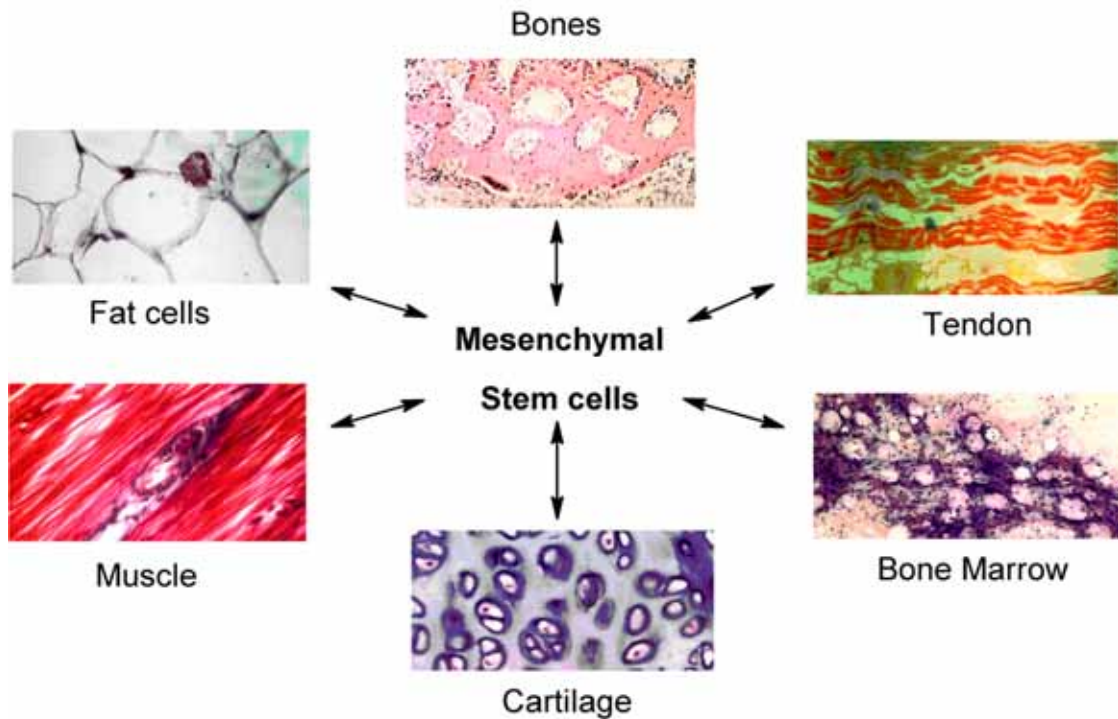
Under the influence of the proteins that they produce themselves, the cells then change into so-called germ layers. These consist of three cell layers: ectoderm (outer), mesoderm (middle) and entoderm (inner). The cells develop their first specialization.

In the adult organism tissue specific stem cells (adult stem cells) produce the new cells needed. These adult stem cells can only develop into one specific cell type. They are constantly renewing themselves and can transform into the tissue cells as needed. They transform into cells for which they already have specific properties (multipotency). For example adults produce around 160 million red and more than 100 million white blood cells from blood stem cells, the haematopoietic stem cells, in the bone marrow. Adult stem cells can be found in the bone marrow, in fatty tissues, skin, brain and liver.

Apart from the haematopoietic stem cells of the blood, the bone marrow contains another important stem cell type, which is derived from the embryonic connective tissue (mesen-

chyme). These mesenchymal stem cells produce fat cells, cartilage cells, bone-forming cells and the connective tissue cells of the bone marrow (stroma cells) (Hacker et al. 2009).

Fig. 4: Differentiation of mesenchymal stem cells



Artwork: ScienceDialogue, adapted from Chen et al 2008

Mesenchymal stem cells can be successfully isolated from umbilical cord blood, from bone marrow, but also from fatty tissue. Mesenchymal stem cells have huge clinical potential, especially for the therapy of defects of joint cartilage and bone defects. With the help of proteins, gene vectors or recently by using messengerRNA (see Chapter 3.2) they can be transformed specifically into the tissue type needed.

For the last couple of years there has also been a new stem cell type, produced in the lab: scientists are able to retransform tissue cells, e.g. from blood or skin back into stem cells. These so-called induced pluripotent stem cells (iPS) then act like embryonic stem cells and can produce other tissue cells (Epping 2010). So far fat cells were turned into iPS cells (T.H.D. 2009) or skin cells were reprogrammed, via iPS, into heart cells (Schöler 2008).

In animal experiments scientists even succeeded in turning skin cells into healthy, mature mice, again this was done via iPS (Gao 2009). In the meantime scientists worldwide have progressed so much that they are now able to transform various mouse tissue cells into other cells without having to employ stem cells. Skin turns into brain (Wernig et al. 2010) or beating heart muscle cells (Srivastava 2010). It is possible that future cell therapies will not need stem cells anymore; however, so far this is only a vision and risks reveal themselves. Recent research has shown that the production of iPS cells from normal body cells causes genetic changes, which could pose a cancer risk (Briseno 2011). Also large segments of the chromosome are not returned to their virgin state. The so-called second genetic coding by methyl groups and histone packages (see Epigenetics Chapter 2.2) cannot be reversed completely (Lister 2011, Osterkamp 2011).

3.1 Stem cell therapy

Since the discovery of stem cells many have seen them as miracle workers and a potential panacea. Millions of patients suffering from diseases such as Parkinsons, Diabetes or Alzheimers hope for a speedy therapy (see Chronicle of Stem Cell Research Chapter 3.2).

Cell replacement therapies, which are based on blood stem cells, are already clinical routine (Hacker et al 2009) – so-called bone marrow transplantations. Blood cancers, such as leukaemia, are treated in this way. However, in this case it is not possible to use autologous stem cells, donors are needed. Autologous stem cells are used for the therapy of lymphomas, tumours or autoimmune diseases (Kiatpongsan et al 2009).

The potential uses of stem cells for the therapy of diabetes, multiple sclerosis or after a heart attack are being investigated in clinical trials. Heart attack patients were injected with stem cells from their own bone marrow into the heart muscle area which was necrotic after the attack (Kutter 2009). In another clinical trial American doctors treated patients with type 1 diabetes with stem cells derived from their own blood. With this type of diabetes the insulin producing cells in the pancreas die. The stem cells are meant to take over their task. This trial proved successful, more than half of the patients did not need to inject themselves with insulin for several months; one patient did not need insulin for all of four years (Dosc 2009).

In future stem cells will play an important role in tissue engineering (Khademhosseini et al 2010). In animal trials a mouse stem cell was successfully grown into a functioning prostate (hach 2008b). Mesenchymal stem cells could be used widely as a source of cells for cartilage and bone (Hacker et al 2009). Scientists at Columbia University used adult stem cells to grow parts of a jaw (Zittlau 2010). Last but not least, a lot of hope is placed in the combination of stem cell and gene therapy, especially for the therapy of hereditary diseases (see Successes and Setbacks of gene therapy Chapter 4.3).

At the same time charlatans are promising unverifiable cures using stem cells. Especially in Russia, China and India these expensive and obscure stem cell therapies are on offer (Schwägerl 2009). But even in Europe there are questionable offers. While neurologists constantly warn against treating Parkinsons patients with stem cells (von der Weiden 2009, Matthes & Kutter 2010), the company XCell-Center in Düsseldorf offered such therapies (it was closed by the authorities in 2011). In October 2010 an 18-month-old boy died of acute heavy bleeding after a doctor had injected stem cells into his brain. This was done completely legally as the German Medicine Law still contains special regulations for drugs containing autologous cells (Berndt 2010, see also Chapter 6.2.1 Law). The XCell-Center issued the following statement: “The complications were caused by the surgical intervention, before the stem cells were administered. Therefore the outcome was not caused by stem cells.” (XCell 2010). Professional associations such as the International Society for Stem Cell Research (ISSCR) are planning to take an active stand against the worldwide proliferation of dubious offers by stem cell companies (o.V. FAZ 2010; see Ethics Chapter).

3.2 Chronicle of stem cell research

1981 Stem cells are detected in mice embryos. They have the potential to turn into any tissue of a mouse body (hach 2008a).

- 1996** The **sheep Dolly** is born. It is the first clone (“identical copy”) of a mammal and was created from an udder cell of an adult sheep. The experiment only came to public attention in the spring of 1997 (hach 2008a).
- 1998** The US biologist James Thomson is the first to succeed in extracting stable stem cells from early human embryos. To this end he destroyed embryos 10 days after fertilization. The **embryonic stem cells (ES)** give rise to the hope that it may be possible to grow every tissue needed in future. At the same time they provoke ethical debates (Berres 2009 and hach 2008a).
- 2000** The German neurologist Oliver Brüstle applies for permission from the German Science Foundation to import ES for his projects. He based this application on a loophole in the **Embryo Protection Act**, which prohibits the creation of ES in Germany but does not explicitly prohibit the import of ES from abroad. This application sparked a political debate (Berres 2009).
- 2002** The team of Rudolf Jaenisch proved that **therapeutic cloning** works in mice: The scientist cloned a mouse with a gene defect, isolated stem cells from the clone embryo, repaired the gene defect with gene therapy and then implanted the healthy cells into sick mice (hach 2008a).
- 2002** The **Stem Cell Act** came into effect in July 2002. It only allows research on foreign ES that were obtained before January 1st 2002. Later the deadline was extended to May 1st 2007 (Berres 2009).
- 2004** The South Korean vet **Hwang Woo-Suk** becomes world famous, when he claims to have cloned a human embryo and to be obtaining stem cells from this embryo. Around the turn of the year 2005/2006 Hwang was exposed as a **fraud** (hach 2008a).
- 2006** The Japanese scientist Shinya Yamanaka produces the first stem cells that require **no embryos**. To this aim he transfected mature specialized mice cells with four genes that produce specific proteins that change the cells back into pluripotent cells i.e. their embryonic state. These new stem cells are called **induced pluripotent stem cells (iPS)** (Berres 2009).
- 2007** The Japanese scientist Shinya Yamanaka transforms **human skin cells** of a 36-year-old woman into **human iPS**. In November of that year two teams independently demonstrated the potential of these stem cells. They utilised these iPS and transformed them into heart, nerve and other body tissues. Unfortunately, these stem cells are not suitable for potential therapies: their production method is perceived as a cancer risk as the gene vectors used incorporate the genes randomly into the DNA (Berres 2009, Schöler et al. 2008).
- 2008** The Californian Company Stemagen announces that they have created a **human clone** and have grown it in the lab for several days (hach 2008a).
- 2008** At the end of the year 20 iPS cell lines for various diseases are available worldwide (hach 2008a).
- 2008** A **single stem cell** was enough for the scientists of the biotech company Genentech to produce a functioning **prostate** (hach 2008b).

- 2009** A German-American research team managed to reprogramme mice cells without genetic engineering, using just a protein cocktail. The method is time consuming, but the **protein-induced pluripotent stem cells (piPS)** are thought to be safer. Only one month later South Koreans and Americans use the piPS method for human skin cells (Berres 2009).
- 2009** American doctors present the results of a trial, during which patients suffering from **Type 1 diabetes mellitus** were treated with stem cells derived from their own blood. These stem cells took over the insulin production in the pancreas – in more than half of the patients this effect lasted for several months. One patient did not need insulin for four years (dosc 2009).
- 2009** At the same time two teams from Beijing reported that they had grown **iPS cells** from healthy adult **mice** (Berndt 2009).
- 2010** The team of Marius Wernig at Stanford University succeeded in transforming **skin cells** in a mouse model **directly into nerve cells** – without the intermediate stage of iPS cells. They called these new nerve cells induced nerve cells (iN) (Wernig et al 2010). Later scientists of the University of California managed something similar: They transformed **tissue cells** directly into actively **beating heart muscle cells** without having to resort to stem cells first (Srivastava 2010).
- 2010** In the US doctors are treating a partially paralysed patient with **embryonic stem cells**. The US drugs agency FDA was the first worldwide to give permission for a treatment of humans with embryonic stem cells (chs/dpa 2010). In November 2010 it granted permission for a treatment with ES cells. This time it is for a therapy treating patients with the hereditary eye disease Morbus Stargardt (ORF 2010).
- 2010** An 18-month-old **boy dies** from severe bleeding, after a doctor had injected **stem cells directly into his brain**. Previously there already had been several incidents at the XCell Center where the death occurred. The German Medicine Law makes special provisions for preparations that contain cells and that are used for the cell donor (Berndt 2010). Professional associations are planning to take an active stand against the worldwide proliferation of dubious offers by stem cell (o.V. FAZ 2010).
- 2010** The team of Derrick Rossi of the Harvard University in Boston reports about a new efficient way to **reprogramme** tissue cells to iPS without using proteins or gene vectors. They are using so-called **messenger-RNA (mRNA)**, which does not enter the nucleus and can therefore not damage the nucleus or the genetic information (Warren et al 2010).
- 2011** Investigations by several independent research teams show that iPS cells carry genetic mutations and could therefore increase the cancer risk. Obviously it is not possible to transform the cell completely into stem cells without epigenetic imprinting: They maintain traces of their methylation and histone modification pattern which are typical for mature cells (see Epigenetics Chapter 2.2; Lister et al. 2011, Osterkamp 2011).

4. Gene therapy

In the middle of the last century it became apparent that the genetic information of all organisms consists of DNA. It was then shown that the genetic information can be transcribed to proteins with the help of the genetic code (see Biological Basics Chapter 2). With the help of various methods it has been possible for more than 30 years to isolate individual genes from the genome (Hacker et al 2009). For the last 20 years genes have been introduced into cells to cause changes.

It does sound convincing: Instead of using pills and creams or a scalpel, genes could be replaced, renewed or complemented, if they are the cause of the problem. This would induce a healing from within. The revolutionary approach was discussed in all media. In the 1980s and 1990s doctors got carried away and made unsustainable promises of cures. Venture capital was pumped into research (Traufetter 2009). But despite more than 1640 clinical trials (see Clinical gene therapy trials Chapter 4.5) in January 2011 there were only three licensed gene therapeutics— none of these in Western countries such as the US or Europe (see Chronicle of gene therapy research Chapter 4.6).

Gene therapy is a targeted transfer of genes or of gene components into human cells to treat or to prevent diseases. The transfer is achieved with the help of gene vectors (BBAW 2008).

4.1 Somatic gene therapy vs. germ line therapy

In the case of hereditary diseases it would be a theoretical option to repair or to amend the defective gene directly in the cells of the germ line, to ensure a recovery spanning several generations. But such germ line therapies are very controversial and very risky (see also Manual Ethics Chapter 5.6).

Therefore present gene therapies only aim at mature adult cells (somatic gene therapy), rectifying defects or adding a therapeutic gene. Changes in the genome of egg or sperm cells are so far taboo.

4.1.1 Functions of somatic gene therapy

In its narrowest definition gene therapy means removing a defective gene from the existing genome of a cell and replacing it with a healthy one. However, this would only be possible with a germ line therapy. In a somatic therapy where millions of blood stem cells have to be transformed simultaneously it is not possible to open every single cell to remove defects and to repair or replace them.

Therefore gene therapy aims at introducing genes with a specific effect into body cells. For this nothing has to be removed from the cells, but something is added:

- For example the genes could take over the function of defect genes that cannot be transcribed with intact copies (genetically modified replacement of defective genes – gene - addition).
- The gene can stop the function of other unwanted gene products, such as cancer causing genes (Anti-Gene Therapy or Antisense Therapy).

- The gene can help to heal a disease by producing additional active substances (therapy with genes). This is the approach that GAMBA is pursuing.

The introduced genes are transcribed in the cells and this leads to the production of the desired or missing protein within the cells. This is supposed to have a healing effect. In this context the genes have the function of therapeutic substances.

4.1.2 Diseases that can be cured by gene therapy

During the development of gene therapy hereditary diseases, where a single gene is missing, defective or cannot be transcribed were the main focus. Such diseases, e.g. monogenetic diseases, are very difficult to treat. These diseases include haemophilia, cystic fibrosis, sickle cell anaemia or severe immune defects such as Severe Combined Immunodeficiency, SCID. Furthermore in the case of monogenetic diseases a long-term effect is desirable to ensure a permanent recovery (see Chronicle of Gene Therapy Chapter 4.6).

But somatic gene therapy is not only suitable for the correction of monogenetic hereditary diseases, but also for the treatment of other serious diseases. At present around 65 percent of all clinical gene therapy trials are aimed at a therapy of various forms of cancer (see Clinical Gene Therapy Trials Chapter 4.5). It is also hoped that there will be progress in cardiovascular and inflammatory diseases, in virology, in respiratory diseases, diseases of the central nervous system or the immune defence (genetic vaccine).

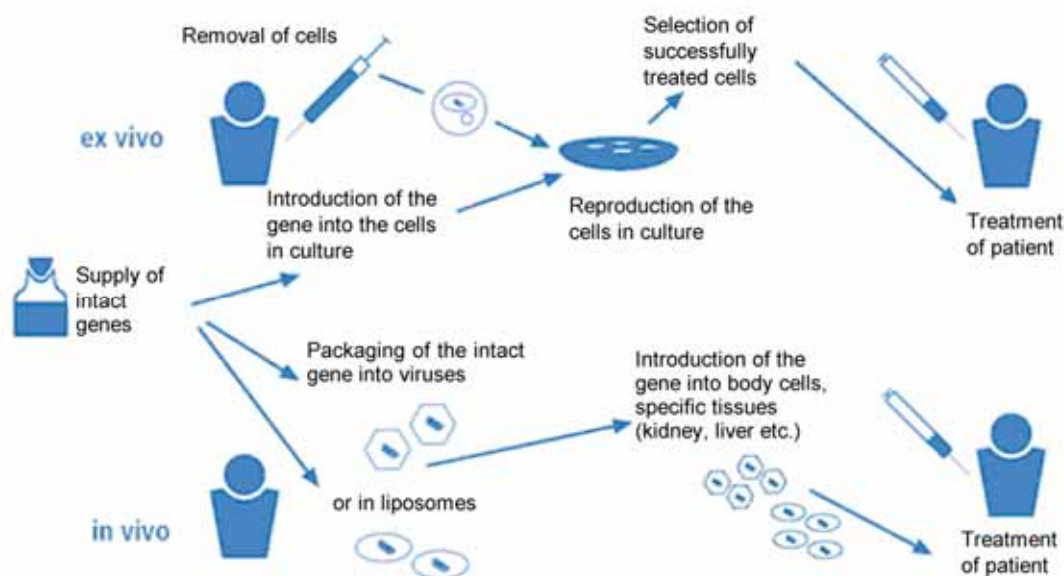
Generally one differentiates between therapies that are meant to reach and affect the entire body over the blood stream, as in the case of monogenetic diseases, and therapies which are meant to have a localised effect – for example in a cancerous organ or in a joint as envisaged for GAMBA.

Cancers are seen as particularly complex, as quite often several genes are defective. Therefore the gene therapeutic approach not only aims at the repair or replacement of mutant genes, but also at a growth inhibition or destruction of the tumour cells, a disruption of the tumour's blood vessels and other measures

4.1.3 Gene transfer in vitro or in vivo

An important prerequisite for the use of the somatic gene therapy are efficient and safe methods to introduce genes into cells. This can be done either outside the body (ex vivo) or directly in the body (in vivo). The insertion of the genetic information into the target cells is usually achieved with the help of so-called gene vectors, into which the gene is packaged (transgene).

Fig. 5: Methods of medical gene transfer



Artwork: BBAW 2008, p. 41, adapted from Winnacker 2002: *Gene technology: Interventions in humans* (translation: ScienceDialogue)

Ex vivo: Cells are extracted from the human body and then the cells in need of therapy are isolated – for example mesenchymal stem cells (MSC) from the bone marrow. A gene or a gene component is then introduced into these cells and added to the existing genes. The treated cells are then cultivated and finally reintroduced into the body. The ex vivo gene therapy is considered to be easiest to control in the case of monogenetic diseases, as it is not necessary to introduce a large amount of vectors directly into the bloodstream of the patients.

In vivo: In the case of in vivo gene therapy viral and non-viral gene vectors (the figure shows liposomes) are administered directly into the blood stream or the individual organs, to genetically change the target cells (transduce). The gene is meant to produce a specific effect.

4.2 Gene vectors

A gene vector is a kind of postman delivering packaged genes into the target cells. It can open the “front door” (cell membrane) and deliver the genes. This is easier said than done. It is still the biggest hurdle, to introduce the therapeutic genes into the cells – especially if this is to happen within the body (in vivo). This is due to the fact that evolution has developed a whole battery of barriers, not only against the absorption of naked DNA, but also against hostile “postmen” such as viruses, to protect the body against diseases.

In order to introduce genes into the target cells, viral vectors, non-viral vectors and physical methods are being tested. Virus vectors are particularly suited as during the course of evolution viruses specialised to transport their gene freight into body cells.

The choice of suitable gene vectors is crucial for the effectiveness of a gene therapy. The choice depends, for example, on whether the gene transfer is taking place in the patient or in a cell culture dish, as these procedures have different requirements regarding the safety and accuracy of the vector (DFG 2006).

Gene vectors for somatic gene therapy must be able to

- Efficiently change specific human cells
- Guarantee a sufficiently strong and sufficiently long-term translation of the gene information into proteins and must have
- The lowest risk profile possible for the desired therapy approach.

Fig. 6: Properties of some gene vectors⁵

	Retrovirus	Adenovirus (GAMBA)	Adeno-associated Virus	Non-viral (GAMBA)
Length of the gene sequences	8 kb*	8 or 36 kb	4,5 kb	Bigger volume of data
Integrates into the genome of the target cell	Yes	rarely	rarely	rarely
Long-term effect	Good	decreasing	good (?)	decreasing
Efficiency	High	high	high	low
Immune reaction to viral proteins	Low	yes	yes	no

**kb = Kilobase = 1000 Bases. This is the length of the gene sequences that can be packaged into the gene vector. Grafik: own compilation*

The choice of the right gene vectors for specific applications will probably require a lot of further research. Gene vectors differ, for example, in the “data volume” (length of the gene sequences) that can be incorporated. Usually a high integration rate and the efficiency of the vector are of importance. Furthermore, depending on the type of therapy, other properties are desirable.

In the case of monogenetic diseases the gene vectors are supposed to integrate their gene freight directly into the genome of the target cell, to ensure a long-term effect. In other therapies, like the osteoarthritis therapy in GAMBA, the effect should decrease over time and not be permanent. Therefore the gene freight should not be integrated into the genome of the cells in this case.

Each disease has particular requirements for the gene vector. In cancer therapies, for example, it may be important that they target specific cells or organs.

⁵ BBAW 2008 et al.

4.2.1 Viral gene vectors

During the course of evolution viruses have adapted perfectly to their host cells and are therefore ideal gene vectors. In most current clinical gene therapy trials, non-reproductive viral vectors are used which are derived from viruses such as retroviruses, adenoviruses, adeno-associated viruses, smallpox viruses, herpes simplex viruses or polio.

Of course it is possible to modify many of the naturally occurring viruses. In the case of naturally occurring viruses, these are usually manipulated in such a way, that they are still infectious (i.e. can still infiltrate cells) but can't reproduce. To achieve this, parts of the virus's genome are removed and they are made receptive for the desired gene freight.

It is planned to use human adenoviruses for GAMBA. These viruses normally cause colds. Almost a quarter of all trials so far used adenoviruses as a transport mode for genes (see Gene Therapy Trials Chapter 4.5). One advantage of these viruses for GAMBA's purposes is that they are not integrated into the host genome. One disadvantage is that they are quite efficient at stimulating the immune system to produce antibodies which destroy the virus. This can be a distinct disadvantage in gene therapy studies; if the patient already had an infection with exactly that type of adenovirus the gene therapy will have no effect. Because they are so common, most people have antibodies against one or several of the almost 50 known virus types (Meichsner 2006). Therefore GAMBA hopes to disguise the adenoviruses with the help of a chemical wrapper.

So far adeno-associated viruses (AAV) have been rarely used in clinical trials but are highly valued by experts. They do not cause diseases and only replicate if a human is infected with an adenovirus. Beside their ability to introduce foreign genes into a variety of body cells, another factor in favour of AAV is that the immune systems only shows a weak response to them (Meichsner 2006) and that they are very stable. However, it was assumed for a long time that adenoviral vectors don't integrate into the genome. Recent research has shown that this is, after all, the case (King & Cohen-Haguener 2008). A lot of risk research is needed to optimise these viruses before they can be widely used in humans.

4.2.2 Non-viral gene vectors

As viral gene vectors can cause problems – they can cause an immune response or could integrate their gene freight into the genome of the target cell (see Risks Manual Chapter 4.2) - researchers are constantly looking for new non-viral possibilities to transport genes into cells. Some genes are directly injected into the muscle or cells as so-called “naked plasmid DNA”. Another possibility is to stimulate the target cells electrically or with shock waves. This causes them to open the pores in the cell membrane for a short time, thus enabling the therapeutic gene to infiltrate the cells.

Plasmid DNA can also be bound to gold coated spheres or packaged into biopolymers of small fat globules (liposomes) (Meichsner 2006). It is planned to utilise liposome packaging for GAMBA.

The biggest advantage of non-viral gene vectors is the fact that they are able to transport large volumes of DNA in one go – multiples of the virus load. Their disadvantage is that they are not as efficient in unloading their gene freight in the cells. Also they are not able to target

specific cells. The way to achieve this is to package them in magnetic nanoparticles which can then be guided to the target area (see also Manual, Chapter Nanoparticles 2.2.4).

4.2.3 Integrating and non-integrating gene vectors

Apart from viral or non-viral, this is another important differentiation of gene vectors: Some integrate their gene freight specifically into the target cells. Some unload their gene freight as genetic elements in a cell. These elements then divide independently from the chromosomes (episomes).

Integrating vectors are needed for monogenetic diseases where a permanent therapy is desirable. Traditionally they are developed on the basis of various representatives of the big family of retroviruses. These include spumaviruses, lentiviruses, oncoretroviruses and others. According to current statistics (see Chapter 4.6.4) various retroviruses were used in 20 per cent of all clinical gene therapy trials up to now. They are the only viruses which have learned, over the course of evolution, to integrate themselves permanently into the chromosomes of their host cells.

However, it is not possible to control at which location in the chromosome the retroviruses integrate their genes. It is likely that they prefer the vicinity of active genes and could therefore be a cause for potential cancer. The leukaemia cases in some SCID children who had been treated with gene therapy seem to be closely linked to the use of retroviruses (see Chronicle of Gene therapy research Chapter 4.6).

A further disadvantage of the oncoretroviruses is that they only penetrate dividing cells, i.e. only a fraction of all cells. One attempt to remedy this is the use of gene engineered amputated Aids viruses – so-called lentiviral vectors. Even though they are closely related to retroviruses they can also reach non-dividing cells (RDB 172). Also, despite the multiple integration of the lentiviral vector into the genome of the cell, it rarely targets cancer causing areas that are so popular with retroviruses.

Non-integrating vectors could be either viruses or non-viral vectors. They are described as non-integrating, because they usually deposit their gene freight outside the genome in the nucleus. Even with targeted attempts to use them to introduce genes into the target genome, they do this only very rarely or not at all. Such vectors are of particular interest when short-term effects are desired or when the aim is to reduce the risk of cancer being caused by a random integration into the genome of the target cell (insertional mutagenesis). However, as recent studies show, integration can never be ruled out completely (Donsante 2007, Stephen et al 2010).

The group of viral non-integrating vectors includes herpesviral, adenoviral, adeno-associated viral (AAV) vectors and so-called integrase-defective lentiviral vectors (BBAW 2008 et al). Even this additional insertion of the genome into the nucleus can have long-term effects – for example when the treated cells divide only rarely or not at all.

All non-viral vectors are considered non-integrating. Generally, it is estimated that the chances of DNA present in the nucleus integrating into the target genome are 1:10,000 – especially when there are breaks in the DNA strands (BBAW 2008, Ledwith et al. 2000; see also Manual, Risks Chapter 4.2).

4.2.4 Production of gene vectors for GAMBA

For the construction of functioning gene vectors two factors are most important: the DNA strand and the vector envelope.

The DNA strand for GAMBA has to contain at least the DNA sequence for the promoter (the switch that enables the copying of the DNA) and the DNA sequence for the desired protein (see biological or pharmacological start, Manual Chapter 2.3.1). For examinations in the lab, sometimes an additional DNA sequence is added, the gene code for a green fluorescent protein (GFP). This makes it possible to check whether the DNA strand has been integrated into a cell and whether the production of proteins works (green fluorescence) or not (no fluorescence).

The DNA sequences for the protein can be obtained in several ways: biochemical reproduction or isolation and cloning.

- For example the sequence for TGF- β (cartilage protein) is stored as a base sequence in data bases and is then produced artificially.
- The gene sequence for BMP-2 (bone protein) in the GAMBA project was originally derived from human cells. The so-called mRNA (see Biological Basics Chapter 2) is transcribed into cDNA (copyDNA). To obtain more cDNA, it is incorporated into bacteria where it is replicated (cloned) and finally isolated again.
- The DNA sequence for the promoter COX-2 was cloned in a similar way as the BMP-2.

To cut and assemble the desired DNA sequences the researchers use proteins which can “cut” and “glue”. These cutting and gluing proteins are important to produce safe vectors. Many years of research have shown which parts of the virus DNA have which functions. This makes it possible to cut out the part of the adenovirus DNA which is responsible for the replication of the cells. In the case of naturally occurring adenoviruses the replication of the viruses causes the destruction of the infected cells. When this gene is not present anymore, the viruses generally cannot cause illness. To make the handling even easier, the virus DNA is split into two sections: a small one into which the gene switch and the gene are inserted, and a big section, which is not modified and is needed for packaging. Both sections are inserted separately into the gene vectors and then replicated in bacteria. In the end both viral sections are reassembled and this results in an adenoviral vector.

4.3 The biggest successes and setbacks of gene therapy

“Gene therapy is ripe for new successes.” These are the words which the journal ‘Nature’ used recently to summarise the progress which was reported in medical journals in the last few months. “After isolated failures, fatalities and years of stagnation, during which the pharmaceutical industry withdrew its support and the researchers were in danger of running out of money for new trials, the sector is now experiencing a boost”, the journalist Helga Hansen wrote in January 2010 (Hansen 2010). By now the first gene therapeutics have been licensed, however, not in Western countries (see licensed gene therapeutics Chapter 4.3.1).

The most publicised fatality during a clinical gene therapy trial was the death of 18-year-old American Jesse Gelsinger in 1999. Gelsinger suffered from the very rare but non-fatal disease OTCD (Ornithine Transcarbamylase Deficiency). An extremely high dose of adenoviruses

was supposed to transport therapeutic genes into his blood stream and his liver. An excessive immune reaction against this attack was the cause of death.

Despite this setback it was possible to prove a possible effectiveness of gene therapies, especially in trials treating severe immunodeficiencies in monogenetic hereditary diseases (see Chronicle of gene therapy research Chapter 4.6). These therapies were beneficial for severe cases of monogenetic diseases, such as the so-called Bubble Kids, who suffer from immunodeficiency and have to live in a kind of bubble to protect them from germs. These children have a very low life expectancy (“Severe Combined Immunodeficiency”, X-SCID and ADA-SCID). For the majority the therapy was successful and the children were able to live a less restricted life. But despite the initial success there was also a sad drawback. The retroviruses used were the cause for leukaemia in some of the treated children. One of the X-SCID children died of leukaemia in 2002.

The treatment of patients suffering from chronic granulomatosis (CGD) looked very promising at first. The immune system of all three terminally ill patients stabilised at first. However, shortly after the researchers had presented their success in 2006, one of the treated patients died. The effectiveness of the therapeutic gene had gradually decreased. The patient died, so to speak, from the effects of his original disease. The therapy had failed for him (see Chronicle of Gene Therapy Research Chapter 4.6).

Apart from the setbacks outlined, these first effective therapies are also a success, because they were beneficial for some patients. Successes were also reported in other areas apart from monogenetic diseases, for example in fighting different types of cancer and in haemophilia B (DFG 2006).

In the area of joint diseases, trials on rheumatoid arthritis are also ongoing. The first trial in 1995 had the aim to curb inflammation in the joint another trial in 1997 had the same aim. However the duration of the therapy was only one and four weeks, respectively, and the number of participants was very low, therefore no significant results could be obtained.

2003 saw the start of a further trial of a therapy for rheumatoid arthritis – again the inflammation inhibition was the main focus. First results were so encouraging that an extension of the study to more than 120 patients was approved in 2005. However, in 2007 a fatality occurred during the clinical trial. A connection with the gene therapy is not very likely, but could not be ruled out altogether. Nevertheless the continuation of the trial was approved later that year (see Chronicle of Gene Therapy Research Chapter 4.6).

4.4 Licensed gene therapeutics

At present gene therapeutic approaches are mostly in the research and development stage and around 1640 clinical trials have been conducted so far. Most of these (69%) are in the first stage, i.e. Phase I trials (see Gene Therapy Trials Chapter 4.5.1). Only 3.5 percent have made it to Phase III and only three gene therapeutics are on the market, although their licensing is very controversial. They are available in China, the Philippines and India:

- An adenoviral gene therapeutic with the gene p53 (Gendicine©) for the treatment of **head and neck cancer** was developed by a Chinese company and licensed in China in 2004.

- A retroviral gene therapeutic with the gene Cyclin-G1 (Rexin-G ©) for the treatment of various forms of **pancreatic cancer** was developed by a US company and was licensed in the Philippines in 2007. In Japan the drug has been available for “Compassionate Use” since 2007.
- In 2007 treatment permission was granted in India for a cell based gene therapy in **cancer therapy**. This therapy was developed by the German biotech company Mologen. Foreign tumour cells are used to activate the patient’s immune system. This enables the body to recognize its own cancer cells and to fight them. The foreign tumour cells are modified before they are injected and are also combined with a vaccine enhancer.

In July 2008 a US company applied for the license for another adenoviral product with gene p53 for the treatment of **head and neck cancer**. However this application was withdrawn in July 2009 due to economic difficulties of the parent company.

In March the European licensing agency EMA rejected the licensing application of a British adenoviral gene therapeutic. It contains the thymidine kinase gene and is meant to be used after the surgical removal of a **brain tumour**. A relevant benefit in relation to the risk could not be ascertained.

4.5 Market figures

Companies and public authorities have been spending millions in the last few years on the research into modern therapies. The following figures will illustrate this:

According to a study conducted by ConsulTech GmbH, the German Ministry of Education and Research, has awarded 2.1 billion Euro as funding for the entire area of life sciences between 2005 and 2009 – 1.6 billion went to research institutes and 468 million went to small and medium enterprises (SMEs). Overall there were 1392 projects by 850 SMEs as well as almost 4200 projects in university and extramural research institutions (such as Helmholtz, Max-Planck, Fraunhofer or Leibniz Society). The share of state-subsidised projects in enterprises is rising steadily. By now the BMBF even funds clinical phase I and phase II trials conducted by small and medium biotech companies (ConsulTech 2010).

The subsidies of the BMBF focus mainly on the area of „Innovative Therapies“, which include nanomedicine, stem cell research and gene therapy. From 2005 to 2011 30 different research projects were given 34 million Euro. In the area of “cell-based regenerative medicine”, which includes many stem cell research projects, the BMBF awarded funding of 18.5 million Euro for 58 projects which include many stem cell research projects for the years 2009 to 2013 (BMBF undated). For the years from 1995 to 2012 around 65 million Euro were awarded as funding for gene therapy research (BMBF 2010).

The German Research Foundation (DFG) also sees a vast potential in these therapies and, as of the end of 2010, has subsidised around 120 research projects on gene therapy and around 390 projects on stem cells – mostly by funding research fellowships (Gepri 2010).

4.6 Clinical gene therapy trials

4.6.1 Clinical trials

Gene therapy is seen as a medical treatment with gene transfer drugs and is therefore subject to the German Medicine Act (AMG). This means that before a new drug is licensed it has to successfully undergo clinical trials on the efficacy and toxicity of drugs in humans (BBAW 2008).

In the lab – pre-clinic

Long before a new substance or a new method is tested on humans, the physical and chemical properties are examined in laboratory and animal experiments. Scientists review the mode of action and then start first deliberations about dosage and compatibility. Only if the results of basic research are convincing the new drug or the new method are tested on patients in several phases.

First reactions – Phase I trials

To answer first questions about the compatibility of a drug or a therapy a small group of test persons is sufficient. This is a first investigation of the efficacy and the reactions in humans. Therefore only those patients may take part for which there is no other effective therapy, either due to the nature or the stage of their disease.

For drugs, one aim is to find the most suitable form of administration – liquid, capsule, injection or infusion. For therapies and drugs it is important to find the right dosage and to record what side effects may occur.

Optimization – Phase II trials

Phase II trials involve a bigger group of patients (up to 100). The main objective is again to strike a balance between the potential risks of the therapeutics used and the risks caused by the disease.

The aim is to show how effective a therapy is for certain diseases and what possible side effects it has. Frequently there are also investigations into the effects of a combination with other drugs or methods on the therapy. Furthermore the dosage is still being fine-tuned.

Comparison – Phase III trials

During this phase there are between one hundred and one thousand participants in various centres, either nationally or internationally. Usually these are randomly divided into two groups and participants don't know for the entire duration of the trial which group they belong to. One group is given the new drug, the other a conventional standard therapy. If there is no standard therapy the comparative group is given a placebo. Quite often not even the doctor knows whether the patient is treated with an old or the new drug. This kind of trial is called "double-blind trial". Only after the successful conclusion of a phase III trial may a new drug be licensed.

Further surveillance – Phase IV trials

Even for licensed drugs or therapy methods it may be useful to achieve further optimization. For example it could be of interest to see which combinations or what timing with other therapies proves to be most effective.

4.6.2 Gene therapy trials worldwide⁶

According to the worldwide data base “Gene Therapy Clinical Trials Worldwide” there were more than 1,630 clinical gene therapy trials worldwide between 1989 and June 2010 (Wiley 2010). Most of these (64%) were conducted in the USA, 29% in Europe, 4% in Asia and 2% in Australia.

Fig. 7: Gene therapy trials worldwide by continents (1989 – June 2010)

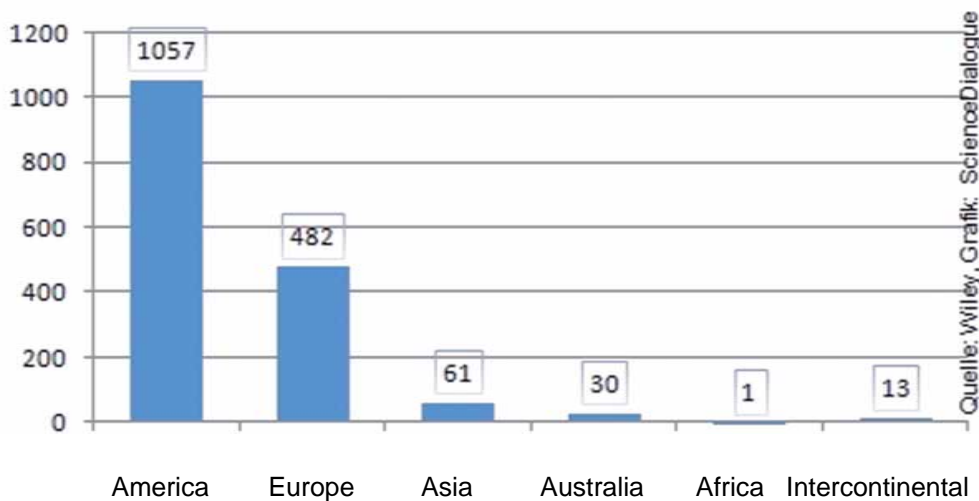


Diagram: ScienceDialogue

So far Great Britain is leading the field in Europe with 195 registered trials. In second and third place are Germany with 79 and Switzerland with 48 clinical trials. 44 trials were registered for France, 27 for the Netherland and 24 for Belgium. It is possible that these numbers from the Wiley database are not complete (Wiley 2010). In future the European Clinical Database Trial (EudraCT) which was announced in September 2010 will supply exact data. This database will list all clinical trials within the European Union.

Only very few clinical trials take the hurdles from phase I to phase III or IV. Many trials are terminated during or after phase I. There are various reasons for this: insufficient or no effect of the therapy, changes in funding or undesirable side effects. Trials by phases: Phase I 61%, Phase I/II 19%, Phase II 16%, Phase II/III 1%, Phase III 3,5%, Phase IV 0,1%.

⁶ Wiley 2010

Fig. 8: Clinical gene therapy trials by phases I-IV (1989 – June 2010)

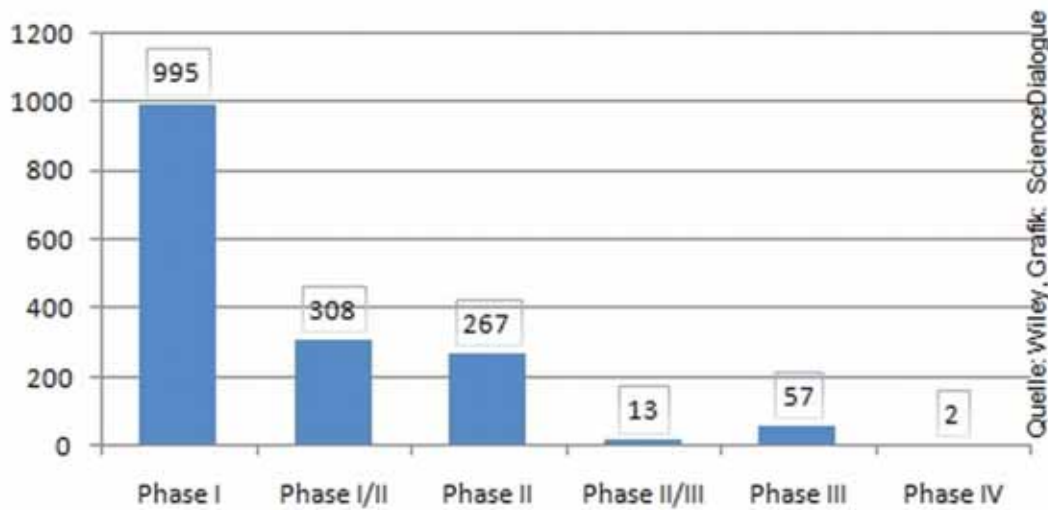


Diagram: ScienceDialogue

4.6.3. Gene therapy trials by diseases

On closer inspection of the fields in which there are the most clinical trials, it becomes apparent that monogenetic diseases, which were the centre of attention in the early days of gene therapy, are not the main focus anymore. Most of the gene therapy trials are focusing on cancers (1060). Next are cardiovascular diseases (143), monogenetic diseases (134), infections (131), neurological diseases (30), eye diseases (18), other diseases (40), gene marking (50) and trials with healthy volunteers⁷ (38).

Fig. 9: Investigated diseases in clinical gene therapy trials (1989 - June 2010)

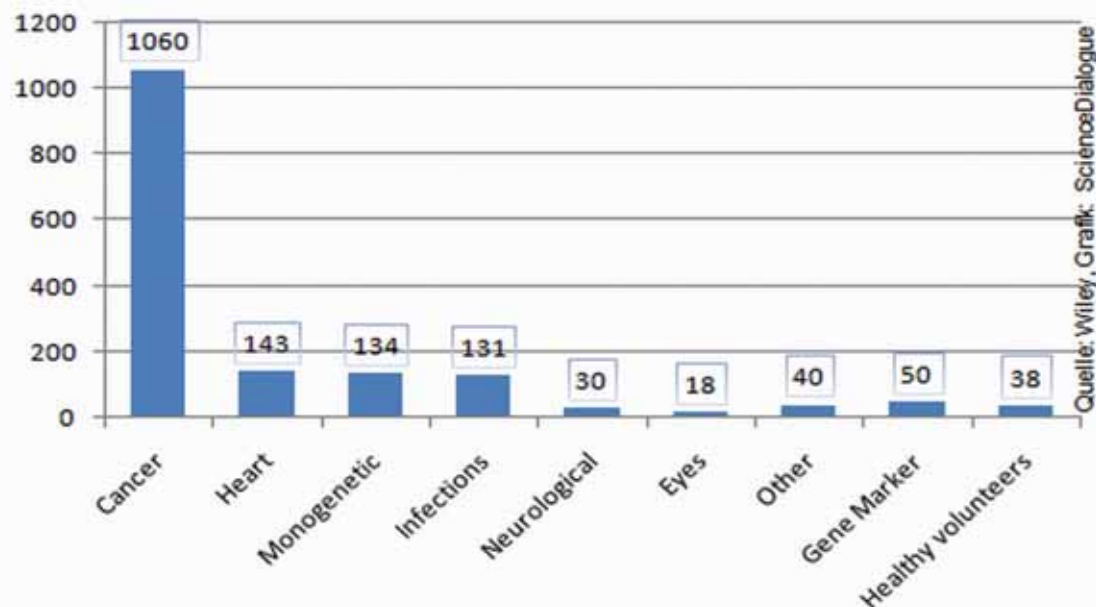


Diagram: ScienceDialogue

⁷ These are dosage finding and compatibility trials for vaccines against HIV, HepatitisB, Japanese Encephalitis, Dengue fever.

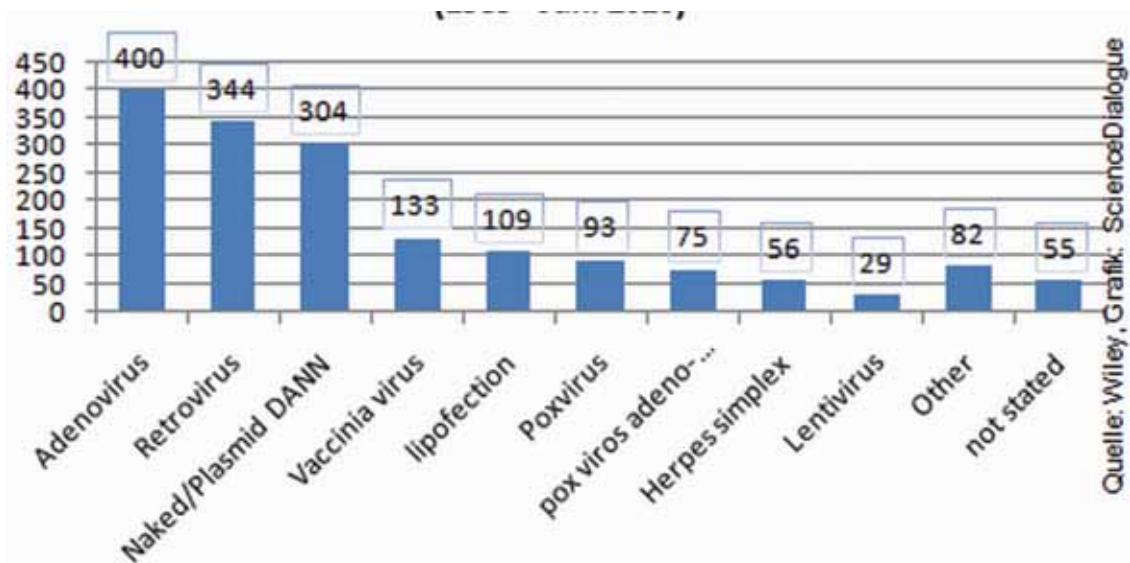
4.6.4 Gene vectors and gene types

The most commonly used viral gene vectors were adenoviruses (24%) and retroviruses (21%). Smallpox viruses (vaccinia virus 8 % and pox virus 6%), adeno-associated viruses (5%), herpes viruses (3%) and lentiviruses (2%) were also tested.

In non-viral gene transfers Plasmid DNA accounts for 18% and lipofection for 7 %.

GAMBA utilises adenoviruses and non-viral gene vectors.

Fig. 10: Gene vectors in clinical gene therapy trials (1989- June 2010)



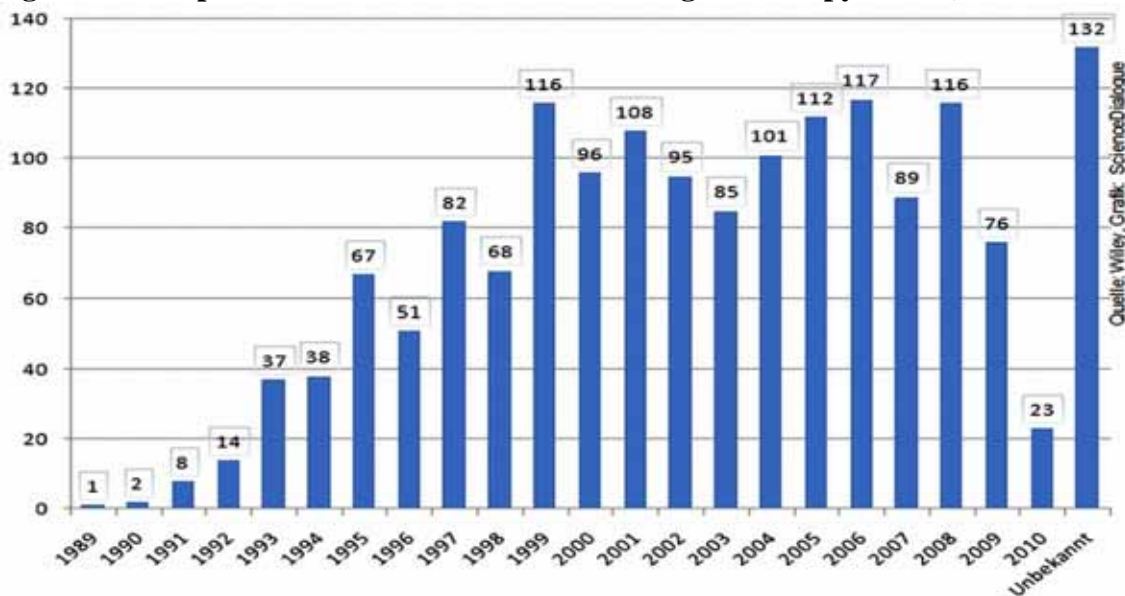
Artwork: ScienceDialogue

The majority of the transferred genes are meant to stimulate the target cells to produce antigens (20%) and cytokines (18%) – the latter are proteins that control immune responses, these also include IL-10 which is used for GAMBA. Next in line are tumour suppressors (11%) and growth factors (7.7%), e.g. BMP-2 and TGF- β that are also used for GAMBA.

4.6.5 Success model with obstacles

During the history of gene therapy there have been various setbacks. One of the most severe was the death of Jesse Gelsinger in 1999. Since then the worldwide number of trials starting per year has been fluctuating between 76 and 117 (Wiley 2010). However the total figure of 9359 ongoing clinical trials worldwide does not exactly indicate a boom in gene therapy (figures from 2010) (ISRTCN 2011).

Fig. 11: Development of the worldwide number of gene therapy trials (1989 – June 2010)



Quelle: Wiley, Cralk. ScienceDialogue
Diagram: ScienceDialogue

4.6. Chronicle of gene therapy research

With more than 1640 clinical trials worldwide there is a plethora of details. The following lists trials, which attracted particular attention or trials concerning joints.

1988 May 22nd 1998 is regarded as the birthday of clinical gene therapy. This was the start date of a study on the genetic labelling of so-called tumour infiltrating lymphocytes (white blood cells) at the US National Institute of Health (NIH). This project did not pursue therapeutic but solely diagnostic purposes. But as the cells were genetically modified, this trial was classified as a gene therapeutic trial (BBAW 2008).

1990 The first gene therapeutic treatment of a human worldwide within a clinical trial was carried out on September 14th, 1990. Due to a gene defect four-year-old Ashanti DeSilva was lacking the enzyme Adenosin-Deaminase (ADA). This meant that her body could not degrade a protein that is toxic for the white blood cells. Consequently the T-lymphocytes which are crucial for the immune response could not mature in the bone marrow. Therefore the little girl had little protection against pathogens. Usually children who are suffering from **ADA-SCID (Severe Combined Immunodeficiency)** do not live to reach adult age, despite treatment and a sterile environment. The medical scientists French Anderson and Michael Blaese of the research institute of the NIH extracted white blood cells from the child. These cells were multiplied in cell culture and healthy ADA genes were added. Even though the therapy was repeated several times it was not successful. Nevertheless Ashanti survived, as she was given the missing enzyme in drug form (Meichsner et al 2006).

1995 In 1995, Chris H. Evans of Harvard Medical School, Boston, commenced the first clinical gene therapy trials on joints. He injected nine patients who were suffering from **rheumatoid arthritis** with recombinant **retroviruses** which were bearing the gene freight IL-1Ra. IL-1Ra is an antagonist of the inflammation promoting interleukin and was injected into the metacarpal joint. One week later these joints were replaced with artificial joints. The removed joints were examined but no effects could be determined (Evans et al 2009).

- 1997** Two years later two patients with severe **rheumatoid arthritis** were treated in the same way that Evans used (1995) with gene vectors for IL-1Ra. The joint treated was again the metacarpal joint. The trial was lead by Peter Wehling of the University Clinic Düsseldorf. But this time the timeframe before the implantation of a joint replacement was extended to four weeks. During this time the patients observed less pain and swelling. In one of the two patients the joint remained pain free even though other untreated joints were affected by further acute phases of the disease. Laboratory examinations of the tissue of the treated joint confirmed that the inflammatory Interleukin IL-1 had been curbed (Wehling et al 2009).
- 1999** On September 17th, 1999 18-year-old American Jesse Gelsinger died following gene therapy. Four days earlier a team around the gene researcher James Wilson of the University of Pennsylvania in Philadelphia had injected the extremely high dose of 38 trillion gene manipulated **adenoviruses** into his blood stream and liver. This seems to have caused an excessive immune reaction against the adenoviruses. The young man was suffering from the rare disease **OTCD** (Ornithine Transcarbamylase Deficiency). Due to a congenital gene defect his body was not able to degrade the ammonia that is produced when proteins are digested. Thanks to a strict low protein diet and additional medication Jesse was in control of his disease and had volunteered to participate in the trial. However in the patient information which had been given to the young man there had been no reference to previous fatal trials on monkeys (Meichsner 2006, Traufetter 2009 et al).
- 1999** Alain Fischer of the Hôpital Necker in Paris, France commenced a clinical trial of a gene therapy for so-called Bubble Kids, **X-SCID** patients without a functioning immune system, who have to live in a special germ-free environment and have a low life expectancy. He treated ten children who were suffering from X-SCID by inserting a piece of healthy genome into blood stem cells taken from the children and then injected them back into the children. The insertion was done with the help of **retroviral vectors**. Following the treatment nine of these children were able to produce antibodies for the first time and were able to leave their protective “Bubble”, and to lead a normal life at home. Initially this was a phenomenal success for gene therapy (Hacker et al 2009, Traufetter 2009).
- 2002** Three years after the **X-SCID** gene therapy in 1999 four of the little patients had developed acute **leukaemia**. Three of these successfully beat the cancer, one patient died. It turned out that the retroviral gene vectors which had been used, had also stimulated cancer genes in the genome of the treated blood cells. Additionally new studies showed that the correction itself may cause cancer. At least this proved to be the case in long-term trials in animal models. To this day it is not clear whether the gene vector or its freight – the substitute gene – caused the cancer (DFG 2006, Woods et al 2006).
- 2003** In October 2003 China licensed **the first ever commercial gene therapeutic for clinical use – Gendicine®**. In approx. 50 percent of all human tumours, the gene p53, which is important for the so-called programmed cell death of defect cells, is mutated. Gendicine introduces a functioning gene p53 with the help of an adenoviral vector. This should lead to a self-destruction of the cancer cells. For a license in China the successful completion of phase I and phase II clinical trials (see Clinical Trials, Chapter 4) is suffi-

cient. Phase III trials which are mandatory in Europe and the US do not have to be completed (BBAW 2008).

- 2003** Philip J. Mease of the Swedish Hospital Rheumatology Clinical Research Division in Seattle tried a different therapeutic approach for **rheumatoid arthritis** than Evans in 1999. He focused on the anti-inflammatory tumour necrosis factor TNFR:Fc. He packaged the gene freight in recombinant **adeno-associated gene vectors** which transported it into the cells in the diseased joint. This construct has the complicated name tgAAC94. 15 patients participated in this first clinical trial. In 2005 Mease was given permission to extend the trial to more than 120 patients (Evans 2008).
- 2004** In Frankfurt (Germany) the gene therapy of a patient who was suffering from the extremely rare immunodeficiency chronic granulomatous disease (CGD) commenced. In the spring of 2005 two more patients were treated, one in Frankfurt and one in Zurich. The most important white blood cells that normally destroy dangerous bacteria (neutrophil granulocytes) are disabled in these patients. For the therapy, blood stem cells taken from the patients were equipped with a functioning copy of the defective gene. Within 50 days there was a marked improvement in the condition of the patients who had been suffering from bacterial and fungal infections, which had not responded to treatment. For the first time the patients were free of serious infections. However, only a few days after the publication (April 2006) of the successful gene therapy trial the patient treated in 2004 died. The number of genetically modified cells in this patient had receded successively. His body was not able to fight germs any more; the gene therapy had failed him (Müller-Jung 2006, BBAW 2008).
- 2004** Alain Fischer and Marina Cavazzana-Calvo of the Hôpital Necker in Paris resumed the gene therapy of children with the immunodeficiency **SCID** (see SCID therapy in 2002). The patients had to be at least 6 months old to qualify for treatment – the children who had previously developed blood cancer had all been younger. Also the quantity of genes introduced per cell was diminished as well as the dose of the genetically modified stem cells (Siegmond-Schultze 2004).
- 2006** A genetic defect is the reason that children with the congenital immune defect **Wiskott-Aldrich Syndrome** (WAS) carry sick blood cells. The gene for the WAS protein (WASP) is mutated and the blood platelets are not able to close wounds like healthy blood cells do. These patients suffer from severe bleedings and frequent infections. So far children with Wiskott-Aldrich syndrome are treated with healthy blood cells from donors, if possible. In October 2006 Christoph Klein from the Medical University in Hannover commenced a trial with ten children for whom no donor had been found and whose life was in danger. Stem cells were taken from the children and the defective WASP gene was corrected with a gene therapeutic **retroviral vector**. The transplantation of the genetically corrected autologous blood stem cells resulted in a fast and marked improvement in nine out of the ten patients. Unfortunately, one of the patients developed **leukaemia** following the therapy, just like some of the X-SCID children. The scientists are not able to control where in the DNA strand the retroviruses used as gene vectors unload their gene freight (Boztug et al 2010, Klein 2009, BBAW 2008).
- 2006** In November 2006 scientists of the University of Pennsylvania reported successes with a gene therapy for the treatment of 74 **Aids patients**. The doctors took blood precursor

cells from the patients and introduced the gene OZ1 into these cells with the help of a **lentiviral** mouse virus that cannot reproduce any more. After two years of treatment the patients had less viruses in their blood and a higher number of CD4-T cells that escaped destruction from the virus. During the phase II trial the patients treated with OZ1 had a lower number of viruses in their blood after one and two years and a higher number of CD4-T cells, when compared to the control group. However the positive effects were not as good as those of the current standard combination (Spektrum direkt 2009).

2007 On July 2nd, 2007 a 36-year-old woman died of multiorgan failure. 22 days before she had been injected with the second dose of the gene therapy tgAAC94 (tumour necrosis factor and adeno-associated viruses) into her right rheumatic knee. This was done during the clinical trial for the therapy of **rheumatoid arthritis** which was extended in 2005 (see 2003). On the day of treatment she had complained about tiredness and a slightly raised temperature (37.6 degrees Celsius). The American Food and Drug Administration (FDA) stopped the trial after her death and investigated the causes as did the relevant advisory body at the NIH (Recombinant DNA Advisory Committee, RCA). It was presumed that a fungal infection, histoplasmosis, was the cause of death. In December 2007 the FDA gave permission to resume the trial, while the RAC stressed that due to the limited data available a causal relation to the trial could not be ruled out completely (Evans 2008). The results of the resumed trial with 127 patients are not available yet.

2007 The Team of Patrick Aubourg of the state-run French Health Institute INSERM are the first to treat a disease of the central nervous system with gene therapy. They used viruses of the HIV family, so-called **Lentiviruses**. The two treated seven-year-old boys were suffering from the fatal disease **Adrenoleukodystrophy (ALD)**, which was the topic of the Hollywood film "Lorenzo's Oil". A defect of the ABCD1 gene causes a disruption of the disposal of fatty acids. Due to this fault ALD patients progressively lose the protective layer of the nerves in the brain. The nerves are degraded. For both boys the search for a donor for the conventional bone marrow transplantation had been unsuccessful. Two years after the gene therapy the scientists reported that the therapy with autologous blood stem cells and modified ABCD1 gene had been as successful as the conventional donor therapy. It was also shown that lentiviruses repeatedly introduce genes into the genome and are less prone than retroviruses to introduce them into regions of the genome that could increase the cancer risk (Cartier et al 2010).

2008 Two research teams are reporting about a gene therapy for the hereditary eye disease **Leber's congenital amaurosis (LCA)**, that results in blindness. Each team treated 3 patients by injecting **adeno-associated viruses (AAV)**, which had been equipped with the correct version of the gene RPE65 under the retina of the eye. This resulted in a regeneration of the cells of the retina and they regained the ability to produce photo pigments. The day and night vision of the patients improved (Kaiser 2008).

2009 In January 2009 Italian scientists inspired with the news of a new form of therapy for children suffering from the severe immunodeficiency **ADA-SCID**. This disease is caused by a gene defect and the resulting lack of the enzyme adenosine deaminase (ADA). Eight out of ten children treated were as good as cured after a gene therapy with autologous stem cells with a **retrovirus** and the gene freight for the adenosine desami-

nase (ADA). After four years the gene modified stem cells were safely integrated. The patients did not require additional enzyme therapy any more (Aiuti et al 2009).

2010 Further eye diseases are the focus of researchers. In patients with the hereditary disease **retinitis pigmentosa (RP)**, which leads to a destruction of the retina and consequently blindness, a little capsule containing gene modified cells that produce the protein CNTF (Ciliary Neurotrophic Factor) is inserted and prevents the destruction of the retina. Scientists reported that the vision of 10 patients had improved or stabilised. A further trial of these capsules was conducted with patients suffering from a form of **age-related macular degeneration (AMD)**. The results of this trial are not yet available (RDB 105 Inno).

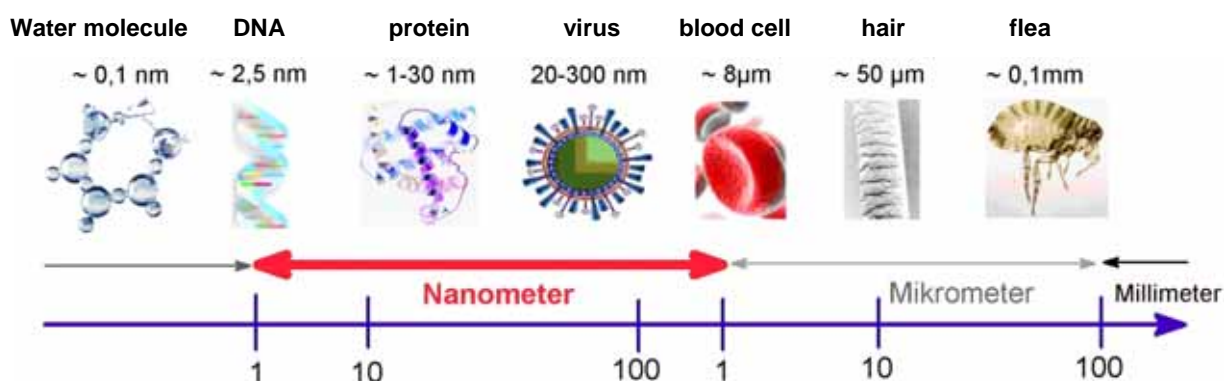
2010 Further progress towards a gene therapy against **HIV** was reported by the American scientist John Rossi and his team. With the help of lentiviruses they modified autologous blood stem cells in such a way that they produce three new molecules. One blocks the production of proteins, which are important for the HI virus. The second leads to the degradation of viral protein in the cell. The third ensures that an important receptor for the HI virus (**CCR5**) is not produced on the cell surface any longer. This means that the viruses cannot connect to and enter the cell anymore. However, after four years the number of the modified stem cells was so low that the four patients had to continue their conventional medication (Zaia et al 2010).

5. Nanomedicine

The world of the dwarves (Greek: Nanos) only became accessible to us thanks to new technologies like the scanning tunneling microscope. One nanometer equals 10^{-9} meter, that's 50,000 times thinner than a human hair. Up to a size of 100 nanometres a particle is considered to be a nanoparticle – this category includes molecules, the DNA, proteins, viruses many fine-grained minerals and more.

Today it is possible to produce particles on a nanoscale and to utilise their specific properties – for example for water-repellent coatings on surfaces, nano impregnation sprays or reflecting nano particles in sun lotions and polishing particles in tooth paste.

Fig. 12: Nano-scale



Artwork: ScienceDialogue

For a long time the image of nanomedicine was characterized by utopian ideas and the most important of these was the brainchild of a technology expert: In 1986 K. Eric Drexler published “Engines of Creation” which contained a vision of nanomachines, which could replicate. He also conjured up a nanomedical vision: nanorobots which move purposefully through the blood stream, to either repair or destroy cells (RDB 519 Drexler). Nanomachines or robots that work in our blood stream rank amongst the utopias of nanomedicine. But particles and molecules on a nanoscale are already in use or are being tested in clinical trials. (RDB 518 Thorbrietz et al):

- For the so-called “drug-targeting” nanoparticles with a special coating are used as drug carriers. The coating ensures that they can only be absorbed by certain cell types. If a drug is attached to such a coated nanoparticle it can be transported accurately and selectively into the sick cells only. There have been first successes transporting anti-inflammatories and cancer antagonists and first licenses have been granted.
- Another form of “drug targeting” involves magnetic drug transporters. The substance and magnetic particles are encased in nanocapsules and these are then directed to the target area with the help of an external magnetic field. Once they have reached their destination they release the active substances.
- A further technique promises advancements in cancer therapy. Magnetic nanoiron particles can be directed to the cancer tissue; once they have reached the target area an external magnetic field is used to make them oscillate which causes a rise in temperature in

the tissue (McCarthy 2009). A short-term warming of the cancer cells up to a maximum of 45 degrees Celsius (hyperthermia) blocks certain repair mechanisms of the cells (Müller-Jung 2009), the cancer cells are destroyed. Because magnetic nanoparticles can penetrate the blood-brain barrier they are a ray of hope for the therapy of brain tumours (o.V. wiwo 2010).

- In modern diagnostics iron oxide particles which are injected into the blood stream are used as contrast agents in magnetic resonance imaging (MRI).
- In the case of implants and in so-called tissue engineering optimised surfaces and structures on the nanoscale ensure a better compatibility and integration in the biological environment.

GAMBA is utilising the special features of the nanoworld in many different ways – with magnetic nanoparticles, with viruses on a nanoscale, and non-viral envelopes as gene vectors; and also with special surface structures of the matrices which have tiny openings to accommodate gene vectors and active agents.

6. Legal classification of GAMBA

6.1 Introduction

The research and development of new therapies and drugs as envisaged for the GAMBA project are naturally subject to legal conditions. Statutory provisions of national and European law regulate the domain of life sciences. Furthermore, general standards and rules are important, such as

- Regulations and guidelines of professional bodies: for example the requirements of good professional practice or recommendations of medical associations on the use of gene therapy.

or

- Specifications in administrative provisions and/or from administrative procedures (e.g. requirements concerning approval procedures and their processes).

Chapter 6.2 lists the aspects which are of importance for the GAMBA project.

All of these formal and informal standards help to regulate the way that society deals with certain topics. Important aspects are:

- What is allowed – and also in what circumstances? What is forbidden?
- Who bears what responsibility?
- How can the highest possible level of security for humans be achieved (this does not only apply to patients, but also to scientists, lab technicians, participants in clinical trials, doctors, nursing staff, and possibly even relatives) and for the environment? Which risks could be deemed acceptable in view of the possible healing chances?

The specific legal regulations are the result of a societal deliberation processes (see Ethics chapters in Manual and Compendium). This means they are also an expression of the historically evolved cultural, religious and moral values of a society. As biosciences touch questions of life, and sometimes death, moral convictions and ethical deliberations play an important role. In this case it is particularly difficult to reach a social and political consensus and this process requires intense and sometimes lengthy. Ireland has adopted the European legislation.

Both developments – on the one hand the often very rapid advancement of the possibilities in biosciences and, on the other hand, the time-consuming national and international consent processes – result in regulatory gaps which may not be closed for several years.

For the GAMBA scientists from the different countries, EU law forms an important basis of the national legal systems: this applies not only to the EU member states but also to Switzerland, as the Swiss legal system has been harmonised with many EU standards. In addition to these basic EU requirements there may be stricter national regulations which are relevant to the German, Swiss and Irish patient and citizen panels.

6.2 The regulatory framework for GAMBA as a basic research project

The development chain of new therapy methods begins with **basic research**. This is the phase the GAMBA project is at. The main aim of the research is to ascertain whether the therapy approach would work in principle and whether the desired effect is feasible (“proof of principle”). This could be done in the lab in test tubes or Petri dishes with living human or animal

cells (“in vitro”) or with removed joints or bone parts, which are cultivated for a certain time (“ex vivo”). Before a new therapy will be tested on humans it has to be tested on a living organism, i.e. in animal models (“in vivo”). First risk and safety aspects as well as undesired side effects and the determination of the right dose are also investigated in animal models also called pre-clinical research.

Important regulations aspects that affect the GAMBA project are listed below.

6.2.1 Origin of the materials used, especially that of human biological materials

The GAMBA research project uses various biomaterials, including human biological materials (see Manual, Chapter 2.5). These are partly sourced from the research labs involved and from donors in the clinics associated to the research network. Some biomaterials such as gene sequences or stem cells are also available commercially⁸. Some of the materials are already in use for human therapy, e.g. the calcium phosphate matrix (see Manual Chapter 2.2.5.1), others are new developments. For the materials that haven’t yet been licensed a licence will have to be sought. However, this won’t be initiated until the basic research project has been completed and once it is clear if a further development of the therapy approach is likely to be successful. Nevertheless questions and requirements relevant to a later license play a role during the basic research phase, as the license is the ultimate goal. Three aspects are particularly important:

- a) In Ireland the licensing of both medicines and medical devices falls into the remit of the Irish Medicines Board. The situation is more complicated in Germany where the licensing process depends on whether we are dealing with **medicines (German Medicines Act – AMG)** or with **medical devices (Medical Devices Act - MPG)**. It will have to be evaluated in legal terms whether the GAMBA approach is aiming a future drug or whether individual components (e.g. the nanoparticles or matrices used) can be classified as medical devices only. Another possibility is that the combination of the therapy approaches needs to be licensed as an Advanced Therapy (see b).
- b) Advanced Therapies, as pursued in the GAMBA project, have been governed by the EU regulation 1394/2007 since December 30th 2008 “Advanced Therapy Medicinal Products, ATMP”. This means that they are now under special regulatory supervision all over Europe. ATMPs are defined as gene- and cell-therapy medicinal products and tissue-engineered products. The main change is that these products are no longer licensed nationally but by the EU commission. These licenses are then valid in the entire EU unless national regulations rule this out (see also Chapter 6.3.2). It is recommended that the researchers seek scientific advice from the authorization and licensing bodies, preferably in the early phases of the development of a new drug or in the run-up to clinical trials or the licensing application. In Ireland the relevant body is the Irish Medicines Board (IMB), on the European level it is the Committee for Advanced Therapies – (CAT) of the European Medicines Agency (EMA). It has been announced that the commission, after consultation with EMA, will also draw up guidelines on Good Manufacturing Practice and guidelines on Good Clinical Practice for drugs in advanced therapies (see also the information provided on the website of the Paul-Ehrlich-Institute – also available in English – of the Irish

⁸See Manual Chapter 2.3.1 and Figure 9 “Overview of the human biological material used in GAMBA” p. 28.

Medicines Board and of the Committee for Advanced Therapies, PEI 2006 and EU 2008).

- c) For the use of human biological materials – e.g. the removal of cells and their use for research – the so-called informed consent has to be obtained (see also Ethics, Manual Chapter 5.5). In the GAMBA project this applies to, e.g. the donors of stem cells.

Apart from point c) there are no further restrictions for research with adult stem cells in Ireland as well as in Germany. All adult stem cells can be obtained and used for any type of research, once the donors have given permission (see Faltus 2011, p. 188).

6.2.2 Requirements for the production and the handling of the materials and agents used

There is a lot to be considered concerning the handling of materials and the use of methods such as genetically modified organisms/gene therapy, nanoparticles or stem cells in the lab. The following apply:

- a) Requirements for a good professional laboratory and manufacturing practice. These ensure hygiene and diligence as well as the quality of production. “Good Laboratory Practice” is regulated by the S.I. No. 4 of 1991 European Communities (Good Laboratory Practice) Regulations implementing EU Directive 88/320. Furthermore, there are guidelines of the scientific associations or of the professional groups that need to be adhered to.
- b) Health and safety regulations for the protection of the employees handling the substances and protection requirements for the environment (e.g. for waste water and waste disposal). These regulations help to ensure that the genetically modified organisms are not released into the environment by accident⁹. For the in-vitro steps in the laboratory the Contained Use (CU) Directive 98/81/EC amending 90/219/ECC) apply in Ireland. The regulatory body in charge is the Environmental Protection agency. Laboratories that handle genetically modified organisms are considered as genetic engineering facilities which need to be classified according to a four-stage safety concept with increasingly demanding requirements. The laboratories working on GAMBA have a security level of no higher than 2 (out of 4). This means that the genetic engineering work, according to the state of science, poses only a slight risk for human health or for the environment. Security level 2 means that only named employees have access to the lab. Within the lab, tailor-made disinfections routines have to be followed.

6.2.3 Requirements for animal tests for research

At present the efficacy and the safety of a new agent are mainly established in animal trials on living animals: Medical animal experiments are performed in basic research as well as in pre-clinical research. According to the German Association of research-based pharmaceutical companies (VfA) approx. 86 percent of all animal experiments carried out in the pharmaceutical industry serve the testing of drugs and are legally required (VfA undated).

⁹Regulation of the security levels and safety measures for gene technology works in gene technology facilities.

The aim is to assess or rather predict the reaction of humans to the new agent. This involves finding the kind of animal best suited. Pharmacological and toxicological trials are meant to ascertain,

- What effect a substance has,
- How it is distributed in the organism and how it is broken down and excreted, and
- What is the highest dosage that does not cause harm.

These investigations are primarily done in vitro and later in animal experiments. However, it is controversial in how far animal experiments can result in reliable results that can be applied to humans (see also Ethics Chapter 7.5). According to the German Federal Animal Protection Report published in 2007 2,265,489 animals were used for scientific and other purposes in 2004 (BMELV 2007). There are no current Irish data available.

The GAMBA project also intends to conduct animal experiments, provided it is possible to find evidence (“proof of principle”) for the effectiveness of the research approach in the laboratory (see Chapter 3.5 in the Manual). Then there are plans for experiments on 165 mice, 92 rabbits and 20 goats. All GAMBA partners have committed themselves to comply with the national and international laws and with the guidelines of the Federation of European Laboratory Animal Science Association.

Additionally the GAMBA partners are adhering to the **3R-Principle: Replace, Refine, Reduce**), which was published by William Russel and Rex Burch in 1959 and is increasingly incorporated into standards and procedures (Russel & Burch 1959, quoted in DRZE 2010). In this context the GAMBA partners will make every effort to,

- **replace** animal experiments – animal experiments are replaced as far as possible by in-vitro experiments,
- **refine** the methods statistically, to minimise the suffering of animals – a limited number of animal experiments will only be conducted for specific and highly relevant questions. This is the reason why only one large animal experiment (goats) has been planned for the end of the research project.
- **reduce** the number of animals as much as possible – only the minimum number of animals needed for a statistically significant result will be used.

Authorisation of animal experiments in Ireland

During the last couple of years the animal protection laws were tightened in most countries of the European Union. To avoid distortion of competition within the EU due to different levels of stringency of the national animal protections standards, the European Directive 86/609/EU from 1986 was revised and a new EU directive was passed in September 2010. This directive has been transposed into Irish national law in November 2012 – this means while the GAMBA research project is still ongoing.

At present the the Cruelty to Animals Act 1876 is the basic law for the application, authorisation and implementation of all animal experiments in Ireland. Additionally, Statutory Instrument S.I. No. 17 of 1994 is the legal document which incorporates EC directive, 86/609/EC into Irish law. The welfare of the animals and proven expertise of the staff are prerequisites. As a rule only animals that have been specifically bred are used for experiments. All animal experiments have to be authorised. The application has to be made to the

Department of Health and Children and needs to be assessed and approved by an animal protection committee. The animal protection committee is mainly made up of researchers whose own experience qualifies them to evaluate the indispensability and the ethical tenability of animal experiments. However, members are also chosen from the general public. However, the vote of the individual animal protection committees is not binding for the approval authorities.

For each individual case there has to be a decision on the authorisation. During the approval process the authority focuses on two requirements:

- the animal experiment has to be essential for the achievement of the planned purpose of the experiment and
- the suffering of the animals has to be weighed against the purpose of the experiment and the expected results and they have to be ethical (see also Ethics Chapter 7.5).

6.2.4 Ethical admissibility of the research project

For certain steps within the GAMBA research concept, the GAMBA partners had to and will have to seek the permission of an ethics committee – for example for the extraction and use of human biomaterials. Usually this falls into the remit of local ethics committees (associated with the individual research institutes). For the sampling of stem cells the respective standards for obtaining an informed consent apply (see Manual, Ethics Chapter 5.5). Animal experiments are also ethically assessed, as described above (see Compendium Chapter 7.5).

6.3 Legal aspects in the case of a continuation of the GAMBA project

Under the condition that there are promising approaches on completion of the GAMBA research project, which should be pursued after the EU funding runs out, the following aspects of patients' safety become important:

- the safe and targeted effect of a drug (dose, unintentional effects),
- the reliability and quality of its manufacturing process and
- the balancing of the possible chances versus the possible risks of use in humans.

It usually takes 10 to 20 years until a drug is licensed¹⁰: First the efficacy (dose-response relationship) and the toxicity have to be further investigated in lab and animal experiments during the pre-clinical phase. The manufacturing process has to be developed and documented meticulously. Only upon completion of these first steps a can an application be made for clinical trial involving humans.

¹⁰ These aspects are also discussed in Chapter 3.5 of the manual „Possible follow-up research in the form of preclinical and clinical studies“.

Specific aspects for a legal evaluation of somatic gene therapy in the case of licensing

The authors of the survey “Gene therapy in Germany” published by the Berlin-Brandenburg Academy of Sciences in 2009, emphasize the following aspects with regard to clinical **trials of gene transfer drugs (BBAW 2008)**:

- Special attentions needs to be paid to the scope that the penal code (StGB §§ 223) grants outside the German Medicine Act (AMG §§ 40, 41): **individual curative trials** and advance therapies are generally admissible.
- The trial of gene transfer drugs in purely scientific experiments on **healthy volunteers** is seen as not justifiable at present (see § 40 AMG) due to the inherent risks. This means that generally the experiments should only be conducted on patients who suffer from the disease which could be cured by the drug to be tested.
- Prior to medical experiments with **patients suffering from the relevant disease** there needs to be **risk assessment** according to §§41, 40 AMG and there must be positive indications for a potential efficacy of the therapy (through animal experiments or experimental basic research). The specific danger must be weighed against the potential benefit for the participating patients, for this assessment alternative therapy approaches have to be considered also.
- Finally, according to §§41,40 AMG **group beneficiary experiments with relevantly sick test persons** may be permissible. These open the possibilities that people who are suffering from a severe disease, might make a contribution to the research of this disease and to the development of new therapy approaches, even if they don't gain any personal benefits. Here it is of utmost importance that the risks and disadvantages of a clinical trial must be balanced against the benefit for the patient as well as against the probable future benefit for medicine. They also have to be justifiable from a medical point of view. The use of a high-risk trial medicinal product on persons which would have no potential therapeutic benefit is therefore not justifiable.
- **Ireland** has no specific national law but follows ATMP.

6.3.1. Requirements on the manufacturing and production process

Requirements on the manufacturing process derive not only from the standards and guidelines of the European and national legislature, but also from the guidelines and recommendations of the relevant authorities, and possibly also their advisory bodies (e.g. the scientific Committee for Advanced Therapies – (CAT) of the European Medicines Agency (EMA)¹¹. The latter are sometimes in a position to react more flexibly to new developments and are thus setting new standards.

Apart from the listed requirements on a “Good Laboratory Practice” (GLP, see Chapter 6.2.2) there are also guidelines with requirements on a „Good Manufacturing Practice“ (GMP, EU directive 2003/94/EG) and a “Good Clinical Practice” (GCP, EU directive 2003/94/EG) (Fuchs 2010 et al). In Ireland the adherence to GCP is monitored by the Irish Medicines Board.

Furthermore, the integration of the competent supervisory authorities and drug developers in Europe, the USA and Japan within the framework of the “International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use” (ICH) adopts guidelines (<http://www.ich.org/cache/compo/276-254-1.html>). These are commonly incorporated into EU legislation. In addition the European Medicines Agency (EMA) and its scientific committees and other organisations set guidelines, for example for the production of certain gene vectors or on the risk assessment of gene therapeutic products (VfA 2009; German Parliament 2009).

6.3.2 Approval procedure for clinical research

Even though the use on patients is a long way away from the basic research project GAMBA, the section below lists background information on the authorization of clinical trials and the approval procedure for drugs.

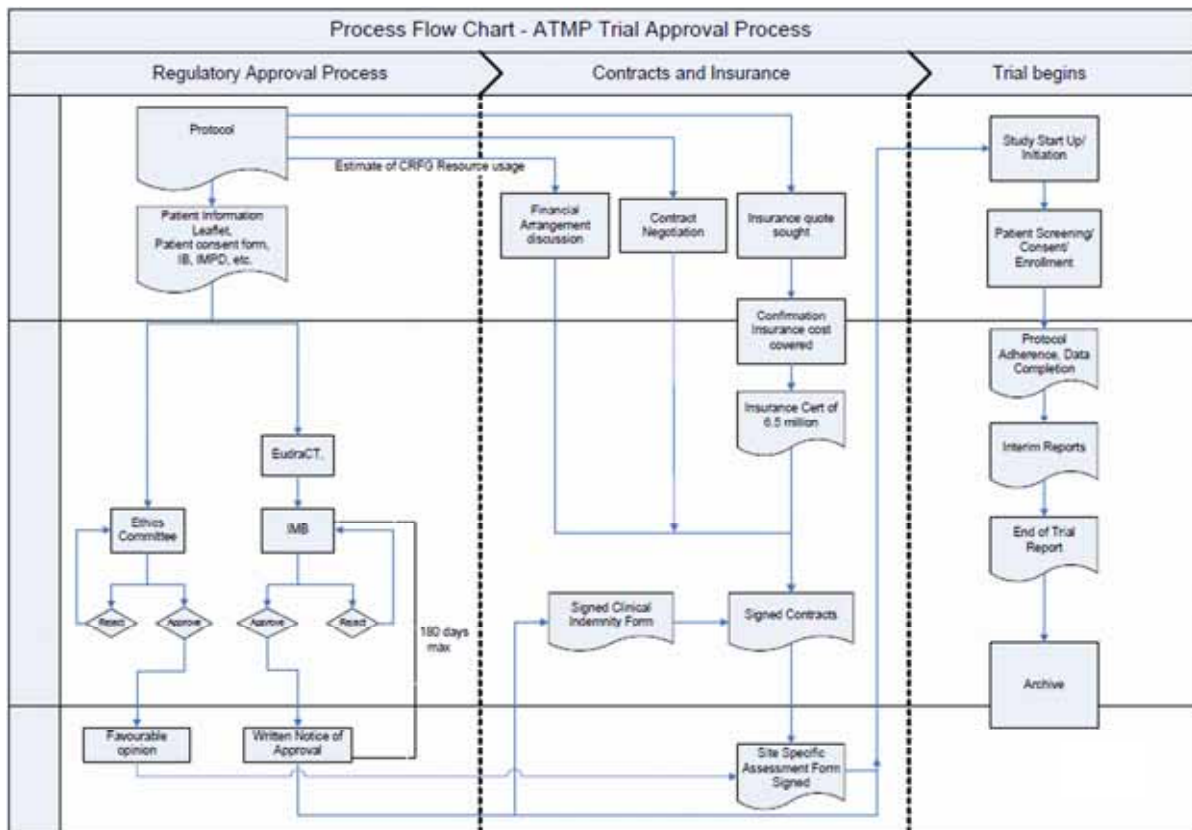
Authorization of clinical trials

Applications for the authorization of clinical trials in Ireland are either submitted by the manufacturers of the product to be tested or by academic researcher (see flow chart next page).

In Ireland the Irish Medicines Board is in charge of authorising clinical trials for novel therapies (e.g. stem cell and gene therapy) as covered by the **EC Regulation 1394/2007 “Advanced Therapy Medicinal Products”** (ATMP) (Krafft 2009). The Irish Medicines Board is also supervising implementation of “Good Clinical Practice” (GCP) during clinical trials of drugs for human use as.

¹¹ See also Guidelines of the German Medical Association on gene transfer into human cells 1989 / 1995 (German Medical Association 1995).

Fig. 13: Approval procedure for clinical trials (according to ATMP)



Source: Clinical Research Office

European approval procedures

Since the ATMP regulation came into force on December 30th, 2008 the licensing of drugs in the field of Advanced Therapies is subject to a European licensing procedure of the EU commission with the participation of the EMA¹².

In the course of the further development of provisions for this European regulation, the Commission, the EMA and the Committee for Advanced Therapies (CAT) will come up with further guidelines and recommendations.

During the licensing procedure it is the task of the Committee for Advanced Therapies, to evaluate the quality, safety and efficacy of the novel therapies and to monitor and advise the scientific development in this field. The main task is the preparation of a draft for a statement on each individual ATMP application, before the Committee for Medicinal Products for Human Use (CHMP) passes a final evaluation - report about the granting, the suspension or the revocation of a licence for the drug in question (CAT undated). The applicants are required to seek scientific advice from the European Medicine Agency (EMA) and from the associated Committee for Advanced Therapies – this may also be useful during the preparation of the application as such. This advice guarantees that the latest scientific developments and recommendations are taken into consideration.

¹² There are transition periods for methods that did not require require permission in Germany before 2008.

6.3.3 Special risks of novel therapeutic methods: Tightening of the conditions after the health of volunteers in clinical trials was damaged

Novel therapeutic methods carry particular risks (see Klug et al 2010), because the main mechanism of action is often still unknown, but nevertheless very potent. This applies particularly to participants in clinical trials (volunteers). After volunteers suffered severe damage to their health during the British trial of the agent TGN 1412 by the German company TeGenero, the requirements for the trial of high risk substances were considerably tightened by the European licensing authorities. The main factor is always the assessment of the benefit-risk-ratio: “The assessment of the risks and the resulting conditions for clinical trials versus the chances of patients to have access to novel therapies sooner and the economic interests of the manufacturer and the national economy, is a difficult task. For patients and users there is the dilemma between a fast improvement of the therapeutic options and the minimisation of risks. For the manufacturers early market access means higher profits, but they also want to avoid endangering the patients and having to recall the product.” (as above p. 7).

6.3.4. Grey area individual therapy trials

As long as there is no explicit ban, doctors may test therapy approaches in individual therapy trials. The German penal law § 223 opens this possibility outside the AMG, to enable medical progress. This is not without problems: “In contrast to clinical trials which aim to gain scientific insight about the efficacy of a drug or a method, the aim of the individual therapy trials is to help an individual patient whose clinical picture is not suitable for existing therapy approaches. Even though the individual therapy trial is an indispensable tool which has greatly contributed to the advancement of medical science, there is still the danger that this principle may be overused to circumvent the strict regulations of the application process which are meant to ensure the safety of volunteers and patients.” (Heyer, undated).

Example of a German regulatory gap: Stem cell therapy

Even before the death of an 18-month-old boy after treatment in the stem cell clinic “XCell-Center” in Düsseldorf (state of North Rhine-Westphalia - NRW, Germany) there had been serious criticism of the respectability of the stem cell therapies of the company, in particular from the Paul-Ehrlich-Institute and professors of the stem cell network NRW (Stammzellnetzwerk NRW undated).

The company utilised legal loopholes in the German regulation of stem cell therapies (see Heyer undated):

- After extensive inspection of the manufacturing process in the plant, the regional administration in Cologne had granted the required production license – but did not grant permission for the use of the product.
- However due to a legal loophole this was not necessary: The utilised therapies fall into the remit of the EC regulation 1394/2007 “Advance Therapy Medicinal Products” (ATMP). But all methods used in Germany before the regulation came into effect were granted an interim period until 2012 when they will need a license from the European Medicine

Agency (EMA). Only then will it be necessary for the companies to prove that the therapies offered are safe and efficient – and that the benefits outweigh the risks.

- Since the 15th amendment of the AMG in 2009 therapies with adult stem cells are subject to the German Medicine Act. Up to then it was not necessary to apply AMG to therapy approaches which retransfer tissues from the body (AMG §4a 3) . This special regulation was severely limited in 2009.

7. Other ethical aspects of gene and stem cell therapy¹³

7.1 Ethics committees

An ethics committee is an independent body made up of members of the health care system and members without medical background. Its task is to ensure that the rights, the safety and the wellbeing of persons participating in clinical trials are protected. This is also a measure to instil public confidence: the committee gives its views on the protocol, the suitability of the investigators and the qualification of the institutions involved. They also assess the methods that are used to inform the participants and to gain their consent (European Guidelines, quoted in Deutsch & Spiekhoff 2008, p. 773).

In Ireland every hospital and university has an individual ethics committee, but the National Bioethics Council was disbanded in December 2010. In 2008 there were 57 so-called Research Ethics Committees in Ireland (see Health Service Executive 2008). In Germany there are presently 52 ethics committees which are associated with the federal states and the university hospitals¹⁴, in addition there is the National Ethics Committee which generally gives its opinion on all current ethical topics which are of concern for politics. The commissions date back to the Helsinki Declaration¹⁵ which defines ethical principles and medical standards for human experimentation. This declaration was amended in 1975. From then on researchers needed the approval of their project by a specifically established, independent committee. This was the origin of ethics committees. However, this name may be slightly misleading as ethics committees usually don't deal with general ethical questions but with the testing of drugs, medical products or novel medical methods such as gene and stem cell therapy (see Druml 2003). **Ethics committees** are quite likely not to have a lot of experience or knowledge of gene and stem cell therapies as these are too new (King & Cohen-Haguenaer 2008).

The committees review the documents submitted with a focus on:

- Suitability of the investigator and qualification of the institution and people involved
- Scientific significance of the trial protocol and risk-benefit balance
- Recruitment of trial participants and the way of informing these about the trial
- Provision of insurance cover (Druml 2003, p. 1352).

For the realisation of clinical trials it is very important that “the assessment of the health risks for the participants should be conducted very carefully and should be risk averse as the participants carry all the risks, whereas the researchers can hope to reap the benefits (i.e. knowledge, publications, scientific reputation and so on)” (Enquete Commission Germany 2004, p. 22). The researchers therefore need to gain the participants' trust, as the participants as lay people can only trust in the fact that the researchers will have the expertise and that everything will be above board.

¹³ This chapter continues the collection of ethical aspects in the manual (there chapter 5).

¹⁴ Source : <http://www.ak-med-ethik-komm.de/index.html> see also Fuchs, M. (2005)

¹⁵ The Helsinki Declaration is a set of ethical principles regarding human experimentation developed for the medical community by the World Medical Association (see <http://www.wma.net/en/20activities/10ethics/10helsinki/>)

Ethics Committees have the task of protecting patients and participants from the danger of bodily harm or even death. They also work towards a fully informed consent and the protection of the privacy of trial participants. Another task of an ethics committee is to make sure that the urge to advance their knowledge does not lead researchers to violate the boundaries accepted by society (Deutsch & Spiekhoff 2008, p. 774).

In this context it is important to strike a balance between the benefits for the patients that participate in the trial and the benefits for society (or future patients). Who decides what is more important? But the key is that nobody may be forced to take part in a trial no matter how high the benefit for future patients may be (Manzeschke 2011). However, it is quite common for patients to participate in clinical trials for altruistic reasons, as they find consolation in the fact that they are helping to advance medical research, even if they themselves do not benefit (Cox & Avis 1996 quoted in Kimmelman & Levenstadt 2005, p. 506).

One problem is that there is no supervision of the activities of the committees (see also Wiesemann & Biller-Andorno 2005, p. 101). Further problems arise from the legally imposed time pressure on ethics committees (they have 90 days to reach a decision for novel therapies): this may lead to clinical trials being started too hastily, no access to pre-clinical results or an over evaluation of these results. There is also the danger that side effects are kept secret (see Fuchs 2011).

7.2 Unrealistic promises of cure / “Hype”

Two aspects are sensitive in this context: 1. Playing with the hope of patients and 2. Public funds that are invested in research, even if there will be no successful results likely for a long time.

On 1. Sick people are especially susceptible to unrealistic promises of cure because they can't wait for an end to their suffering and are grasping at every straw. Therefore they are particularly vulnerable and it would be morally wrong to “bait” these people with unrealistic promises. Especially in the field of stem cell therapy there are numerous promises of fast healing (associated with high costs). For example, the highly controversial and risky Parkinsons stem cell “therapy” offered by the former German company XCell¹⁶ cost approx. 26,000 Euro (Ruhstroth 2009). In 2010 a toddler died after stem cells had been injected into his brain (Berndt 2010; see also Chapter 4.2, Chronicle of Stem Cell Research). In Innsbruck, a supposed incontinence “therapy” was offered without the knowledge of the local ethics committee. The effectiveness of this therapy is very controversial (similar trials were aborted without results in Munich, Mainz and Vienna; see also Sturm 2008).

On 2. Public research funding runs the risk of becoming a “perpetuum mobile”: if a “breakthrough” is imminent it would be wrong to stop funds, especially as all money invested so far would be lost. However, it is usually very hard to judge if the breakthrough is really imminent or whether each corner that is being turned results in even more questions (such as after the decoding of the genome, see Chapter 2.2 Epigenetics).

¹⁶ The company does no longer exist due to scandals surrounding fatalities.

7.3 Conflicts of interest

Conflicts of interest during the introduction of new therapy forms, such as gene therapy, result from the fact that the people involved quite often have several roles which should be kept separate to ensure an unbiased assessment. For example doctors may also be researchers, conduct gene therapy studies or may hold shares of companies which produce products needed for gene therapy. They may be members of an ethics committee that assesses and approve trials or may use their influence as reviewers for scientific journals to influence what is published (and may suppress critical reports in this way). This happened in the case of Jesse Gelsinger (see Chapter. 4.6; Simon 2004, p. 19).

By now it is common practice to inform scientific journals about possible conflicts of interest, e.g. when a researcher is shareholder of a company that makes a profit in the area.

A further conflict of interest is the fact that negative research results are usually not published (King & Cohen-Haguenauer 2008). This means that researchers are denied the chance to learn from the experience of others and to avoid risky techniques from the start. However there is a new journal, the “Journal of UNSolved Questions” (www.junq.info) which aims to do just this. Up to now it is not clear if the research community will be using it.

7.4 “Enhancement”

The term “enhancement” means “Interventions to improve the constitution and functionality of humans, in excess of what is needed for good health” (Parens quoted in Kettner 2006, p. 9). “Mankind is dreaming an old dream: not to feel the burden of age, to maintain a young, attractive body and a lively mind ...” – this is how Peter Suter, president of the Swiss Academy for Medical Science, put it (quoted in Lenk 2006). Wouldn’t it be nice to use stem cells and healthy, functioning genes to make this dream a reality? Genes as a legitimate aid, just like glasses or coffee? Such prospects could give rise to a “quasi-religious euphoria” that wouldn’t have been deemed possible in our time (Kettner 2006).

First of all every individual has the autonomy to make decisions concerning their body; therefore they also have a certain right to use enhancements, as long as this doesn’t harm their fellow citizens (Lenk 2006) or causes costs for the community (e.g. through subsequent damage). However, the question arises whether the people interested in enhancement are really in a position to assess the consequences, as there are a lot of unknown factors (see also Manual Chapter 5.5 Informed Consent) or they may be falling prey to unrealistic promises (see Chapter 7.2). Also, there is the danger that an individual may lose the ability to differentiate between desires that are profound and justified and those that are shallow, self-destructive or otherwise questionable (Kettner 2006, p. 13).

It is helpful to consider the differentiation between therapy and technical assistance when passing a moral judgement on enhancement. The term therapy applies to medical treatments and procedures which serve to cure a disease or to maintain health. “Technical assistance” is the term for all aids that support the sick or those restricted in their everyday life. These aids include glasses, wheelchairs, stair and bath lifts, and technical systems that make life easier for the patients.

In contrast, the term enhancement is used to characterize biomedical interventions that surpass the healing of diseases or the maintenance of health: surgical interventions for the realization of cultural or individual beauty ideals, pharmacological manipulation to increase performance or higher conformity in school or job. This may one day lead to genetic intervention to create certain psychological and physical characteristics which do not improve an individual's health, but bring him closer to an ideal determined by culture or subculture (Lenk undated).

A potential use for **enhancement with gene therapies** is treatments for patients with muscle diseases. However, these treatments are also abused for the doping of athletes and for anti-aging treatments (King & Cohen-Haguenauer 2008). Other possible uses could be stronger joints for top athletes, a prominent chin for “manlier” men or surgical alterations of the cheek bones.

7.5 Animal ethics

After extensive in vitro experiments in the lab, GAMBA will also conduct animal experiments (see Chapter 6.2.3) to prove that the principle of protein production through gene transfer is viable.

Animal ethics is a branch of bioethics. It focuses on the questions of the legitimacy of using animals in the interest of humans, for example for animal experiments in research. “The answer to the question of whether animal experiments are ethically acceptable does not automatically follow from the fact that they are beneficial to many humans (e.g. patients) or even lifesaving. Instead, we have to ask ourselves if and how human interest justifies the suffering and death of animals. This depends largely on what moral status is given to animals in comparison to humans: “The debate about the moral status of animals is ... demonstrated with the help of three diverse positions : (1) animals have no genuine moral status and are therefore not worthy of protection for their own sake, (2) all living beings that have the potential to suffer and are able to have own interests (whether human or animal) have a comparable moral status and (3), the "middle ground": animals have a genuine moral status, but this is ranked lower than the moral status of humans“ (drze undated).

One major problem of animal experiments is the fact that animals often have completely different reactions than humans to therapeutics, such as drugs. Viral gene vectors could cause a different immune response in humans; the effect of gene vectors could also be different to that in animals (King & Cohen-Haguenauer 2008). “Mice tell lies” – is a catchphrase frequently used by scientists. As far as immunology is concerned, mice are “lousy models”“ for the development of new drugs (Davis quoted in Blawat 2010). There are several examples from Alzheimer research or from neurological diseases to prove this. On the cell level, mice differ unexpectedly and considerably from humans. Also quite often only male animals are used as their physiology is much more predictable than that of females. However, there are gender specific differences in many diseases. Therefore, there is a demand to introduce similar standards for animal experiments to those used for clinical trials on humans (as above).

Furthermore, viruses have a specialisation for certain cell types (tropism). If the virus finds this cell type during the animal experiments but they are not present during later clinical trials

on humans, the virus might attack the immune system instead. This is what caused the death of Jesse Gelsinger in 1999 (see Chronology of Gene Therapy, Chapter 4.6). Also tropism and the effectiveness of the penetration of the cell depend on “whether the cell is present in the body or in a culture dish” (Simon 2004, p. 10). This means that it is very difficult to agree on suitable animal models or to draw conclusions from animal models that are also valid for humans. However, the German Research Foundation (DFG) assumes that animal models make it possible to predict “desired and about 70% of undesired effects which affect humans” (DFG 2004 quoted in Fuchs et al 2010, p. 84).

7.6 Research politics

By allocating research grants, research politics have a considerable influence on the areas in which research is strengthened. For example, the EU is drawing up research framework programmes (GAMBA is funded by the EU). In Ireland, there are programmes of the Science Foundation Ireland and the Health Research Board. Targeted funding priorities are set. This means, however, that 90 percent of the money is spent on health research and only 10 percent on health problems (in Germany, no Irish figures available) (Grüber 2005, p. 52). Politicians concerned with development aid have been deploring for years that diseases which mainly occur in developing countries are rarely investigated.

Funds for medical research are mainly spent on areas that work with molecular biological and gene engineering. The only exceptions are AIDS, malaria and tuberculosis. “Researchers, who see the origin and the healing of diseases as a complex process,... receive very little funding” (as above).

Critics are calling to “measure progress on the extent in which it benefits all of society. ... The weakest groups in society should be the benchmark for the ethical quality of decisions” (as above, p. 55). Progress should not be judged solely by technical measures and possibilities.

In this context there is also a lack of transparency in research funding: why is what funded? What are the criteria of the politicians: is it the severity of the disease, the prospect of therapeutic success or economic interests (see also Schmidt 1995, p. 225)?

7.7 Patents on the building blocks of life

The German patent law allows a patenting of genes and gene sequences in principle (§1. 2). The human body, as such, and the mere discovery of its components cannot be patented. An isolated component (including a gene sequence) can be patented, even if the structure of this component is identical to the natural component, as this component – isolated and copied – does not exist in nature. This poses a problem as “the patent protection of the “nature-identical” substances effectively extends to the “natural” substance” (Fuchs et al 2010, p. 144, RDB 643). This becomes apparent in the example of so-called “jumping genes”¹⁷ (transposons) DNA sequences, that are isolated and then reintroduced, possibly with additional genes, at a different location. Such a jumping gene can be patented, even though the “inventor” has no way of knowing which functions this gene has or may have (as above, p. 145).

The initiative “No patent on Life” of the network for gene ethics, criticizes that “almost 20,000 patent applications for human genes have been filed with the European Patent Office (EPO), 2.670 of these applications have already been approved” (Kein Patent auf Leben undated, RDB 656), even though the genetic information has only been discovered, not invented.

7.8 Ethical phase model for biomedical intervention in humans¹⁷

This ethical phase model was developed by the Technology-Theology-Life Sciences Institute (TTN) in Munich in 1997 and refined in 2008. Case studies are used to demonstrate and assess the severity of the gene engineering intervention in humans. The aim of the TTN was to contribute to an objective discussion of the topic. Renowned German biochemists, biologists, physicians, theologians, legal experts and philosophers have contributed to the stage model.

Stage 1: Ethically and medically justifiable: fundamentally justifiable and controllable risk, routine, reversibility of the intervention; no cell proliferation in the body; no serious legal or ethical conflicts. Example: Cartilage transplantation.

Stage 2: Ethically and medically justifiable within limits: justifiable, usually controllable risk, partial irreversibility of the intervention, cell proliferation in the body; Application only in terminal diseases or when there are no alternatives. Example: **Somatic gene therapy.**

Stage 3: Presently not ethically or medically justifiable: unjustifiably high risk at present, irreversibility of the intervention; cell proliferation in the body, severe legal and ethical conflicts. Example: Disease prevention through germ line therapy.

Stage 4: Ethically and medically not justifiable: unjustifiably high risk, irreversibility of the intervention; cell proliferation in the body, severe legal and ethical conflicts; no relationship to the illness and no medical indication. Examples: Treatment of “deviations from the norm”, reproductive cloning.

¹⁷ According to Hacker et al. 2009: A stage model for the assessment of gene and cell therapy.

7.9 Pros (+) and Cons (-) of somatic gene therapy

The arguments listed in table 13 in the manual are treated here in more depth (Source: Schmidt 1995, extended and modified).

Please note: The following arguments were described by ethicists and adhere to a black-and-white scheme. This is a way of showing the extreme positions, which are needed to define the debate. In reality, there is hardly anybody who would unequivocally take one or the other extreme position. Instead, people have mixed or less extreme views. For the assessment of GAMBA it is important for the participants to explore which position they veer towards and why. Therefore, the pros and cons shall serve as a starting point for discussions within the panel groups.

7.9.1 Ethical principle arguments (“deontological arguments”)

a) Man’s mandate

+ **Obligation to act:** “Man has the moral obligation to use his knowledge and the striving for insight for the benefit of mankind. Therefore there is an obligation to develop and use gene therapy as a medical procedure” (Schmidt 1995, p. 176).

– **Violation of boundaries:** “With somatic gene transfer man violates his boundaries in an irresponsible way. He is <playing god>. ... With modern gene technology man (has) gained an authority over nature ..., which surpasses by far his ability to take responsibility (see also Jonas 1987, p. 104, quoted as above). ... Man touches things that are better left untouched” (as above p. 176f.). Gene therapy is defined by great complexity, great insecurity, great level of intrusion and further acceleration (see also Graumann 2000).

b) Image of Nature

+ **Naturalness:** “As gene transfer follows nature and does not violate taboos, this form of therapy is justified” (Schmidt 1995, p. 179). “The warning that somatic gene transfer can cause unwanted mutations in (other) cells is answered with the fact that there are permanently natural mutations in the reproductive cells and that approx. 10 percent of all fertilized egg cells contain gene mutations ...” (World Council of Churches 1982, quoted as above p. 179).

– **Artificiality:** Due to the method and the speed of change, gene transfer is an artificial process and violates the taboo zone of human nature (Schmidt 1995 p. 180). Mutations in the body are reactions that are imbedded in the system of living beings and can be seen as a response to disturbances. An intervention from the outside through gene transfer upsets the natural balance in and between cells and is therefore not comparable to natural mutations.

c) Human dignity

+ **Protection of self-determination:** “As long as a patient has given permission and the principles of “informed consent” were adhered to, the right to self-determination of the individual has been protected and a somatic gene therapy is justifiable” (as above., p. 182). This is about the dignity of the individual.

– **Threat to human dignity:** “As soon as human life becomes available in its genetic predispositions, the dignity of man is in danger. There is a threat, that the human species is

downgraded to a technically planned and manufactured product” (Rifkin 1986, quoted as above p. 185). This is about the dignity of man as a species.

Furthermore, a gene transfer is usually irreversible and the risks are almost impossible to judge. It is also important to ask whether „the assessment of the quality of life impacts negatively on the respect for life (Starlinger & Löw 1989, quoted as above p. 186).

7.9.2 Medico-ethical pragmatic arguments

a) Level of innovation

+ **Similarity to other therapies** (e.g. organ transplantation, medication, stem cell therapies): Just like other therapies, somatic gene therapy is a form of medical intervention and is therefore comparable to existing therapies (as above p. 188). Like all other therapies the somatic gene therapies carry the risk of potential side effects.

– **Novelty:** Gene transfer, for the first time, opens up the opportunity to make direct changes to the human genome within a cell (as above p. 193). Such an intervention does not take place on the level of organs and tissue, as such, but on the control level, the nucleus. Unlike in no self-contained functioning units are transferred, but gene sequences. The function and effect of these sequences in the entire system is determined by the integration location and the conditions that are prevailing there (as above p. 189). Also with somatic gene therapy there seems to be a much greater possibility of abuse and manipulation of human traits than in organ transplantation (as above p. 191).

b) Duty of Care (see also Chapter 5.2. in the manual: medico-ethical principles “benevolence” and “prevention of damage”)

+ **Medical duty to assist/heal:** A gene transfer is justified if it is a further possibility for the physician, to fulfil his primary duty as a doctor (as above p. 194).

Commentaries:

- Other therapies such as chemo or radiation therapy against cancer may well harbour even higher risks (as above p. 200).
- “No matter, how much effort is taken to reduce the risks of gene therapeutic treatments, from a medical point of view unwanted side effects are always to be expected.” (as above p. 203).
- Whoever delays the introduction of gene therapy is responsible for the prolongation of human suffering (as above p. 195). Even the decision not to introduce a new therapy is a decision for which responsibility must be taken and which cannot be reversed. The patients who need a treatment now may die soon because they could not avail of the therapy (Rehmann-Sutter 2003. p. 14).
- For diseases with insufficient treatment options, it is enough to be able to improve the disease even slightly through gene transfer (as above p. 23).

– **Risk of damage:** The somatic gene transfer is to be rejected if a possible damage that the patient may suffer through the intervention is not sufficiently counterbalanced by the expected benefits (Schmidt 1995, p. 195)¹⁸.

Comments:

- “The principle to avert damage to humans has ethical priority to the obligation to do the possible good” (Eibach 1980, quoted as above p. 201).
- The danger of a gene therapy treatment must be less significant than the danger of not treating the patient (as above p. 201).
- So far there have been more than 1600 clinical trials of gene therapy, but these have only resulted in three licensed medical products. This means that gene therapy is not effective in many cases or the risks are too high¹⁹.
- Also the obligation to help should not be accompanied by compassion, as the argument of compassion “could be used to bypass the supervision through the approving agencies” (as above p. 196). This is already possible in the case of “individual curative trials”, (they have to be approved by the local ethics committee only; see also Chapter 6.3.4).
- There is also the danger of an abuse of the term “incurable” to justify dangerous experiments (Fletcher quoted as above p. 197). In the case of the first gene therapy trials on ADA deficiency (see also Chronicle of Gene Therapy, Chapter 4.6) it was suspected, “that it wasn’t so much the difficult lot of the patients, but rather scientific and technical interest” (Ritzert quoted as above p. 198) that was decisive, even though there were alternative treatments available.
- Only extensive long-term trials can give comprehensive indications of the medical risks. However this also applies to other therapies, but as gene therapy is associated with a particularly high degree of insecurity, it is especially important in this case. Unfortunately, long-term trials are time-consuming and expensive and it is very difficult to trace diseases that occur later specifically to an earlier gene transfer (as above p. 203).

c) Effectiveness (Result)

+ **Causal Therapy:** “Gene transfer, especially for hereditary disorders, promises a high effectiveness, because, for the first time, we have the possibility to fight the very cause of the disease. For this reason it should be possible to utilise somatic gene therapy. Scientific medicine sees the human organism as a ... ‘machine’, and therefore disease seems to be a malfunction which has a causal explanation ...” (as above p. 205). Although this view has been repeatedly criticized (see also Chapter 5.3 in the Manual), that criticism is being weakened by first successes (see Chronicle of Gene Therapy, Chapter 4.6).

– **Alternative Therapies:** As gene therapy is partly responsible for the fact that the research into other methods of healing and disease control is neglected, its use must be restricted. Other forms of therapy are not being developed with equal attention due to the “biomedical fixation” and the fact that less funds are available for the alternative research (as above p. 206).

¹⁸ See also Manual, Chapter 4 on Risks .

¹⁹ Most of the clinical trials were discontinued after phase I; this phase focuses on efficacy and toxicity. However funding problems are often to blame, as clinical trials are extremely expensive.

7.9.3 Socio-political Arguments

a) Public Opinion on Benefits / Risks

+ **Benefits:** If a gene transfer is responsible and justified, the public should be informed about these benefits at an early stage to combat possible unjustified fears. The aim is to initiate a social discussion, which establishes standards and guidelines (as above p. 216).

– **Risks:** There is the danger that a public discussion is avoided to conceal possible risks of a gene transfer. Another danger is that these risks are played down if a discussion takes place after all. It is a possibility that the information supplied by scientists turns out to be nothing but propaganda which plays down the risks that are associated with a gene transfer (as above p. 217).

b) Regulation (see Chapter 6)

+ **Limitations:** If the application of a somatic gene therapy can be regulated it should be allowed (as above p. 218). The mere possibility of a misuse is not sufficient to forbid the application (as above p. 223).

– **“Opening of the flood gates”:** Somatic gene transfer has to be rejected, as this therapy which was developed for the therapy of body cells, could pave the way for other irresponsible interventions, such as genetic enhancement (see also Chapter 7.4 on “Enhancement”) and eugenic practices (correction of “undesirable” characteristics, such as the race improvement programmes of the Nazis). Also the somatic gene transfer could play the role of a <Trojan Horse> (Klees 1989, quoted as above p. 221) for a future licensing of germ line intervention, a manipulation which would also affect the descendants of the treated (Dt. ev. Allianz 1992, quoted as above p. 219). Even the use of hormones can’t be reliably regulated and monitored as is demonstrated by doping cases in sport (as above p. 218).

c) Distributive justice (see also Manual Chapter 5.2 “Medico-ethical principles”)

+ **Investment into the future:** “Gene therapy can contribute to a juster distribution of medical resources and should therefore be developed further“ (as above p. 224). If therapies can be targeted to be effective on the molecular DNA and cell level it is no longer necessary to treat an entire group of patients with the same drug that may only be effective for part of the patient group (for the other part the therapy is at best ineffective but could also have serious side effects).

– **Unjust distribution:** “Investments in somatic gene therapy may lead to injustice in the distribution of medical resources and should therefore not occur” (as above p. 224). High-tech experiments such as gene therapy bind financial resources that could be better used by spending them on the public health system. In the US, the country that is leading in gene therapy, a big part of the population does not even have access to basic medical care. At the same time people in the so-called “developing countries” die from diseases that have long since been curable in industrialized nations. Furthermore people in “developing countries” are often used as “guinea pigs” for clinical trials, because the level of regulation is lower and also the possible claims for damages are low (as above p. 225f.).

d) Social Impact

+ **Harmonization:** Gene therapy can contribute to the harmonization of social life and should therefore be supported. In future it will be possible to administer gene therapy treatment to

children with life threatening diseases before birth or within the first weeks of life (as above p. 228).

– **Stigmatisation/Discrimination:** The possibilities of gene therapies intensify an already existing tendency to discriminate against sick and disabled persons and to stigmatise them. Therefore gene therapy should be rejected. The introduction of gene therapy would lead to a society that would further marginalise and exclude illness, disability and differentness (as above p. 229). Intolerance in society would increase.

e) Commercialization

+ **Advantages:** As healing patients or even the possibility of healing makes money, the development of gene therapy will be promoted and there will be attempts to offer a cure to patients (as above p. 230).

– **Dangers:** The commercial interests of the parties involved are endangering the adequate development of gene therapy. If a new method has the potential to earn money it will be marketed, irrespective of its benefits. It is helpful in any case, if the researchers disclose their commercial (conflict of) interest (as above p. 231).

f) Target and Medium Quality

+ **High Target and Medium Quality:** The quality of the target is of utmost importance as healing is always an ethical imperative, as long as there is hope of a cure²⁰. The resources used (gene therapeutics) are adequate, as they can fight the root causes of the disease.

– **Low Target and low quality of means:** The target as such is already questionable, as diseases are caused by more than genetics. Also the funds used are excessive (basic research and clinical trials are very expensive). In contrast there is not enough money for basic medical care, even in highly developed countries like the USA. Even in Ireland there is a two-tier medicine – not everybody gets the best possible treatment.

g) Various, different interests

Many diverse groups have a vested interest in gene therapies: Patients and their associations, researchers, medical doctors, biotech companies, the media and others.

+ **positive:** All groups which have an interest in gene therapy, contribute to the diversity and ensure a quick and efficient advancement of new therapy options.

– **negative:** These stakeholders have different levels of influence; individual patients are of relatively little importance. And the public interest is not represented by lobby groups. Who should decide which new therapies are funded by the state? Politicians are often influenced by the powerful interests of industry and don't allocate the funds fairly.

²⁰ There are however moments, when the order of the day is no longer healing, but end-of-life care. If the prognosis is “terminal“ healing is not important any more, but the focus shifts to pain relief and care (Manzeschke 2011).

GAMBA Glossary

NB: Here you will find explanations for terms that are used in the manual and compendium and which are only explained on the first occurrence. Arrows (→) refer to further terms mentioned here.

ACT (Autologous Chondrocyte Transplantation) – Osteoarthritis therapy, the body's own cartilage cells are taken from cartilage areas that haven't been affected yet. They are then grown in the lab until there are enough to implant in the damaged cartilage area.

ADA-SCID (Severe Combined Immunodeficiency) – due to a gene defect the enzyme Adenosine Deaminase (ADA) is missing. This means that the body cannot digest a protein which is toxic to the white blood cells (T-Lymphocytes); therefore these blood cells which play an important role in the immune defence do not mature in the bone marrow in sufficient numbers or not at all. Affected children have almost no protection from pathogens and even when treated and kept in a sterile environment, only rarely reach adulthood (→ X-SCID).

Adeno-associated Virus (AAV) – An adeno-associated virus needs the help of an adenovirus to grow its DNA in infected cells. These viruses are generally not pathogenic.

Adenoviral gene vectors – Adenoviruses (→) do not incorporate their gene load into the genome of the cell, but leave it as a so-called episomal unit (→ Plasmid-DNA, pDNA) in the cell nucleus. Therefore the adenoviral vectors are only transferred to daughter cells during cell division as long as there are DNA copies present.

Adenoviruses – are causing cold symptoms in humans. They can take a relatively high gene load, when they are being used as → gene vectors. If used in high doses the viruses can trigger strong immune responses as the body has learned to fight cold viruses.

Adult stem cells – Tissue specific cells, in the fully developed (adult) organism which have the ability to self-renew and to differentiate into different mature body cells types (→ somatic cells).

Advanced Therapies Medicinal Products Regulation (ATMP) – European Regulation, which deals with innovative therapies such as gene and stem cell therapies. In Ireland the ATMP is fully implemented, the regulating body is the Irish Medicine Board → IMB

AMG (German Medicines Act) – regulates the licensing and registration of pharmaceutical products in Germany.

Amino acids – building blocks of → Proteins. The human proteome, the entire set of all proteins, contains 20 different amino acids.

Animal Experiment – Experiment with animals in research. Before a new drug can be used on humans, it has to be tested in animal experiments. Before animal experiments are conducted extensive laboratory experiments must have delivered fundamental evidence (→ Proof of principle) that a new therapeutic substance has a promising healing potential. Animal experiments must be approved of by the relevant authorities.

Antibiotics – (Greek anti: against; bios: life): substances with growth inhibiting or eliminating effect. Generally used to inhibit and kill off bacteria.

Antibodies – When the immune system comes into contact with foreign material it produces specific defence proteins, the antibodies, which circulate in the blood stream and neutralize the foreign material.

ATMP – Advanced Therapies Medicinal Products Regulation (→). Ireland has fully implemented the ATMP.

Autologous – Deriving from the own body belonging to the same individual.

Bases – Opposite of acids.

Basic research – Research that is not primarily aimed at a practical use of the results; instead it is delivering general findings, which may in turn be useful for later applications.

BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte = German Federal Institute for Drugs and Medical Devices) – is an independent higher federal authority within the portfolio of the German Federal Ministry of Health. It is in charge of the licensing and registration of medical products and medical devices on the basis of the German Medicines Act → AMG. It also conducts its own research to review the efficacy, safety, and adequate pharmaceutical quality of the finished medicinal products (→ PEI)

BMPs (Bone Morphogenetic Proteins) – are a subgroup of the → TGF- β family. BMPs play an important role in early embryonic development and the formation of organs. They are important signal molecules. If certain BMP effects are disrupted or increased this can lead to short fingers or a fusion of the finger bones.

Cartilage – Only 5 percent of the mass of the cartilage is made up of cartilage cells (chondroblasts → Chondrocytes and Chondroblasts). The rest of the matrix consists of proteins, especially collagens, which were produced by the cartilage.

cDNA (complementary/copy DNA) – A cDNA strand is a copy of a copy. First the information of the DNA is transcribed into an → mRNA. Then the information of the mRNA is transcribed again into DNA with the help of a viral enzyme (reverse transcriptase). This DNA is then called cDNA.

Cell differentiation – In developmental biology **Differentiation** is the development of cells or tissues from a less specialised (stem cells) into a more specialised cell type (skin, nerves, bone ect.)

Chondrocytes (cartilage cells) – cells derived from → chondroblasts and found in the cartilage tissue. They are embedded in a thick, voluminous extracellular matrix within the → cartilage and are not connected to the blood stream. Unlike cells in the bones and other tissues they also have no direct contact with their neighbours. Also they are not regularly visited by immune cells, which patrol the body to find harmful foreign substances and aged cell structures.

Chromosome – The human genotype (DNA) is about two meters long. In order to accommodate it in the nucleus, the DNA is wrapped around proteins (histones). The resulting structures are called chromosomes.

Chronic granulomatous disease– Due to a genetic disorder the phagocytes in the blood of granulomatosis patients can destroy pathogens, but they are lacking the toxin superoxide, to kill them. This means that the patients are very prone to infections.

Clinical research – (→ Clinical Trials).

Clinical Trials – Studies to establish the efficacy and toxicity of drugs in humans. These trials are subject to stringent regulations. In Phase I the toxicity and compatibility of the new substance is tested on a small group of healthy test persons. Due to the unpredictable risks of gene and cell therapy trials, the researchers focus on patients which did not respond to conventional therapies instead of healthy volunteers. The approval of the trial is subject to strict criteria. Based on the results of Phase I, the trials in Phase II include a greater number of participants and have the aim to establish the optimal dosage. In Phase III the actual effect is determined in a trial with a sufficient number of patients with certain inclusion and exclusion criteria to ensure a statistically significant evaluation. This includes, if necessary, the comparison with a → Placebo without active substances. The licensing of a new drug is only possible after the successful conclusion of a Phase III trial. Then the effects of a new therapy in its licensed use can be further investigated or observed. These further trials are called Phase IV trials.

Code – The genetic code within the → DNA is written for the production of proteins and consists of a sequence of three → Bases. Such a triplet (three bases) represents one → amino acid. Specific sequences of amino acids form the → Proteins.

Collagen – a structural protein, that is mainly found in the connective tissue. The protein is an essential organic component of the connective tissue as well as of bones (Collagen Type I) and cartilage (Collagen Type II).

Cox-2 inhibitors – non-steroidal anti-inflammatory drugs. These inhibit the enzyme. Cyclooxygenase-2 (Cox-2), which plays an important role in inflammation and pain.

Cytokines – Proteins which regulate the growth and the differentiation of cells (→ cell differentiation). Some cytokines also play an important role in immune reactions.

Cytoplasm – the basic structure filling the cell around the nucleus. Many metabolic processes of the cell take place within the cytoplasm. These metabolic processes are regulated by enzymes.

Differentiation – Specialisation of embryonic or adult stem cells from the undifferentiated state into a specialised body cell.

DNA (Deoxyribonucleic acid) –The DNA is the carrier of genetic information. It is a double helix which consists of sugar molecules, phosphate groups and four different → Nucleobases: Adenine, Guanine, Cytosine und Thymine. The DNA stores all genetic information. The sequences of these four bases encode the genetic information.

DNA Sequence – coding sequence of → DNA segments. These do not have to be located close to each other, but they ultimately lead to the production of certain proteins (→ Gene).

Doxycyclin – An antibiotic of the Tetracycline class. Apart from the antibiotic effect, tetracyclines also have an effect on degenerative joint diseases. They are used in human medicine to alleviate the symptoms in osteoarthritis or rheumatoid arthritis and to slow the progression of the joint deterioration. Tetracyclines inhibit, amongst other things, a range of → Enzymes, such as collagenases, metalloproteinases (MMPs) and gelatinases, which cause joint damage. For GAMBA Doxycyclin is used as a control element for gene vectors.

EMA (European Medicines Agency) – Drug licensing agency of the EU.

Enzyme – Proteins (→), which act as biological catalyst to accelerate certain chemical reactions.

Epigenetics – Special field of biology, which looks into cell characteristics which are passed on to daughter cells but which are not defined in the → DNA sequence.

Epigenome – all epigenetic marks of a genome (→ epigenetics).

Ethics – philosophical discipline which discusses criteria for good and bad actions.

EudraCT – a database of all clinical trials carried out within the EU, the database is maintained by → EMA. This database will be available to everybody in the near future.

Exon – information-bearing, coding segment of the → DNA. Only exons are translated into → Proteins (→ Introns are not).

Ex vivo (“out of the living“) – living biological material, in particular cells, tissue or organisms, which have been taken from a living organism are cultured → in vitro in the lab over a certain period of time. This makes it possible to carry out treatments, changes and investigations under controlled conditions.

GAMBA (Gene Activated Matrices for Bone and Cartilage Regeneration) – EU project researching new therapeutic avenues for the treatment of osteoarthritis which are meant to induce a self-healing process from within.

GCP (Good Clinical Practice) – International quality standard for the rules for the preparation and conduction of clinical trials taking into consideration ethical and practical aspects on the basis of up-to-date scientific findings. Details can be found under www.emea.eu.int/pdfs/human/ich/013595en.pdf

Gelsinger, Jesse – first patient to die during a → clinical gene therapy study. His death resulted in an intense debate on the justification of gene therapy trials; especially as the 18-year-old was not fatally ill and had not been comprehensively informed about the risks involved (→ informed consent).

Gene – Segment(s) of a DNA molecule, responsible for the expression of a hereditary trait. A gene contains the building plan for a protein or a functioning RNA. It consists of the entire functioning unit made up of coding (→ Exon), non-coding (→ Intron) and regulatory segments. Each body cell contains the same genes. Depending on the purpose of the cells different sets of these genes are transcribed.

Genetic Code – The genetic code is a translation key, which contains the genetic information for the production of proteins. Three consecutive nucleotides (from the bases (→) A, G, C, U) contain the code for an amino acid. Such a trio (the scientific term is triplet) is also called a codon. All in all there are $4^3 = 64$ different codons. Each codon is associated with one of the 20 amino acids that occur in natural proteins. Additionally there are 3 codons which function as a stop signal.

Genome – The entirety of the hereditary information of a cell or an organism.

Gene expression – Reading of a gene (→ Transcription) and production of the corresponding protein molecule (→ Translation).

Gene technology – The entirety of all methods that concern themselves with the isolation, characterization, reproduction and new combinations of genes, even between different species.

Gene therapy – Attempt to cure diseases by introducing genes into the bodies. There is a differentiation between → somatic gene therapy, which only manipulates body cells and → germ line therapy, which makes changes to the germ cells that can then be passed on to the descendants of the patient. Germ line therapy is banned in Germany.

Gene transfer – Introduction of foreign genes into cells with the help of either → Transduction or → Transfection.

Gene vector – (→ Vector)

Germ cells – reproductive cells of an organism, egg and sperm cells. They can pass on the genetic information to the next generation and form the so-called germ line (→ germ line therapy).

Germ line therapy – Gene transfer into → germ cells (egg or sperm cells or their precursors). The changes in the genetic information would be passed on to future generations. In Ireland, as in most countries worldwide, germ line transfer is ethically unacceptable and therefore not used.

GLP (Good Laboratory Practice) – International rules and standards for quality assurance of the organisational processes and conditions of non-clinical health and environmental tests. Further details can be found under http://ec.europa.eu/enterprise/chemicals/legislation/glp/index_en.htm.

GMP (Good Manufacturing Practice) – International protocols and guidelines for quality assurance which are meant to guarantee the safe handling, implementation and production of medical products and substances. Further details can be found under www.emea.eu.int/Inspections/GMPHome.html.

Growth factors – proteins produced by the body, which have various effects on the → Cell differentiation and the function of cells. They also enable the communication between cells.

HSP (Heat Shock Proteins) – support the correct folding and maturation of proteins within the cells and they also help the proteins to maintain their spatial structure in stress situation, e.g. after a heat shock.

Human biological material – blood or cells used for research, this material was donated after → informed consent.

Hyaluronic acid gel – a biomaterial used for GAMBA to bind → Stem cells and → vectors; the main component is → Hyaluronic acid.

Hyaluronic acid – Main component of the synovial fluid (→ Synovia) acts as a lubricant in all joint movements.

IMB (Irish Medicines Board) – The mission of the Irish Medicines Board (IMB) is 'to protect and enhance public and animal health through the regulation of medicines, medical devices and healthcare products'. The objective of the IMB is to ensure in so far as possible, consistent with current medical and scientific knowledge, the quality, safety and efficacy of medicines available in Ireland and to participate in systems designed to do that throughout the

European Union. Before a medicine can be authorised for use, an application must be made to the IMB and this must contain all of the necessary data supporting its quality, safety and efficacy. The IMB is also the Competent Authority for the regulation of Medical Devices and Cosmetic products in Ireland. In addition, it is responsible for the implementation of EU and national legislation relating to Blood and Blood Components and also for Tissues & Cells.

Immune system – System to ward off foreign substances and to clear the body of sick and non-functioning cells at regular intervals.

Informed consent – Consent after detailed information to either donate human biological material (e.g. cells) or to take part in a clinical trial → test person.

Insertional mutagenesis – Integration of the therapeutic gene at an unfavourable location in the → Genome, which may lead to a degeneration of the cells and consequently to cancer.

Integrase – viral or bacterial enzyme, which ensures that the transfer gene which is introduced into the cell by the viral gene vector is integrated into the genome of the treated cell.

Interleukins (IL-x) – Signal substances, whose main task is to facilitate the communication between white blood cells (leukocytes). They also enable the communication between other cells which contribute to an immune reaction. They are the natural messenger substances of the cells of the immune system and belong to the group of the → Cytokines. The noun interleukin is derived from Latin: *inter* = *between* and Greek *leukos* = *white*. They are divided into several subgroups which are labelled with numbers in the order of their discovery.

IL-10 (Interleukin-10) – is an → Interleukin; it has numerous functions concerning the regulation of the immune system. It protects the organisms from destroying itself with excessive inflammation processes and has an inhibiting and limiting effect on immune reactions that could lead to a septic shock. Along with → TGF- β it is one of the most important anti-inflammatory cytokines and plays an important role in the development of the immune tolerance. IL-10 is produced by connective tissue cells and → Macrophages and plays an immunosuppressive role by limiting the production of pro-inflammatory cytokines.

Intracellular – within a cell.

Intron – non-coding segment of the → DNA. Introns are not translated into → Proteins (→ Exon).

In vitro – outside of a living organism in the lab, e.g. in a test tube, Petri dish, incubator ect.

In vivo – in the living organism.

iPS (induced pluripotent stem cells) – derived from specialised tissue cells such as skin cells → Stem cells retransformed from cells.

Legality – lawfulness (applicable law).

Lipid – Fat or fat-like substance.

Liposomes – spherical vesicles, which are surrounded by a double layer of fat molecules die (Greek Lipos = fat). Such measure-made liposomes are used as → non-viral gene vectors in gene therapy.

Matrix – In tissue engineering the term matrix (plural: matrices) is used for the carrier material of structure to which the desired structures and tissues are attached and which are then inserted into the body.

MBCP (Micro Macroporous Biphasic Calcium Phosphate) – absorbable carrier material → Matrix for tissue, which has micro- as well as macropores. According to clinical trials this is bone substitute is 100% absorbable.

Membrane – here cell membrane, consists of phospholipids, a special form of → Lipids and → Proteins. It separates the cell from its environment and controls the exchange of substances. There are also membranes within the cell, for example the outer wall of the nucleus.

Mesenchymal Stem Cells (MSC) – precursor cells of the connective tissue (soft tissues). They are present in the bone marrow and can differentiate into cell types such as bone cells (osteoblasts), cartilage cells (→ chondrocytes), fat cells (adipocytes). They ensure a steady supply of new cells for the maintenance and regeneration of the supporting and connective tissues, such as bones, cartilage, muscle, ligaments, tendons and fatty tissue. This happens when the cell-cell-contacts signal a need for new cells and certain → growth factors and → cytokines stimulate them.

Methylation – Methyl groups (-CH₃) attach to a specific component (the base cytosine) of the DNA strand or to proteins.

microRNAs – tiny → RNAs, which control many processes within the cells.

Monogenetic hereditary diseases – diseases which are caused by the mutation of a single gene.

mRNA (messenger RNA) – Base sequence which is produced through → Transcription of the DNA and the subsequent → Splicing. mRNA is able to leave the nucleus and can be translated into proteins by the ribosomes.

MSC – Mesenchymal Stem cells (→)

Multipotency – The ability of an adult stem cell to differentiate into a limited number of cell types or into closely related family of cells.

Musculoskeletal disorders – Osteoarthritis, osteoporosis, rheumatoid arthritis

Mutation – hereditary natural variation of a DNA sequence. Such variations are the result of replication errors or of external influences such as radiation or chemicals.

Mutagenesis – a mutation triggered artificially (e.g. through UV radiation, chemicals).

Nanoparticles – tiny particles which as size of less than 100 nanometer (nm; 1 nm = 1 billionth of a meter).

Non-viral vectors (e.g. → liposomes) can be used in gene therapy to transport foreign genes usually in the form of → Plasmid-DNA, without having the risks of viral vectors. However, they are not as efficient as viral vectors in introducing their gene freight into the target cells.

Nucleobases (→ Bases) – alkaline building blocks of the genome, whose sequence represents the → genetic code for the production of → proteins. Two nucleobases each are connected in a DNA strand. These connections bind the two strands of the DNA double helix. The base

Guanine (G) forms a pair with base Cytosine (C), and the base Adenine (A) pairs with the base Thymine (T) as well as with the base Uracil (U).

Nucleotides – tiny molecules, which form chains in nucleic acids such as DNA and RNA.

Oncogene – a gene, that normally plays a role in the cell cycle control. If this gene is activated through mutation it can aid the development of cancer or even cause it.

Osteoarthritis – a chronic degenerative disease affecting joints which bear a lot of strain. It is characterized by a progressive degradation of cartilage cells and of the cells of the surrounding tissues. Finally this leads to changes in the neighbouring bones. The joint surface is destroyed. After several years this leads to a complete and usually painful destruction of the joint and stiffness.

Paul-Ehrlich-Institute (PEI) – The PEI is also called the "Federal Institute for Drugs and Medical Products" and is in charge of approving applications for → clinical trials and lately also for the licensing of vaccines, tissues and medical products for gene therapy and somatic cell therapy (→ ATMP) in Germany. Further tasks are scientific advice on the development of drugs, experimental batch testing and the batch release as well as the evaluation of adverse drug effects. To this end the PEI conducts independent research. In Ireland the Irish Medicines Board is in charge of the above tasks (→ IMB).

PEG (Polyethylene glycole) – is used as a component of nanoparticles in GAMBA. Depending on the chain length PEG can be liquid or solid and is a chemically inert, water soluble and non-toxic polymer. Amongst other uses it is used as a carrier for pharmaceutical agents.

PEI – Paul-Ehrlich-Institute (→).

Petri dish – shallow, round, see-through dish with an overlapping lid and is used in biology and chemistry labs.

Phagocytes – are „digestive cells“ which have the ability to engulf and digest living and dead foreign particles (microorganisms, blood cells tissue fragments ect). This process is called phagocytosis.

Pharmacology – Science of the kinds, development, mode of action and area of application of remedies/drugs.

Placebo – simulated treatment.

Plasmid – Small extra-chromosomal, circular DNA molecule which can multiply independently of the chromosomes in bacteria. Certain DNA fragments or genes can be incorporated. Frequently used as a → Gene vector.

Plasmid DNA (pDNA) – DNA, which is not incorporated in a genome, but is present in the cells in the form of independent circular structures. During cell division this is normally not doubled and therefore gets lost after several cell divisions, unless the plasmid DNA is permanently incorporated into the genome.

Pluripotency – the ability of embryonic stem cells to differentiate into any cell type.

Polyethylenimine – synthetic material highly charged with positive ions, which is used as a component for → non-viral vectors.

Preclinical research – Research in the lab and with animal models, conducted before a new therapy or drug is used on humans. Preclinical research ensures efficacy and safety (→ clinical trials).

Promoter – DNA sequence before a gene, which initiates the → Transcription of the gene.

Proof of principle – Proof of the feasibility and efficiency of a method or an idea.

Proteins – In living organisms proteins enable all reactions that are necessary to sustain life. They consist of a chain of amino acids. The sequence of these amino acids is determined in the genome (→ Genes). The sequence of the amino acids determines the structure and the function of the proteins. One important group of proteins is called → Enzymes.

Recombination – Distribution and rearrangement of genetic material (→ DNA, RNA) of different origins.

Retroviruses – Viruses which contain → RNA (e.g. HIV). After a cell has been infected a viral enzyme (Reverse Transcriptase) transcribes the RNA into → DNA with and is then integrated into the genome of the infected cell.

Retroviral Vectors – Gene vectors which are derived from retroviruses. Retroviral vectors integrate their genome into the genome of the host cell. Only the therapeutic gene is integrated into the cell DNA. It can produce neither viral RNA nor viral proteins, only the desired protein. The integration occurs at a random location in the genome. Retroviruses are presently the focus of gene therapeutic research and are, due to their properties, the second most common vectors after → adenoviral vectors. At the moment recombinant viruses are the only way to heal monogenetic retroviruses permanently. Retroviruses are RNA viruses. The problem is that they can cause malign tumours if they proliferate in the body.

Ribosomes – small complexes in the cytoplasm where amino acids are strung together according to the code of the messenger RNA (mRNA) .

RNA (Ribonucleic acid) – a single-stranded molecule consisting of the four bases Adenine, Guanine, Cytosine and Uracil, the sugar Ribose and phosphate residues. The main function of the RNA is to transport the base sequence for the production of a → Protein from the nucleus into the → Cytoplasm.

SCID (Severe Combined Immunodeficiency) – Severe immunodeficiency which is due to a defect in the „blueprint“ for a receptor. Without this receptor the cells of the immune system are unable to play their role in the defence against pathogens (→ADA-SCID).

Somatic gene therapy – Application of gene transfer on → somatic cells. Genetic modifications are not passed on to descendants. Somatic gene therapy aims to heal hereditary or acquired gene diseases by introducing normal (healthy) genes into certain target cells in the body.

Somatic cells – all cells of an organism, apart from germ cells and germ precursor cells. Their genetic information is not passed on to the following generations. This means they are no → germ cells.

Splicing – non-coding → introns are cut out of a → RNA- transcript and coding → exons are assembled to an mRNA which is then translated into proteins.

Stem cells – Cells which can divide repeatedly, where one daughter cell is a differentiated cell type (e.g. skin or cartilage cell) and the other another stem cell.

Superparamagnetic Nanoparticles – Magnetic → nanoparticles are used in bioengineering, medical diagnostics and in preclinical and clinical trials. They consist of iron oxide structures which are surrounded by biocompatible copolymers which protect them from degradation. The nanoparticle can be useful to concentrate → gene vectors and to guide them into the target cells → in vitro and → in vivo with the help of magnetic fields. This increases the number of gene vectors introduced.

Synovia – produced by the Membrana synovialis (joint membrane); a clear, mucous, stringy liquid in the joint.

Synthesis – Assembly of a substance from simpler materials.

Test person – participant in a → clinical trial.

TGF (Transforming Growth Factor) – These growth factors belong to the group of signal molecules (→ Cytokines) and play an important role in the growth of cells and tissues. They are sub-divided into the TGF-alpha, → TGF-beta and the BMP group.

TGF-β – the TGF-β polypeptides are multifunctional. Studies show that the properties of bones (elasticity, hardness) can be influenced by TGF-β. Low TGF-β levels make bones more elastic, higher concentrations of calcium phosphate make them harder. Apart from that they can influence → cell differentiation and other functions in a wide range of other cells. This includes the direct anti-inflammatory effect of → TGF-β. TGF-β is a local → cytokine, which is associated with healing processes and fibrosis of tissues and has, for example, relevance in the case of heart failure after a myocardial infarction.

Tissue Engineering – Living cells are cultivated as replacement material – partially on prefabricated three-dimensional matrices. Skin replacement, cartilage replacement (e.g. cultivated on biodegradable polymers). Central technique of regenerative medicine.

T-Lymphocyte – Group of white blood cells, which are part of the immune defence.

TNF factor (Tumour necrosis factor) – multifunctional signalling substance (→ cytokine) of the immune system, which is involved in local and systemic inflammations.

Toxicity – poisonousness, harmfulness.

Transduction – A virus introduces the therapeutic gene into the cells. The → viruses used can be DNA viruses, RNA viruses or retroviruses.

Transfection – chemical, physical methods to transport a therapeutic gene into an cell (see also →transduction). One method of transfection is the use of nanoparticles which are made up of the gene and other substances. → polyethylenimine, → liposomes or calcium phosphate are often used for this. Physical methods such as microinjection, electroporation or magnetofection can be utilised for a transfection. With a microinjection a very fine needle under a microscope to inject directly into a cell. During electroporation an electric surge is used to make the cell membrane permeable so the gene can enter it. For the magnetofection the gene is bound to a magnetic nanoparticle and the introduced into the cells with the help of a magnetic field.

Transgene – the transferred gene sequence. This can cause unexpected surprises: The cell incorporates it in differing areas within the genome and by doing this switches genes on and off, this might contribute to the development of tumours.

Transcription – Copying of a DNA strand into a complementary RNA strand (→ m-RNA messenger RNA) by the enzyme RNA-Polymerase.

Translation – Translation of the mRNA into the amino acid chain of a protein.

Triplet – Combination of three subsequent → bases of a nucleic acid, which in turn is the key for the production of an → amino acid.

Tumour Necrosis Factor – TNF (→).

Vector – Vehicle (Virus or Plasmid), which is used to introduce DNA into a cell or into an organism. Apart from various viruses, mostly incapable of reproduction, → plasmid-DNA in its pure form or mixed with other substances is used as a non-viral vectors.

Viral Vectors – to introduce genetic material into target cells with the help of a viral → Vector (→ transduction) the desired DNA sequence in the genome of the viruses needs to be cloned. In most cases certain DNA areas of the viral genome are replaced to prevent the viruses from further reproduction.

Viruses – Collective name for particles which consist of a nucleic acid (→RNA or → DNA) and a protein envelope. To grow and produce they need host cells as they are lacking the enzymes needed. Often their effect is pathogenic, as they re-programme the cells that they intrude and possibly kill them. The intrusion into the cells is called infection.

Wiskott-Aldrich Syndrome – Hereditary immune defect, which occurs with a frequency of 1:200.000 in the population. It leads to autoimmunity, eczema and coagulation disorders.

X-SCID – congenital immunodeficiency. The so-called Bubble Babies have no white blood cells and therefore no immune defence. They have to live in a sterile environment and have a low life expectancy (→ ADA-SCID, → SCID).

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