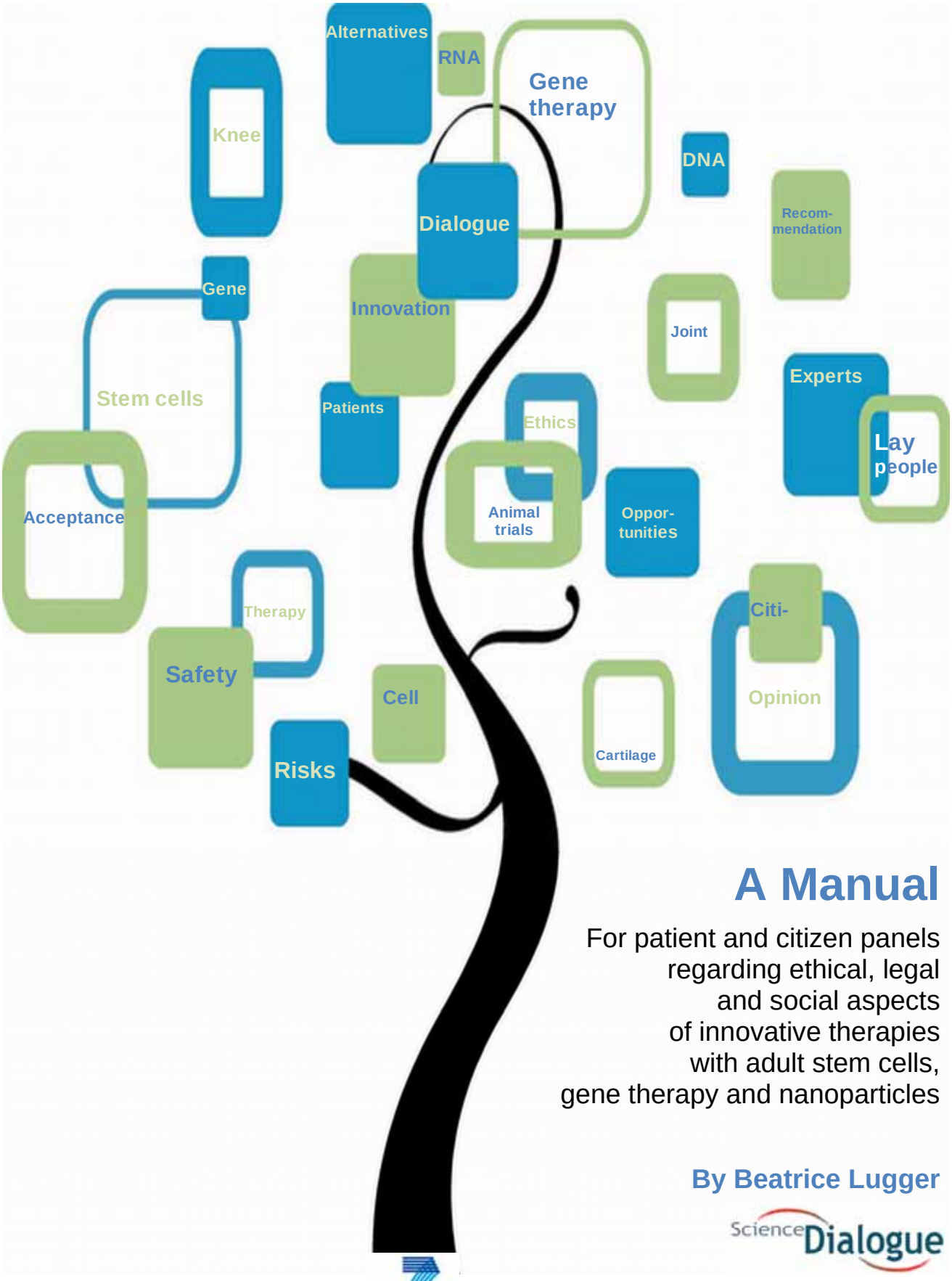
EU Project GAMBA

Basic Research into Regeneration
of Bone and Cartilage in Osteoarthritis



A Manual

For patient and citizen panels
regarding ethical, legal
and social aspects
of innovative therapies
with adult stem cells,
gene therapy and nanoparticles

By Beatrice Lugger

ScienceDialogue

Funded by the 7th framework program of the EU [NMP –2009-2.3-1]

How to read this manual:

This brochure is structured like a "Manual". You don't have to read it in one go; you can read it chapter by chapter (or parts thereof). If you have trouble understanding something or if you have questions please make a note. If you are not really interested in a chapter or you have the feeling that you don't understand enough, skip to the next chapter. During the panels we will make sure that all your questions will be answered as comprehensively as possible. Further information can be found in the compendium which also includes a glossary that explains the medical and scientific terms. You can also email us your questions: christine.ritter@nuigalway.ie

Key messages
The most important messages relating to GAMBA within a chapter are highlighted in orange.

Reference
The text has been kept to a minimum on purpose. Further information (e.g. in the compendium) is highlighted in blue.

Medical and scientific terms
When you come across a term you don't understand, you will more than likely find it in the glossary in the compendium. There you will also find further references (marked →)

Imprint

Publisher: ScienceDialogue Dr. K. Zöller, M. Schüpphaus, S. Siebert GbR
Zöpfstr. 25, 82362 Weilheim, www.sciencedialogue.de

Text: Beatrice Lugger; Ethics Chapter: K. Zöller

Originally published in Germany as "*Ein Handbuch für Bürger- und Patientenforen zu ethischen, rechtlichen und sozialen Aspekten innovativer Therapien mittels adulter Stammzellen, Gentherapien und Nanopartikel*"

Translation: Christine Ritter, Galway, Ireland

Editors: K. Zöller, M. Schüpphaus, S. Siebert
Layout: S. Siebert
Print : Clondalkin Group, Dublin

Kerry Hardie "Flesh"*

Sitting in a doorway,
in October sunlight,
eating
peppers, onions, tomatoes,
stale bread sodden with olive oil –

and the air high and clean,
and the red taste of tomatoes,
and the sharp bite of onions,
and the perpper's scarlet crunch –

the body
coming awake again,
thinking,
maybe there's more to life than sickness,
than the body's craving for oblivion,
than the hunger of the spirit to be gone –

and maybe the body belongs in the world,
maybe it knows a thing or two,
maybe it's even possible
it may once more remember

sweetness,
absence of pain.

*This poem was chosen by the Irish team. The German version of this manual featured the poem "The Doubter" by Bertolt Brecht

Table of contents

List of Illustrations

Preface

The systematic manipulation of the genome touches and alarms people in a special way. Because this means gaining immediate access to the basis of all life. The timescale is no longer dictated by the drawn-out function of natural evolution.

BBAW 2008, p. 5

Will we be able to grow bones back in 20 years time? Will it be possible to grow cartilage in the body and to stop inflammation in joints effectively? What risks and ethical aspects are connected with these visions? Could modern alternative treatments be more effective, less risky and cheaper and should these be given preference? What is it that people want and need?

Normally the **assessment of the opportunities and risks of new health technologies** is left to the experts. New therapy methods usually only come to the attention of patients and society when the new technology is being tested in clinical trials or when the first products are marketed. But there is a lot to be said for discussing new medical therapies when they are at the basic research stage, especially as a lot of tax money is invested in these therapies. During this project we aim to incorporate the views and ideas of patients and citizens into the early stages of research.

Therefore affected and interested citizens in Germany, Switzerland and Ireland will participate in so-called patient and citizen panels to discuss the opportunities and risks as well as the ethical and social aspects of the innovative osteoarthritis research. The discussion will include the scientists of the GAMBA team and other experts from various fields with whom the patients and citizens will meet as equals. The **scientists involved** gain an early insight into the aspects that interest and worry the public and are able to implement these insights into their ongoing research and communication strategies. The participating **citizens and patients** benefit from the direct communication with the scientists and become advisers in the development of new therapy approaches

Osteoarthritis is a very common joint disease which often severely affects the life quality of the patients. Approximately every fourth Irish person has symptoms of the disease; half of those who are over 65 years old are affected. Almost twice as many women as men are affected. With osteoarthritis the joint cartilage is worn down over time, depending on strain, until bone rubs on bone. Symptoms like limited mobility and pain can be alleviated but up to now there is no cure for this disease.

Should the research approaches taken in the EU project **GAMBA** (short for "**G**ene **A**ctivated **M**atrix for **B**one and Cartilage Regeneration on **A**rthritis") prove to be successful, there could be new therapies within the next couple of decades that may heal the affected joints. Biomaterials in combination with patient's own stem cells, gene vectors and nanoparticles that are inserted directly into the affected tissue could become a new treatment in the future. These enhanced biomaterials could lead to a regeneration of the joints. Therefore the key topics in this dialogue will be **adult stem cells, gene therapy, new biomaterials and components of nanomedicine.**

To overcome the classical one-way communication with scientists in the role of experts providing information and citizens in the role of lay people receiving information, ScienceDia-

logue will facilitate an intensive two-way dialogue at 'eye level' between scientists and patients/citizens. **First** the participants in the patient and citizen panels are introduced to the field of innovative research on osteoarthritis, for example with the help of this manual, through expert presentations and a hearing with experts selected by the citizens themselves. In a **second step,** participants will discuss the opportunities, risks and the ethical/social aspects of the topic. This will enable participants to give an evaluation of GAMBA's field of research as seen from their particular vantage point as patients or interested lay people. They draw up **recommendations** for the scientific world as well as for other sectors of society, such as industry and politics.

ScienceDialogue, with its team of experienced facilitators and moderators will be responsible for the conception and **implementation of the patient and citizen panels**. Special attention is given to ensuring that the information provided is balanced and unprejudiced. The ScienceDialogue Team is not involved in the research of future therapies, but is only in charge of organising and facilitating the patient and citizen panels as neutral partners. The researchers and advocates of stem cell and gene therapy will be heard as well as the critics, and experts on the risks and on ethical aspects.

During this dialogue, both "parties", i.e. researchers and lay persons, will learn from each other. By addressing the results to decision-makers in politics, administration, industry and other areas, these too will benefit from the ideas and assessments of the participants. The lay persons' views and ideas will give an early indication of the acceptance of the new technologies being discussed.

The ethical aspects of GAMBA described in this manual are intended to be guidelines for the participants of the panels to enable them to come to their own assessment of the subject matter. Some of the questions to be explored might include whether it is a good idea to focus on gene and stem cell therapy. If yes, what should be the conditions? Does this field need restrictions and if yes, which ones?

To the participants of the patient and citizen panels:

It might be useful to imagine that you have been offered to participate in a therapy study as described in this manual. What questions would you have? What would you need to know to come to a decision?

This manual 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.

Curiosity always comes first, when there is a problem that needs to be solved.

Galileo Galilei

1. Osteoarthritis – a widespread disease

Osteoarthritis, also known as worn joints, is the most common joint disease and almost everybody has a relative or friend who is affected by it. In osteoarthritis, the joint cartilage, the adjoining bones, ligaments, the joint capsule and the synovial membrane are damaged.

Osteoarthritis is defined as a "degenerative joint disease, which occurs due to an imbalance between the strain the joint parts and tissue can bear and the strain they are actually exposed to" (Pschyrembel, Germany 2007). The cartilage layer of a joint is destroyed and this leads to changes in the bones. The mobility of the affected joints decreases more and more. It is inflamed, swollen and painful.

Osteoarthritis can affect all joints, but most commonly it affects the joints that have the most everyday wear – the knees (gonarthritits), the hips (coxarthritis), the spine and the hands. Osteoarthritis can lead to years of joint pain and a restriction of mobility. The joints can become deformed and eventually ossify. This leads not only to difficulties with everyday tasks, but also results in changes in social life and a reduction in quality of life (RKI 2007).

1.1 Large numbers and high costs

There are several ways to slow down the progression of osteoarthritis. The main priority is to alleviate pain and to improve the mobility of the joint (see chapter on Therapy, page 14 f). The most efficient therapy at present is an artificial joint (prosthesis). Thus the aging population and the increasing trend to surgery leads to more and more new hip and knee joints being implanted. In Ireland, based on VHI statistics, knee replacements have increased in number by 173.4% between 1999 and 2004. Osteoarthritis is the reason for most of these surgeries.

Osteoarthritis can strike at any age, but is more common at an advanced age. Only 9 percent of 20-year-olds are affected, already 17 percent of all 34-year-olds. This number rises to 90 percent of over 65-year-olds (Pschyrembel, Germany 2009). So far there have been no representative screening tests of the population, i.e. where the diagnosis is backed up by x-ray (RKI 2007). Estimates range from five to 20 million patients in Germany, out of a population of approx. 80 million (idw 2010). Previous X-ray screenings of over 65-year-olds show typical symptoms of osteoarthritis in 2 out of 3. But this doesn't necessarily mean that those affected suffer physical complaints. Quite often the disease remains undetected (Techniker Health Insurance 2002).

Overall, the German Federal Statistics Office calculated the health costs for the treatment of osteoarthritis to have been around 7.6 billion Euros for the year 2008. This compares to overall health costs of 254 billion Euro; i.e. osteoarthritis accounts for 3 percent of all health costs. The rate of women affected by osteoarthritis is twice that of men (Federal Statistics Office Germany 2010). Why women are more commonly affected is still the subject of ongoing research. One possible reason is hormonal factors – there is a known connection between osteoporosis and menopause. Other possible factors are lesser muscle power, the different distribution of muscles in the body and the additional burden caused by pregnancies.

To get an impression it is it is possible to extrapolate the number of affected adults using the data of the so-called „Herner Arthrose-Studie (HERAS)", a survey conducted with more than 8,000 over 40-year-olds. On the basis of this survey the number of affected adults in Germany amounts to 8.5 million people (Schlingensiepen 2006). About 400.000 people are affected in Ireland (Arthritis Ireland).

Fig. 1: Joints most commonly affected by osteoarthritis in percentages (2005)

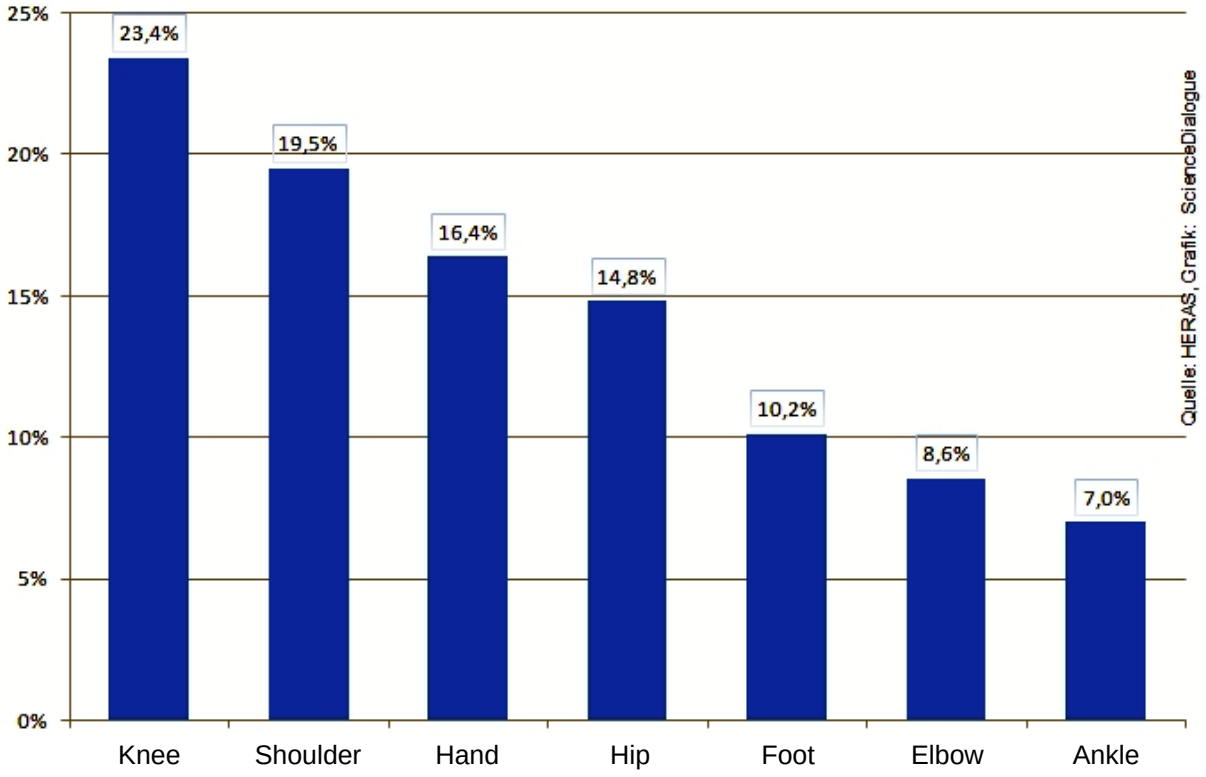


Diagram: ScienceDialogue

Musculoskeletal disorders are increasingly the centre of attention worldwide, not least due to the rising life expectancy. In view of demographic development the World Health Organisation, WHO, estimates that the number of patients will double within the next 20 years (WHO 2003). The WHO had declared the years 2000 to 2010 to be the "Decade of bones and joints" to draw attention to the health and economic implications of this disease and to strengthen research in this area.

1.2 The difference between osteoarthritis and rheumatoid arthritis

There are several forms of cartilage and joint diseases. We can distinguish between two main groups: osteoarthritis and rheumatoid arthritis.

Osteoarthritis is a form of joint wear and affects one or a couple of joints as a result of excessive strain. Osteoarthritis is a local, incurable, degenerative condition of a limited number of weight bearing joints, especially hips and knees. In contrast, rheumatoid arthritis is a systemic autoimmune condition that affects the whole body. This autoimmune disease causes inflammations and affects multiple joints – (this can be compared to allergies where the immune system attacks the own body) (Evans 2009).

With osteoarthritis the joint wear leads to inflammatory processes but, according to present knowledge, these are not the actual cause for the disease of the individual joint.

The most common joint disease by far is the above-mentioned osteoarthritis. It is also the main subject of this manual. The main vision of the European research project GAMBA (see page 15ff) is to find a treatment for this disease in the long term.

1.3 Varied and often still unknown triggers of osteoarthritis

The reasons for the development of osteoarthritis are not yet completely understood. The Health Report 2007 published by the Robert Koch Institute states: “The causes for this disease are varied and still partially unknown. Joint injuries, congenital and acquired joint misalignments, metabolic disorders, a genetic predisposition as well as mechanical strain on joints contribute to the development of osteoarthritis.” (RKI 2007). It usually begins with an injury of the cartilage in the joint.

In general a distinction is made between a purely degenerative disease - which can occur sooner or later depending on disposition and the daily strain on the joints (primary osteoarthritis) - and a disorder which is triggered by a specific cause (secondary osteoarthritis)

- In the case of **primary osteoarthritis** the reasons for the degradation of the joint are undetermined; they are described as a degradation process in the joint. However, current research explores whether this is purely down to joint degradation or whether there is a disease causing process at the root of it (idw 2010). For example changes in the synovial fluid (Brandt 2010) or changes in the cartilage (DGrh 2010) could cause joint damage in the long term.
- In contrast **secondary osteoarthritis** is, without a doubt, a consequence of another disease or accidents. Joint misalignment, either congenital or caused by accidents, such as knock-knees or bowlegs or so-called compression fractures close to the joint can damage the cartilage and thus cause osteoarthritis. A meniscus injury or an inflammation of the joint can also lead to misloads and destruction of the cartilage tissue – please note that not all cartilage damage necessarily results in osteoarthritis. Further causes can be metabolic disorders (such as diabetes), infections, cruciate ligament injury, surgery, instable joints, weak muscles and overuse e.g. due to hard physical work (Techniker Health Insurance 2002).

Body weight has a very significant influence on the severity of osteoarthritis – this is true for both primary and secondary osteoarthritis. An Australian study with more than 32,000 par-

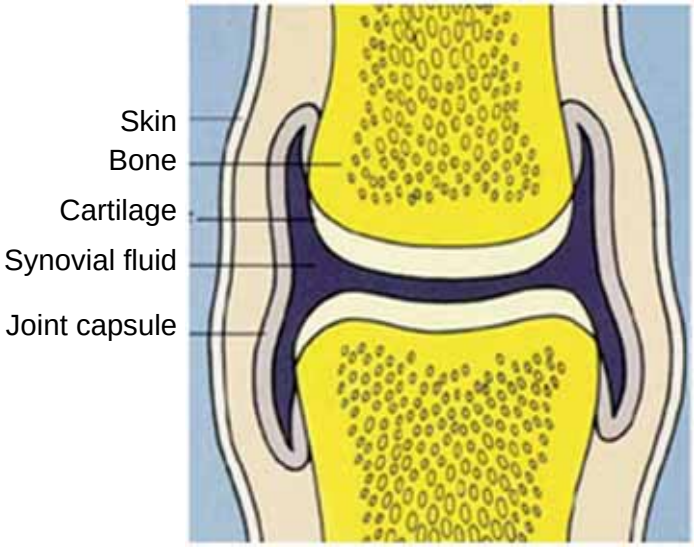
ticipants showed that higher body weight significantly increased the risk of developing osteoarthritis. The risk of very obese participants was shown to be increased fourfold (BioMed Central 2002).

1.4 The Joint

Joints are flexible connections at the ends of bones. Joints enable us to move. They absorb sudden and hard movements and give support. The following example of a knee joint shows how a joint changes during an arthritic process.

1.4.1 Structure of a healthy joint

Fig. 2: Healthy knee joint



Source: Evans et al. 2004

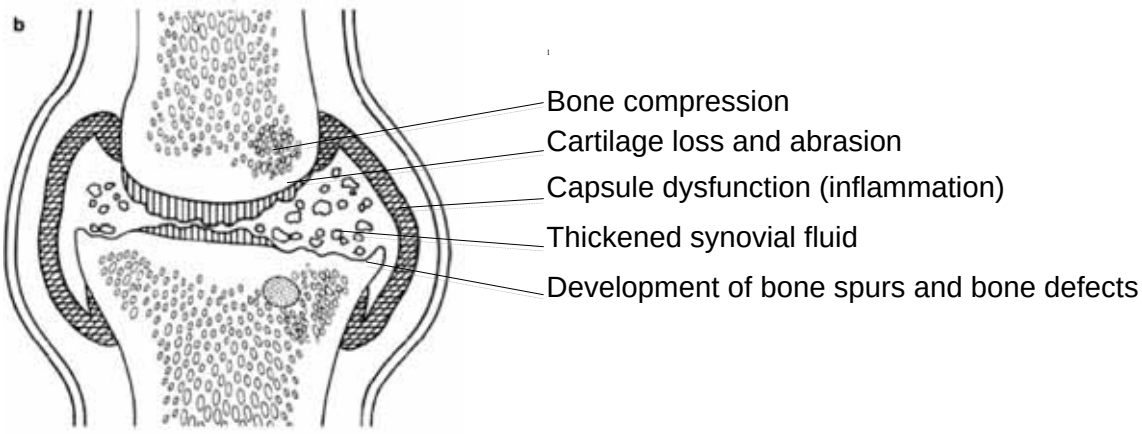
Synovial fluid: The synovial fluid improves and facilitates movement. This liquid film is formed by the inner joint mucosa.

Joint cartilage: Cartilage is the shock absorber in the joint. The smooth and elastic surface at the end of bones has a thickness of between two and five millimetres and protects the joints during each movement. Cartilage consists mainly of two components. The main one is water (65 to 80 percent) in the form of a watery gel that is embedded in a resilient network of collagen fibres. The cartilage cells (chondrocytes) account for only 5 percent of the cartilage mass, but they play a central role, as they produce collagen fibres and certain proteins (aggrecans), which interact with water molecules to form the gel and therefore the cartilage (Groß 2010). Cartilage does not contain nerves and blood vessels. The cartilage cells are maintained by the synovial fluid.

Joint capsule: The joint capsule is a covering of connective tissues enclosing a joint. It encloses the joint space which is filled with synovial fluid and is lined with synovial membrane. It consists of two layers of tissue.

1.4.2. An arthritic joint[1]

Fig. 3: Arthritis in a joint



Source: Evans et al. 2004

During the course of osteoarthritis the first step is an injury and degradation of joint cartilage. The starting point is commonly a defect in the cartilage layer. Quite often this is no more than some superficial damage of approx two square centimetres that progresses to more extensive changes in the cartilage. Additional **changes of the bone** are an important sign for the **early stages** of osteoarthritis. Without these changes in the bone we are dealing with damaged cartilage but not with osteoarthritis. This means that osteoarthritis is always a combination of cartilage damage and changes in the bone.

In the long term there will be **destruction of soft tissue** such as mucous membrane, capsules and ligaments as well as bone close to the joint. This process can take many years. Fissures in the cartilage layer lead to cell fragments. The irritation of the joint occasionally leads to joint effusion (increased volumes of synovial fluid or influx of liquids such as blood or pus). In the **later stages** the joint cartilage is not only diseased and damaged but completely worn off. Then the **exposed bone** rubs directly on the bone on the other side.

The bones themselves become much denser and harder. On the edges of the joint big bony bulges develop. These so-called osteophytes lead to a broadening of the joint. The mobility of the joint deteriorates further.

However, there are no firm rules for the progress of osteoarthritis. It is very individual and cannot be predicted. It is even possible for the disease to come to a standstill at a certain stage.

[1] Techniker Health Insurance 2002, RKI 2007, www.arthrose.de

1.5 Modern Therapies

In the case of osteoarthritis the perception of the patient is very important. It takes some patients many years to notice their osteoarthritis, even when x-rays show that they are suffering from it. Only 15 percent of all patients who's x-rays showed that they were suffering from arthritic knees actually complained of pain (Michael et al. 2010).

You can find more information on the typical symptoms of osteoarthritis, diagnostic procedures and therapies of osteoarthritis in the **Compendium** to this manual in **Chapter 2**. You will also find information on self help groups.

Because osteoarthritis starts with a degradation of cartilage, this is an important starting point for a possible osteoarthritis therapy and prevention. But cartilage has very limited healing powers. The cells that are needed for the maintenance of cartilage amount to only 5 percent of the cartilage mass. They produce the collagen which in turn incorporates water and thus forms the elastic cartilage proper.

The cartilage cells are embedded separately in this dense, voluminous structure and have – in contrast to the cells in the bone and in other tissues of the body – hardly any direct contact with their neighbours. Also, they are not connected to the blood circulation and get their nutrients through the synovial fluid. Because they are not connected to the circulation, there is no regular check by typical immune cells which patrol the body to find harmful substances and aged cell structures. Furthermore, the cartilage cells divide at a much slower rate than other body cells or blood cells. This means that not only does the joint have hardly any blood supply but it has limited self healing capabilities; this disadvantage exacerbates with age.

Although the first cell therapies for gene defects were already deployed in the year 1994, up to now there are no methods that show a marked improvement on conventional therapeutic approaches – especially with regard to medium- and long-term effects (Osch et al. 2009). The three most common used techniques to heal cartilage in young people are:

- Drilling into the bone to improve the migration of stem cells from the bone marrow (see Microfracturing, Compendium Chapter 1.3.5.1);
- Injection of precursor cells of cartilage and bone cells (see stem cells p. 17f and Compendium Chapter 3); or
- The transplantation of one's own cartilage cells (see ACT, Compendium Chapter 1.3.5.1)

All of these methods improve the symptoms, but it is not yet proven whether they lower the risk of joint degeneration (limited functionality/joint wear) (Osch et al. 2010) in the long run.

The option of transplantation of one's own cartilage cells, where healthy cartilage cells are taken from undamaged and non-weight-bearing areas in a joint and are then cultivated in the lab before being inserted into the damaged cartilage, is limited. With increasing age it becomes more difficult to find intact cartilage cells and the cells are more and more difficult to grow in cell culture as their capability to divide decreases.

I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me.

Isaac Newton

2. GAMBA

Within the EU project GAMBA (**G**ene **A**ctivated **M**atrices for **B**one and Cartilage Regeneration on **A**rthritis) scientists are searching for new therapeutic approaches for osteoarthritis which can induce a kind of self healing process from within. Until now, the best we can do is to slow down the development of the disease. However, the degenerative disease which progressively damages cartilage and bones can't be stopped. Current treatments are maintenance therapies and give short-term relief but are not effective in the long term. The disease progresses until a joint replacement is required (See Therapies, Compendium Chapter 1.3). Therefore, and also because of the huge numbers affected, it is logical to think about new approaches.

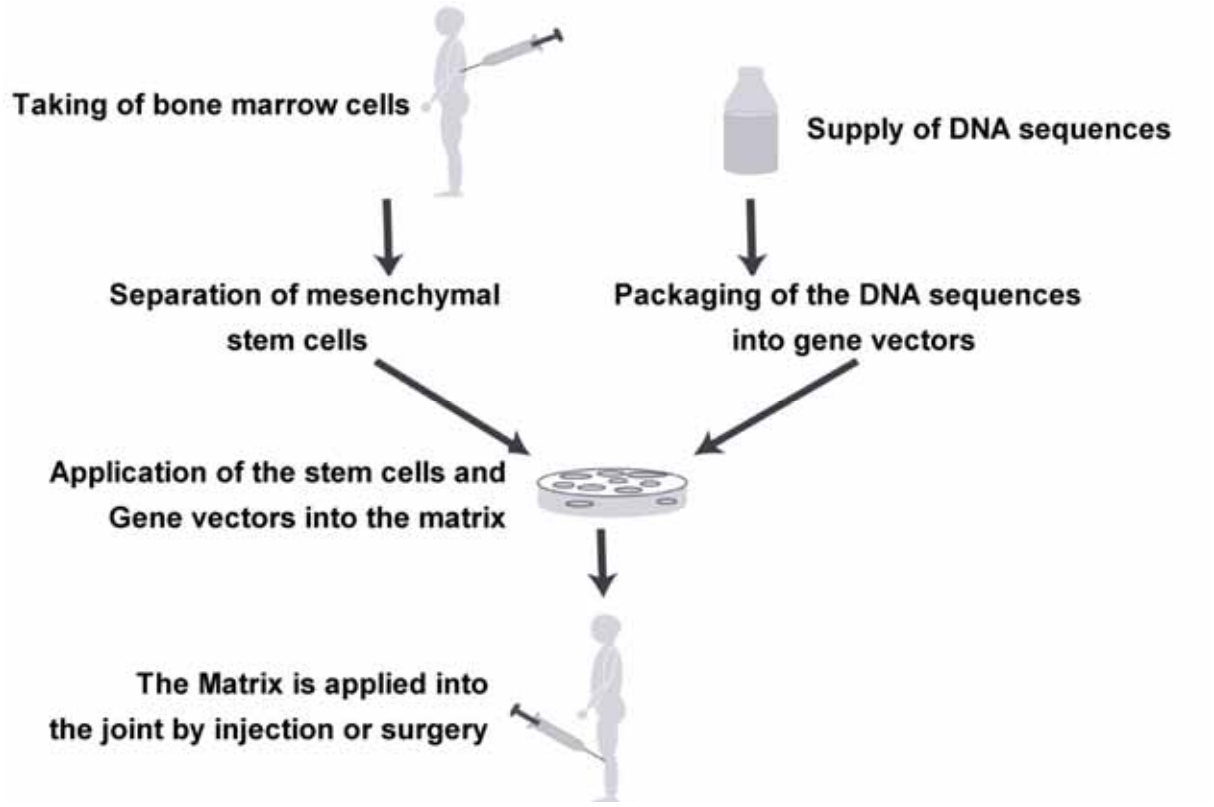
2.1 Healing from within with appropriate means

Instead of cartilage cells the GAMBA project now focuses on the healing potential of mesenchymal stem cells (see Stem Cells, Compendium Chapter 3). Like all stem cells they can divide and certain proteins can be used to encourage them to develop into the desired cell type (such as bone or cartilage cells). These stem cells can be harvested from the patient's bone marrow or fatty tissue (autologous stem cells).

To achieve the desired effect the stem cells will be isolated in the lab and then cultivated until they are eventually embedded in a so-called gene activated matrix (GAM). The matrix (plural "matrices") is made of a biocompatible material – either tiny ceramic beads with lots of very small pores or special gels or a scaffold. Apart from the stem cells, so-called gene vectors will be embedded into this biomaterial (for more information on gene therapy see Compendium Chapter 4). These gene vectors can introduce selected therapeutic genes into the cells, which in turn lead to the production of specific proteins within the cells. These proteins are the therapeutic substance in the GAMBA project. Certain proteins can have an anti-inflammatory effect. Other proteins can induce stem cells to change more specifically into bone and cartilage cells. This would mean a healing cell supply for the cartilage and bones damaged by osteoarthritis.

It is GAMBA's vision that such gene activated matrices for the regeneration of bone and cartilage in osteoarthritis will be introduced into the affected joint either through surgery (in the form of a three-dimensional structure) or injected in the form of dissolved beadlets. Only when the matrices have reached their destination within the joint will the healing process be started and regulated from the outside, either chemically or physically. The expression of the genetic code (information) and the production of healing proteins begin at the same time stem cells are released.

Fig. 4: Stem cells: from the own body back into the joint



Stem cells are taken from the bone marrow. Then those that can change into cartilage and bone cells over several generations are isolated (mesenchymal cells). DNA sequences for the production of healing proteins are packaged into gene vectors and then embedded into ceramic beadlets, ceramic structures or gels alongside the stem cells. Finally the matrix is inserted into the affected joint, either during surgery or with a syringe. Diagram: ScienceDialogue

During the experimental stages GAMBA aims to identify a combination of the following healing processes:

- Firstly, to **stop** inflammation,
- Secondly, to **heal the cartilage** and
- Thirdly to **heal the bones**.

It is envisaged that through laboratory and animal experiments the best combinations of substances will be found for each of these individual healing processes. GAMBA will also seek to prove their feasibility. However, it is possible that the combination of agent, matrix and cells identified by GAMBA will address only one or even none of these healing processes. GAMBA places special emphasis on the **appropriate control of the reaction period and location** which is necessary for the production of the active agents.

GAMBA is a basic research project. Initial research is with animal and human cells in test tubes (in vitro), animal organs such as bovine joints in the laboratory (ex vivo) and finally in a living organism (mouse, rabbit, goat). Clinical trials (see Compendium Chapter 4.5), which

focus on the effects and side effects on humans, are not part of the GAMBA project. They will be conducted at a much later stage and only after preclinical trials have concluded and only if the therapeutics developed by GAMBA have been proven to be effective in laboratory and animal experiments (see Ethics page 42ff). Nevertheless, the researchers are eager to start the dialogue with people now, so that their views about the opportunities and risks can be considered in their research.

2.2 GAMBA's therapeutic research spectrum

GAMBA is pursuing a form of innovative medicine which aims to combine several novel approaches, which are seen as potentially effective, in an ideal way:

- Autologous stem cells
- Gene therapy with gene sequences in gene vectors
- Biologically active proteins (growth factors)
- Nanomaterials
- Biomaterials for tissue engineering
- Pharmacological agents

2.2.1 Stem cells

The stem cells used for the GAMBA project (mesenchymal stem cells) are precursor cells of the connective tissue (soft tissues), which can change (differentiate) into bone, cartilage and fat cells over several generations (Stoddart et al. 2009). They show promising potential for osteoarthritis therapy (Charbord 2010). These stem cells are continually producing cartilage and bone cells even in an adult organism. However, the production rate slows with age (see Stem Cells Compendium Chapter 3).

Theoretically, it would also be possible to take cartilage and bone cells from the patients and cultivate them in the laboratory before returning them to the diseased joint. However, the number of cartilage cells within a joint is very low and they have an extremely slow division rate. Bone cells are very difficult to extract. Therefore, GAMBA wants to ensure supplies for these cells with the help of stem cells which can easily be isolated from the body's own bone marrow. This means that the stem cells are encouraged by the self-produced proteins to turn into cartilage and bone cells (see Opportunities of GAMBA p. 29ff).

The aim of GAMBA is to induce stem cells with the help of two proteins (in this case growth factors) to turn into either cartilage or bone cells. For the differentiation into cartilage cells an elaborate interaction of several proteins is required. So far this is not fully understood. But the most important factors are known (Chen et al. 2008).

During the research project GAMBA, animal and human stem cells will be tested in combination with various gene vectors, matrices and nanoparticles. The human stem cells can be commercially bought from laboratory suppliers or they are provided by the research teams at the National University of Ireland Galway or from the Erasmus Universitair Medisch Centrum Rotterdam, Netherland (see organization chart on p.28). The stem cells were donated by volunteers or patients, who gave consent for their use in research (see "informed consent" in the ethics chapter p. 45).

More information on stem cells can be found in the **Compendium** in **Chapter 4**

2.2.2 Gene Vectors

"Osteoarthritis … remains difficult to treat and provides an attractive target for … gene therapy, especially because it is a local disease of relatively few joints… (which are) discrete, accessible cavities that can be readily injected", the gene researcher Christopher H. Evans, one of GAMBA's advisors, states in a publication (Evans et al. 2009).

To achieve a better result, the proteins will not be injected directly into the diseased joint but rather certain DNA sequences are used that promote the production of the proteins within the stem cells themselves.

To this end the DNA sequences for the production of the proteins are first isolated as so-called cDNA (copy DNA). Researchers then combine this cDNA with specific start and stop sequences and then package the entire DNA sequence in gene vectors, which can penetrate the cell envelope and then deliver the gene freight into the cell. With this gene freight the cells are meant to produce the healing agents under controlled conditions.

The gene vectors used for the GAMBA project are non-viral (e.g. miniscule fat globules) and adenoviral gene vectors (inactivated cold viruses), which host the cDNA. The gene vectors transport the DNA sequences into the cell nucleus. Normally these sequences are not incorporated into the genome of the target cells, but they are only present in the nucleus temporarily (exceptions: see Risks p. 34f). During continuous cell division the number of cells with the inserted gene vectors halves with every division.

Fig. 5: Gene vectors

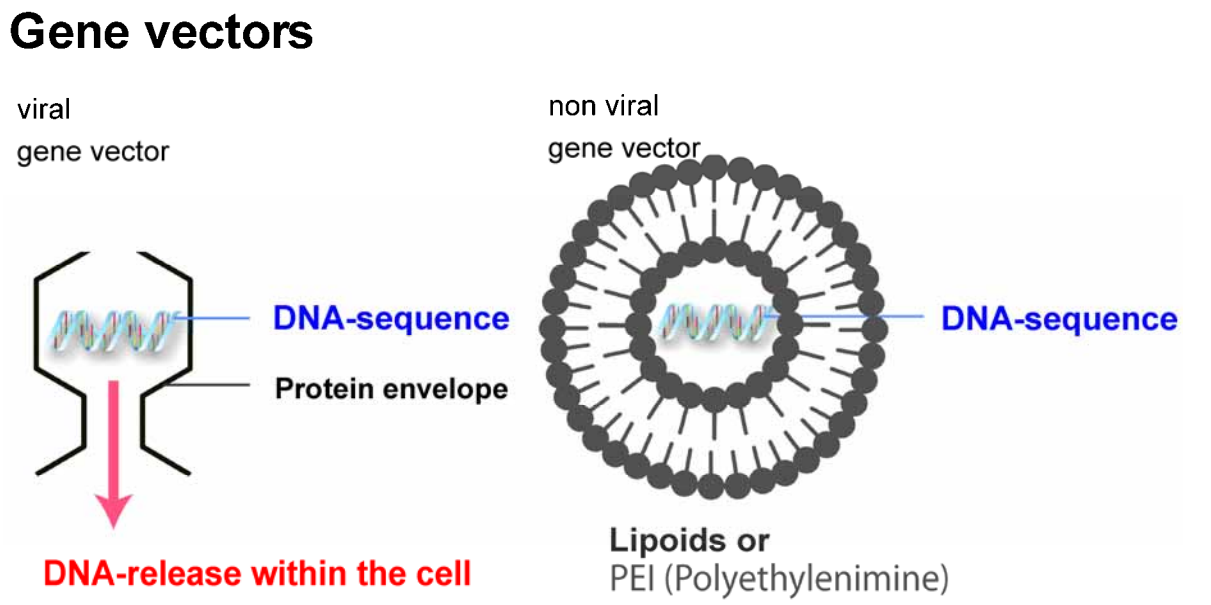


Diagram: Science Dialogue

Adenoviral gene vectors are being used for GAMBA because of their superior efficiency in transporting genetic materials into cells. They are derived from cold viruses which are biologically specialised to unload their gene freight in the target cells. Before they are used, they are genetically depleted and are then filled with the new desired genome. Adenoviral gene vectors are of use especially during the test phase in the laboratory as they are particularly effective gene vectors. They can help to show whether the different projects are feasible, i.e. the cells produce the desired proteins as planned. However, it is not very likely that the adenovirus model will be used as a gene vector in osteoarthritis treatment in the future. After all, adenoviral gene vectors harbour certain risk factors for humans even if their use is limited to specific time periods and locations (see Risks p. 38f). But they are very useful in basic research as they very effectively introduce new genes into cells and thus enable the scientist to see whether the gene expression works in principle. Once the feasibility is proven (Proof of Principle), it will be time to look for gene vectors better suited for future therapies.

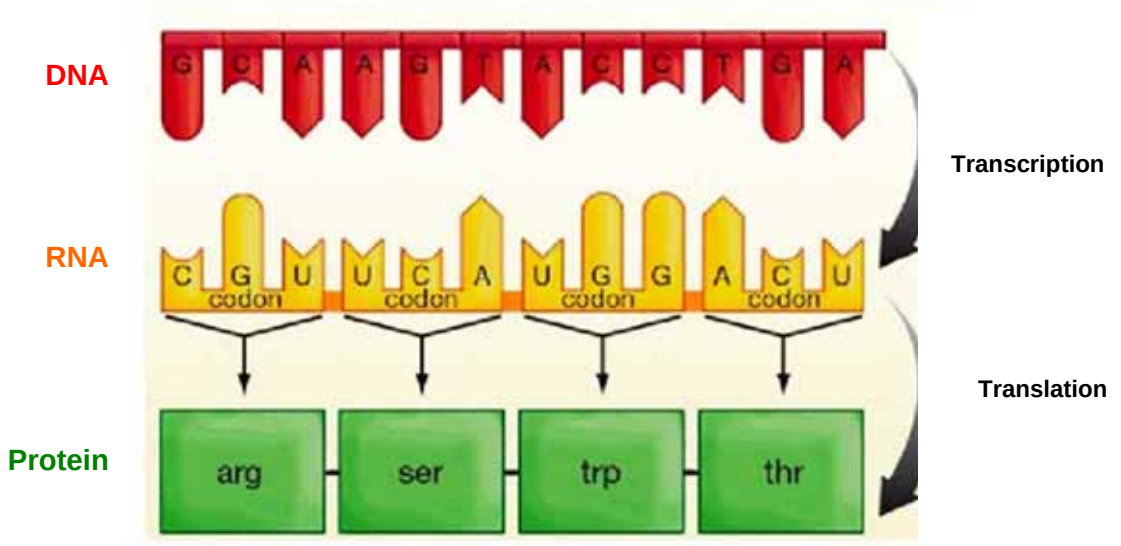
For future use with humans GAMBA is therefore concurrently researching the potential of **non-viral vectors**. The research teams involved already have a lot of experience with two forms of non-viral DNA packaging: Polyethylenimine (synthetic material) and biodegradable fat globules. They are not as efficient as adenoviruses in unloading their gene freight in the target cells, but the rates are nevertheless satisfactory.

More information on the opportunities and risks of the individual gene vectors and on the successes and setbacks of gene therapy can be found in **Chapter 4 of the Compendium**.

2.2.3 Biologically effective proteins

Proteins are part of most components of life. They are responsible for all life functions. They are produced in the cells by expressing and transcribing the genes (See Biological Basics, Compendium Chapter 2).

Fig. 6: Expression and transcription of the genetic code for the production of proteins



Graphic: Plank

For Explanation see next page.

*Explanation Fig 6.: Healing from within is the great potential of the GAMBA project. Therefore DNA sequences are inserted into certain stem cells with the help of gene vectors. These DNA sequences will be transcribed into the so-called RNA (*Ribonucleic acid*) within the cell. The information of the RNA is finally translated to build the desired proteins. These* ***proteins*** *are the* ***actual therapeutic agents*** *within the joint. They are expected to inhibit inflammation and encourage the healing of cartilage and bone.*

For more information on the biological basics see **Compendium Chapter 2**.

Today approximately 20,300 different proteins in the human body are known. They are in constant exchange with each other and can stimulate new processes of the cells. Intensive research is still going on to further distinguish the proteins in the human body (proteomics) and to understand their interactions, various functions and control mechanisms (see Epigenetics, Compendium Chapter 2.2).

There are numerous proteins involved in the disease process of osteoarthritis. After an injury of the cartilage, scientists have so far identified almost 700 proteins which may be produced in the joint as a reaction to the injury in varying numbers (Pitzalis et al. 2008). Some of the proteins play a central role in the disease but others can counteract the effects of these proteins. For example the treatment with the endogenous protein 1Ra is already established (see Compendium Therapies Chapter 1.3.3).

More Information on proteins and DNA sequences (genes) as basic building blocks of life can be found in the **Compendium** in **Chapter 2**.

GAMBA is testing **three different proteins**:

- One protein, the so-called Interleukin 10 (IL-10) is believed to stop the inflammatory process which often is associated with osteoarthritis. IL-10 is one of the most important anti-inflammatory proteins. The interleukins act locally. The affected cells change their metabolism and thus promote their renewal. They also influence neighbouring cells to do the same. Below we will talk about the “anti-inflammatory protein”.
- Another protein is believed to encourage stem cells to change into **cartilage cells** – it is called the Transforming Growth Factor-ß (TGF-ß). TGF-ß is known for this effect and a broad range of other functions – including effects in inflammatory processes. Below we will talk about the “cartilage protein”.
- The third protein is believed to encourage stem cells to turn into **bone cells** – it is called the Bone Morphogenetic Protein 2 (BMP-2). BMP belongs to the same group as the above-mentioned TGF-ß. These proteins play an important role in the embryonic development of cartilage and bone. Below we talk about the “bone protein”.

In a first step the cartilage and bone proteins will be tested in the lab to establish the minimal amount needed to induce the production of cartilage and bone tissue. Also, the best possible time window needs to be established. Furthermore researchers will figure out which gene vectors are best suited for the transport of the genes and which biomaterials are best suited to store and dispense stem cells and gene vectors.

Finally, the cartilage and bone production will be examined in animal and human cartilage and bone samples in the laboratory. If these tests are successful, first trials in small animal models (e.g. with mice) could follow.

2.2.4 Nanomaterials

Generally all gene vectors used in GAMBA have a size of less than 100 Nanometers (1 Nanometer equals 1 billionth of a meter) and are therefore nanoparticles (Nanos = dwarf, see Nano Medicine, Compedium Chapter 5). To ensure they are well protected they are then wrapped in a protective layer of water soluble non-toxic Polyethylenglycol (PEG); this results in so-called COPROGS (Copolymer Protected Gene Vectors). This protective layer means that the gene vectors are not as easily eliminated by the phagocytes of the immune system.

Furthermore the GAMBA research project utilises iron oxide particles, which heat up in a magnetic field (so-called superparamagnetic nanoparticles). It is hoped that it will be possible to control the release of certain gene sequences with their help from the outside (see Temporal and Localised Control p. 22f). Similar iron oxide particles are already approved as contrast media in magnetic resonance scans and are in daily use in clinics all over the world (see Nanomedicine, Compendium Chapter 5).

During the course of the project we will find out where these tiny iron oxide particles are best located:

- They could be spread in one of the matrices, the gel.
- They could be directly connected to the gene vectors and could be brought into the target cells with their help.
- They could be inserted into the mesenchymal stem cells before these are embedded in the matrix.

An overview of the current state of nanomedicine can be found in **Compedium Chapter 5.**

2.2.5 Basic matrices for Tissue Engineering

As described above, during the course of the GAMBA project the various building blocks – stem cells, gene vectors and nanoparticles – that are meant to enable healing from within are incorporated into functional materials that may degrade in the body. For the bones the most suitable materials for the matrices are ceramics, for the cartilage gels are best suited. In the lab these matrices are built in layers, similar to the joint. The best way to actually introduce them into the body is part of the GAMBA research.

2.2.5.1 Calcium phosphate matrix for bones

Calcium phosphate is a mineral and an essential component of bones. It can be used as a matrix for bone and can be produced in a way that its surface forms pores of a certain size – ideal niches for stem cells and gene vectors. The Research Institute Biomatlante which is involved in GAMBA (see organizational structure p. 26f has many years of experience generating calcium phosphate with certain micro- and macropores (Micro Macroporous Biphasic Calcium Phosphate, MBCP). The material can be absorbed and degraded in the body and is already approved for clinical use (Goyenvalle et al. 2010, Sohier et al. 2009).

The miniscule granulates of MBCP are inserted into polymers such as hyaluronic acid gels (see Hyaluronic acid gel p. 22). These combinations of MBCP and gel can be produced in various forms:

- Powdered MBCP can be dissolved in the gel and can then be injected into the joint. In this case it is very likely that the size of the powder particles will be the determining factor for the success of bone augmentation (Layrolle et al. 2009).
- MBCP can be surgically inserted as a three-dimensional, malleable implant (Cordonnier et al. 2010).
- Also available are combinations with so-called tissue adhesives (fibrin glue) or bone paste.

Project GAMBA aims to optimise the pore size and the surfaces of the calcium phosphates for combination with cells and gene vectors. It is also important that the structures ensure a targeted release of the gene vectors in the body, so that the desired reaction can commence.

2.2.5.2 Hyaluronic acid gel for the cartilage

Hyaluronic acid is one of the main components of synovial fluid, but also a building block of the cartilage structure in the joint. The acid can bind a lot of water and thus forms the shock absorbing and lubricating hyaluronic acid gels. Hyaluronic acid gels have been used in osteoarthritis therapy for quite some time in so-called augmentation injections, which lead to a temporary improvement of symptoms (See Therapies, Compendium Chapter 1.3.3). Hyaluronic acid is also frequently used as an antiwrinkle agent in cosmetics.

The hyaluronic acid used for GAMBA can be distributed and layered as needed; cells and other materials can be embedded (Khademhosseini 2010). There is a lot of promising research ongoing into this material, also in combination with cells. For example a gel mix composed of hyaluronic acid and autologous cartilage cells and supporting agents has shown good results in experiments in mice and bovine models (Pereira et al. 2009).

The hyaluronic acid used for GAMBA reacts to changes in temperature. It is planned to raise the local temperature in the joint to 42°C. This will lead to a swelling of the gel which in turn leads to the release of the gene vectors into the surrounding tissue. This is one of the possible ways to achieve a timed control (see below) of the healing process.

During the GAMBA project hyaluronic acid gel will be tested and optimised as an injectable carrier material for gene vectors, MBCP matrices, stem cells and nanoparticles. The stability of the gels will be examined and the efficiency of gene vector release and the amount of desired proteins produced and degraded in the gel assessed.

2.3 Temporal and localised control of the healing process

An essential part of GAMBA is the attempt to gain control over the healing processes from the outside. After embedding the matrices into the joint, the sophisticated mechanisms of the individual healing processes such as anti-inflammatory processes and the bone and cartilage formation will start. It might even be possible to repeat such a start reaction, if needed, after the first reaction has slowed down.

A targeted deactivation of the reaction is not planned: the reaction will slow down gradually. Excess gene vectors should be degraded in the body over time. Depending on the stability of

the healing protein produced, the production of this protein stops in and the proteins will be degraded as well.

It is planned to keep the stem cells, gene vectors and nanoparticles localised, e.g. by incorporating them into a matrix as well as through the relative isolation of the joint. This temporal and localised limitation will help to prevent possible side effects, such as excessive growth of tissue und immune reactions (Salzmann 2005) (see Risks p. 34ff).

Within the framework of GAMBA there will be no research into whether the gene vectors, growth factors or nanoparticles spread in the body beyond the cartilage bone area. Such toxicological tests would be absolutely necessary as a next step should the GAMBA experiments prove to be successful (see Clinical Trials, Compendium Chapter 4.5).

The release of the gene vectors as well as the introduction of the DNA sequences into the stem cells and therefore the production of the desired proteins will be started either biologically, medicinally or physically, depending on the system (see below).

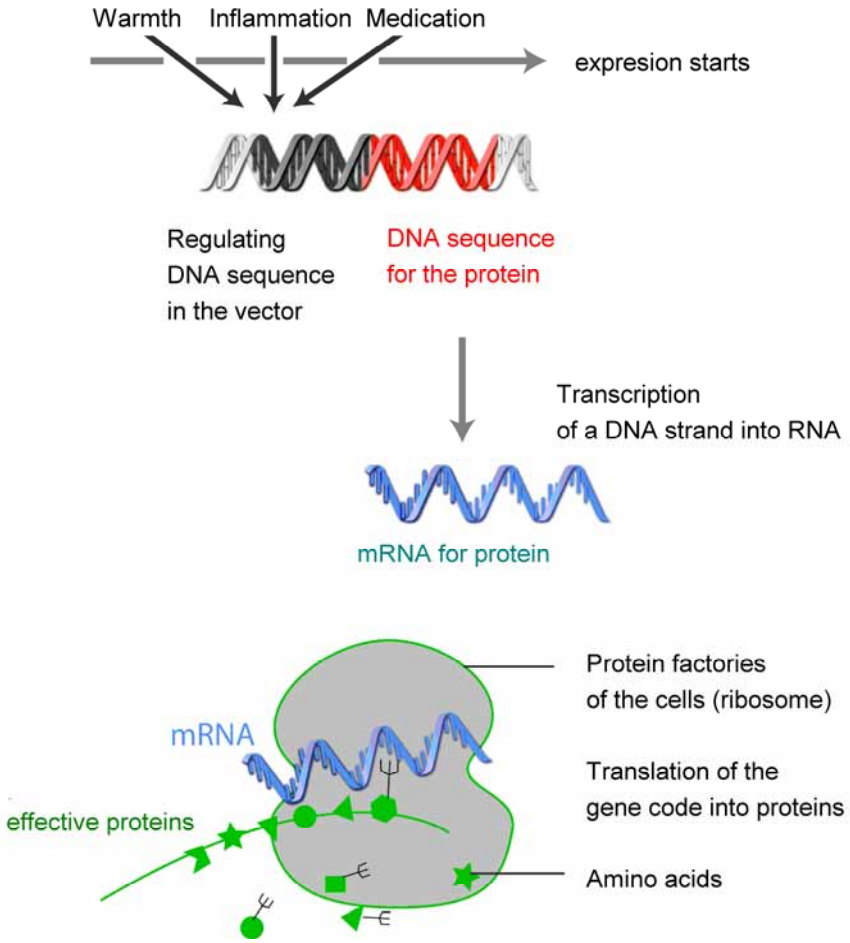


Fig. 7: Control of gene vectors

In GAMBA a DNA sequence will be placed in front of the gene sequence for the desired effective protein. This DNA sequence reacts to certain stimuli (the body's inflammation proteins, medication) or to warmth. This means: by stimulating this sequence the expression and the production of the effective protein in the protein factories (ribosomes) within the cell can start.

Figure: ScienceDialogue

Therefore, the gene vectors not only contain genetic information (code) for the proteins which promise a healing effect, they also carry gene sequences which react to certain biological or pharmacological signals and which start the expression of the gene sequences. This code is read in the protein factories and the therapeutic proteins are then built by assembling various amino acids (gene expression).

2.3.1 Biological or medical start

The construction of DNA sequences from separate components in the lab is common practice. The researchers of the Technical University Munich, who are participating in this project, do this routinely. The individual DNA-sequences needed can be isolated from human and animal DNA or produced artificially on demand. The world market leader, GeneArt AG, based in Regensburg, Germany produces artificial DNA sequences on a large scale – in early 2010 this amounted to approx. 3000 artificial genes per month (Grolle 2010).

For a biological or pharmacological start GAMBA can avail of two modes of action:

- **Biological mode of action:**
  A naturally occurring protein (Cox-2) that is produced by body cells as a **reaction to an inflammation** ultimately leads to the **need-based production of the protein that inhibits the inflammation.** To achieve this, researchers place a sequence (Cox-2 promoter) in front of the sequence that is responsible for the production of the anti-inflammatory protein. This first sequence will only be activated if Cox-2 is produced as a reaction to an inflammation. The gene sequence that regulates the expression in this way is called a promoter.
- **Pharmacological mode of action:**
  Another possibility to start the expression of the gene sequence could be active agents, which are given as drugs. For example the antibiotic agent Doxycycline is used in this way to initiate the production of the bone protein BMP-2. The bone protein induces the stem cells to transform into bone cells. A further possibility is a combination which ultimately leads to the production of the cartilage protein TGF-ß. Animal experiments have already shown such a Doxycycline regulation in combination with non-viral gene vectors works in a cartilage defect model (Ueblacker et al. 2004).

  In lab experiments the drug is added to the culture medium for the cells or injected directly into the animal joint. In subsequent animal experiments it is added to the feed. Doxycycline is an antibiotic that is licensed for human use. The doses needed to induce the reaction desired by GAMBA would be significantly lower than the dose needed for an antibiotic effect. So far there has been no decision if and how this active agent would be used in humans.

2.3.2 Physical starting options through heat

A further option to start the activation of the gene vectors could be superparamagnetic iron oxide nanoparticles, which start to resonate in a magnetic field and thus produce warmth. The target DNA sequences are controlled by a heat sensitive promoter (HSP70) that starts the expression and the production of the **cartilage protein** TGF-ß.

For the activation of the protein production it is planned to generate a temperature of 42 degrees to a maximum of 45 degrees Celsius in the target area. A fairly short heat impulse of just a few minutes is sufficient to start the reaction. This is important to prevent possible heat damage to the cells. It is possible to repeat this impulse a couple of times to induce the reaction.

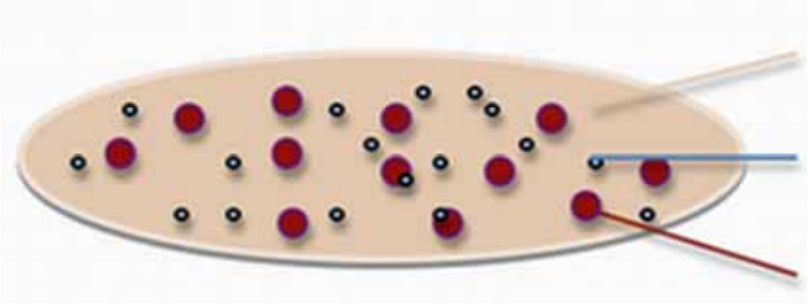
The temperature rise achieved with the help of the iron oxide particles can also create another effect. The hyaluronic acid gel could swell or shrink, depending on temperature. This in turn could lead to the release or binding of gene vectors or stem cells (see hyaluronic acid gel p. 22). It is one of the objectives of GAMBA to develop such a measure-made temperature-dependent gel.

2.4 Potential therapeutic modules (building blocks)

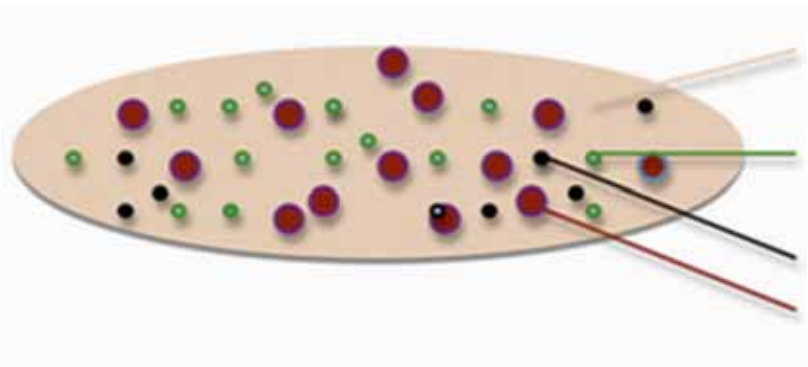
With the selected proteins (anti-inflammatory proteins, cartilage and bone proteins) the GAMBA researchers want to demonstrate that the different control techniques are feasible. To this end, different combinations of matrices with stem cells and gene vectors with the individual start systems will be investigated.

Fig. 8a-c: Therapeutic modules

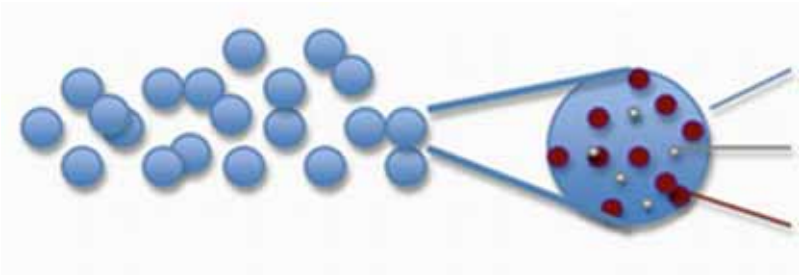
a) An option for an **anti-inflammatory,** active layer would be a hyaluronic acid gel module, which contains stem cells as well as gene vectors that react to inflammations present. This reaction then starts the production of the anti-inflammatory protein.



b) A module composed of hyaluronic acid gels with stem cells and the heat-reactive promoter is a possibility for the **cartilage-building layer**. Warmth induces the expression of the DNA sequence for the cartilage protein. The warmth is generated by superparamagnetic nanoparticles.



c) A **bone producing module** could consist of calcium phosphate beadlets, which contain stem cells and gene vectors for the bone protein in their pores.



Calcium phosphate beadlets

Gene vectors with DNA sequences for protein for the production of bone cells

Stem cells

Diagrams: ScienceDialogue

In the lab such modules can be layered on top of each other to be able to assess the timeframe and spatial distribution of the reactions. It is not yet known what the individual modules will look like, before they have been tested in animal models. Several combinations are possible. Also it is not yet known whether they will be inserted into the joint separately, layered as gels or as a matrix.

2.5 Organisational structure and costs

The research project GAMBA commenced in August 2010 and received funding of 3.2 million euro from the European Union. A total of nine institutes is involved in the GAMBA project which will run for a total of 3 years. Project coordinator Dr. Martina Anton and co-initiator Prof. Christian Plank of the Institute of Experimental Oncology and Therapy Research (Technical University Munich, Germany) have put together a team of international specialists with work groups in Germany, France, Ireland, Italy, the Netherlands and Switzerland. All groups involved contribute their specific expertise to the overall project. Galway has specific expertise in stem cells.

Many of the separate research steps are taken concurrently in several linked-up institutes, but each institute has its own specific emphasis, and contributes its specific and specialist know-how. An overwiew of the human biological material used in GAMBA cann be found on page 8.

The entire project is coordinated from the **Technical University of Munich (TUM).** They also supply the gene vectors. The TUM already has a lot of experience in the development of gene vectors and also has access to biocompatible magnetic nanoparticles. The function and control of the gene vectors is being analysed in cooperation with other partners.

The Swiss **AO Research Institute (ARI), Davos,** develops the thermally sensitive hyaluronic acid gels and optimises them for the embedding of stem cells and gene vectors. ARI will also be involved in potential trials in a large animal model (goat). However, these trials will not take place until GAMBA has shown in the lab and in small animal models (e.g. mice) that it is feasible.

Biomatlante (BIM) in Nantes, France is developing and testing the calcium phosphate granulate (MBCP matrices) for use in GAMBA. In cooperation with the **Institut National de la Santé et de la Récherche Médicale[2] (INSERM)** in Nantes, Biomatlante is aiming to improve MBCP as carrier of gene vectors. INSERM itself focuses on the research of bone production in animal models.

The **OZ Biosciences (OZB)** in Marseille, France is responsible for the packaging of non-viral gene vectors such as liposomes, nanoparticles, polymers and other materials.

The **National University of Ireland (NUI Galway)** focuses on the inhibition of inflammation with the controlled production of the anti-inflammatory protein IL-10. It is here that patients donate stem cells for research. Also, human joints which are no longer required after replacement surgery will be used for so-called ex vivo trials (with permission of the patient).

The Italian **Istituto Nazionale per la ricerca sul cancro[3] (INRC)** in Genoa will be doing research into the localised and timed control of the production of the bone protein BMP-2 (in the lab) and in vivo (in animal models). Like NUI the INRC also works with donated human mesenchymal stem cells.

The **Erasmus Universitair Medisch Centrum Rotterdam (EMC)** researches the effectiveness of the protein TGF-ß on the production of cartilage in joints in culture medium (ex vivo) and in an animal model (in vivo). They are also trying to establish the ideal time window. Pilot trials indicate that a short stimulation with the protein is only effective after the stem cells have been released into the cartilage. The EMC also isolates mesenchymal stem cells from consenting patients.

Science Dialogue (SCID) in Weilheim near Munich is in charge of the coordination and implementation of the citizen and patient panels in Germany, Switzerland and Ireland. They are responsible for the dialogue between scientists and citizens about the opportunities, risks and ethical aspects of GAMBA as a neutral partner and will evaluate the panels. This manual has also been compiled by ScienceDialogue.

[2] National Institute for Health and Medical Research
[3] National Cancer Research Institute

Fig. 9: Overview of the human biological material used in GAMBA

Human biological Material			
Stem Cells	Healthy bone marrow donors after informed consent[4] (NUI Galway) or commercially available	Removal of tissue from patients during surgery after informed consent (NUI Galway, EMC)	Removal from the bone marrow of animals (NUI Galway, TUM, ARI, INRC)
Adenoviral Vectors	Basic structure based on own and others' preliminary work; cloned; commercially available (TUM)		
Non-viral Vectors	DNA-structures based on own and others' preliminary work; cloned; commercially available (TUM)	Lipoplexes/Copolymers: biochemical production, components are commercially available (TUM, OZB)	
Genes	Originally isolated from cell DNA, then multiplied (TUM, NUI Galway)	Synthetic: bases are connected according to information from data bases (commercial)	
Gene switches/ Promoters	Originally isolated from cell DNA, then multiplied (TUM)		
Biopolymers	Biochemical synthesis, components are commercially available (ARI, BIM, INSERM)		

Source: own compilation

More information on the production of DNA sequences and GENE vectors for GAMBA can be found in **Chapter 4.2.4** of the **Compendium.**

[4] See Chapter 5.5.1

Concepts like absolute certainty, absolute accuracy, ultimate truth and so forth, are figments of the imagination and have no place in science.

Max Born

3. Opportunities of GAMBA

The vision for GAMBA is to find new methods and agents that will make a cure for osteoarthritis possible or at least a significant alleviation of the disease. This could lead to a lower number of surgeries and would significantly delay the necessity of joint replacement. The therapy should be limited in terms of time and location to avoid possible negative effects (see Risks p. 34ff).

3.1 A basic research project

Because GAMBA is a basic research project, it is very possible that individual results may well be different from what is anticipated. It is indeed possible that the three years of GAMBA research will not result in a defined product that will be usable in the near future. However, as a basic research project, it will help to establish new and better definitions of tools, which in turn could be the basis for the development of future therapies. Even if the actual aims can't be achieved, a failure would provide valuable new insights for basic research.

Two points are critical for GAMBA:

- To achieve a localised and time-limited healing process.
- To combine the components stem cells, gene vectors, growth factors, biomaterials and nanoparticles in new and experimental ways. In principle, all possible combinations of the various components could be an option. The vectors could be viral or non-viral, they could carry the gene freight for one of the three proteins or the gene expression could be controlled with a chemical or thermal reaction (see p. 17ff).

3.2 Tested components of GAMBA

Many of the components used for GAMBA have already been tested in the lab, either individually or in initial combinations; therefore there is a good chance of success in individual areas.

a) Several past trials with **mesenchymal stem cells** provide reason for hope.

- In goats with meniscus damage a therapy with stem cells induced a healing process of the meniscus and slowed down the progress of osteoarthritis (Murphy 2003).
- In clinical trials with 24 osteoarthritis patients, 12 of whom were given a placebo drug, a therapy with autologous stem cells resulted in no significant improvement of the clinical symptoms compared to the control group. However, according to the findings of arthroscopies and tissue analyses, there was an improvement (Wakitani et al. 2002).
- Another study shows that stem cells can not only specialise, but are also able to interact with the area surrounding them leading to an increased production of healing proteins in the case of an inflammation. (Coleman et al. 2010).

Through various lab trials and trials in animal models, GAMBA will make an important contribution to a better evaluation of the healing potential of stem cells.

b) The **bone and cartilage proteins** (BMP- 2 and TGF-ß) have been tested in numerous studies.

- Detailed research into the bone growth in fingers, has confirmed the central role of the relevant protein (Witte et al. 2010).
- The bone protein (BMP-2) is already commonly used in the treatment of osteoporosis.
- Different gene vectors (adenoviruses, plasmids and liposomes) used to package DNA sequences that produce the healing proteins (BMP-2), have been tested in numerous animal models. In small animals the bone healing results were good, in larger animals the results were mixed (Evans et al. 2009).
- In the case of cartilage healing there have also been trials with rats and rabbits, which tested the DNA sequences for the healing proteins (BMP-2 and TGF-ß) in various gene vectors. In these trials it proved to be beneficial to carry out the gene therapy treatment of the autologous cartilage or stem cells before they are introduced into the joint (Evans et al. 2009).
- Other trials have also shown potential negative results. An overdose of the bone protein (BMP-2) could result in an unwanted ossification (see Risks p. 39). However, during these trials the gene expression was not regulated – regulation is an explicit aim of GAMBA.

GAMBA's aim is to find an exact definition of how much of which protein is needed over what time frame, to achieve the desired healing effect with stem cells that change into cartilage and bone cells.

c) The **basic structures for tissue engineering** have also been tested in numerous studies over a long time or are already licensed.

- A gel mix consisting of hyaluronic acid gel, autologous cartilage cells and supporting agents that can be injected into joints, has achieved good results in mice and bovine models (Pereira et al. 2009).
- The calcium phosphate matrix is already being tested in combination with stem cells (Cordonnier et al. 2010) or proteins (Sohier et al. 2009).

Through further research and new combinations GAMBA will also contribute to the advancement of Tissue Engineering.

d) Some of the **gene vectors** used for GAMBA have been used in clinical trials for quite some time. But the new ways of packaging, their precise effectiveness, as well as the matrix they are incorporated into, could have a very decisive influence on the activity of the vectors.

GAMBA is also expected to result in crucial indicators for promising combinations of gene vectors, stem cells and matrices, which could under certain circumstances prove to be useful for other therapeutic areas.

3.3 Possible results of GAMBA

A specific aim of the project is the definition of new gene vectors. These non-viral and adenoviral vectors could open up new paths to transport DNA sequences precisely into the target cells. Tests are being carried out, to see whether the DNA sequences really lead to the production of the desired proteins. Additionally, there will be tests to see whether the gene vectors really react to body signals, as in the case of an inflammation.

Furthermore, it is planned to develop new hyaluron-hydrogels, which change their properties depending on the temperature. Another specific aim of GAMBA is a composite of several layers of calcium phosphate granulates that resemble biological structures.

3.4 Possible medium term innovations and long-term results

In the medium term it is expected that new gene vectors will be employed. Furthermore, innovative, mutually supporting basic mixes of stem cells, biomaterials and gene vectors are a possible result. Another possibility is a localised and timed control of the production of cartilage and bone with the help of gene-activated stem cells. Ideally, the reconstruction of the joint would not only be induced but could also be slowed down again, depending on the state of cartilage and bone, to prevent excessive growth (see Risks p. 39).

Findings from GAMBA could also be used for other bone diseases, such as osteoporosis or for the improvement of tooth implants. They could also lead to new methods of healing wounds and tendonitis.

Halfway through the project and again at the end, the partners involved in the GAMBA project will compile suggestions on the areas that the GAMBA findings could be used in future.

3.5 Possible follow-up research in the form of preclinical and clinical studies

Gene therapy is seen as a medical treatment with gene transfer drugs and is therefore subject to regulation by the Irish Medicines Board. Therefore all new drugs have to be tested successfully in clinical trials to determine efficacy (BBAW 2008). First, there are preclinical trials (lab and animal models) and clinical trials (with human volunteers).

Long before a new substance or a new method is tested in humans, their physical and chemical properties are determined in the lab and in animal models (preclinical research). Scientists evaluate the mechanism and deliberate on possible dosage and on compatibility.

After a successful conclusion of GAMBA further preclinical trials would be necessary which could be carried out by the institutes involved, i.e. ARI, NUI, EMC, INSERM and TUM (see organizational structure p. 26f).

The aim of the preclinical drug trials is to confirm the efficacy of new substances and to rule out undesirable side effects on humans as far as possible. This means efficacy and compatibility of the substances should be tested. Researchers are also looking for a possible dosage. After the conclusion of GAMBA there could possibly be trials to establish how much the gene vectors and the proteins produced spread beyond the joint.

Finally, before any clinical application, the quality of the individual components needs to be established, by drawing up Good Manufacturing Practice (GMP) protocols and guidelines that describe in detail how their safe use and implementation can be guaranteed. Only once the safety of all of these components has been established (see Clinical Trials, Compendium Chapter 5.5.1) can clinical trials with human volunteers commence. The permission is granted by a national authority. In Germany this falls into the remit of the Paul-Ehrlich-Institute (PEI) (see Law, Compendium Chapter 6). In Ireland, the Irish Medicines Board fulfils this function.

Fig. 10: Possible further developments after the conclusion of GAMBA

GAMBA	preclinical trials	drug trials		GMP	clinical trials	new therapies
2009 – 2013	2016		2018	2020	2025	**2030?**

Table: ScienceDialogue

3.6. Gene therapy of the joint in clinical trials

Gene-therapeutic clinical trials with patients with rheumatoid arthritis with the aim to produce proteins that counteract inflammation have been ongoing since the 1990s (see Chapter 5.5). So far, there have been no clinical gene therapy trials dealing with osteoarthritis.

- The first clinical gene therapy trials commenced in the US in 1995 with a gene for the protein IL-1Ra packaged in a so-called retrovirus (see gene vectors, Compendium Chapter 4.5.4) to treat rheumatoid arthritis of the metacarpal bone joint in 9 patients (Wiley 2010). Interleukin 1 is seen as an important factor in the development of inflammation, pain and cartilage degeneration. After one week, artificial joints were implanted as planned and the treated joints could be removed and examined. However, no effect could be seen (Evans et al. 1996).
- A German-American team used the same combination in 1997, but extended the time-frame to four weeks. They modified the autologous connective tissue cells taken from the synovial fluid of the patients, treated them gene therapeutically and then injected them into the metacarpal joints of two patients. An examination of the lining membrane which was removed from both patients after four week confirmed that there had been an increased production of the healing protein. One of the two patients also reported less pain and swelling during the four weeks (Wehling 2009).
- A second approach, that is still being pursued, aims to block the errant inflammatory reaction in the joints, this time with a transmitter of inflammation signals (tumor necrosis factor receptor) using adeno-associated viruses as gene vectors (see gene vectors, Compendium Chapter 4.5.2). However, in July 2007 there was a fatality during one of these trials; a 36 –year-old woman died (see Chronicle of gene therapy, Compendium Chapter 4.7). Due to insufficient data, a causal link with the trial could not be ruled out. Presumably a mycotic infection was the cause of death. The trial with 127 patients was continued the same year.

- There have been two more trials, taking a similar approach to GAMBA, i.e. trying to increase the production of the cartilage protein TGF- ß, the gene vector used was a retrovirus. However, so far no results are available (Evans et al. 2008).

All these trials are phase I clinical trials (see clinical trials, Compendium Chapter 4.6.1) and are therefore a long way from a possible application.

All research is built on an ethics of uncertainty.

King & Cohen-Haguenauer 2008, S. 437

4. Risks of the technologies used

GAMBA is a new approach in research with the vision to help osteoarthritis patients by improving their symptoms or healing them. The aim is to achieve this with a time and location controlled therapy of the diseased joints. This will not be achieved during the term of the EU project GAMBA, as the project will end at a stage well before clinical trials with patients. However - as the individual research approaches of GAMBA have the potential to reach the phase of human trials and because these trials will involve gene therapy, nanoparticles, growth factors and stem cells - it is important to evaluate and discuss potential risks in the run-up.

4.1 General risk factors of innovative therapeutics

All medications and surgical treatments carry an inherent risk. Package leaflets and information sheets for surgery draw attention to all known side effects. Accordingly, negative effects are to be expected with all kinds of new approaches to personalised medicine. These new methods of personalised medicine usually require a removal of autologous cells or tissues, their cultivation in the laboratory or the extraction of specific proteins from these cells and subsequently the re-administration of the isolated or modified material or tissue. For these steps there are rules to be adhered to: “Advanced Therapy Medicinal Products (ATMPs) are a heterogeneous group of medicinal products, which can include gene and cell therapeutics as well as engineered cells and tissues. The innovative therapy approaches are often based on very recent research results. This means however, that there is a general lack of knowledge about the risks that are associated with using these medicinal products on or in patients” (Klug et al. 2010, p. 58).

One of the main safety goals is to prevent the transmission of pathogens. Therefore, the labs and all materials used for the culture of, for example, stem cells are subject to a very specific code of hygiene. Culture media, which are used to grow the cells, have to be tested thoroughly and their safety has to be proven. Furthermore, all source and raw material, including cells and tissues, have to be traceable to their source and clearly attributable on their own and in combination with patient data. Additionally there are special recommendations for the clinical observation of patients, who participate in clinical trials, such as those with gene therapeutics: a long period of follow-up observation is deemed very important to be able to recognise possible delayed undesired effects (s. Chapter Ethics, Patient data protection p. 45f).

Often side effects of therapeutics are caused by an undesirable distribution in the body, the so-called biodistribution. GAMBA aims to limit this distribution in the body as much as possible. It is planned to introduce the therapeutics exclusively into the joints which are seen as relatively closed systems: the cartilage has no blood supply and the cartilage cells obtain their nutrients from the synovial fluid. However, no system within the body is completely closed. Even the almost impenetrable blood-brain barrier can be passed by nanoparticles. Even bones

which are, in the true sense of the word, ossified and rigid systems do communicate with the body. A study has shown indications that the bone and organ metabolism are interconnected, the substance of some bone components such as osteocalcin can influence the insulin balance and vice versa (Katsnelson 2010).

4.2 Risk factors of the therapeutics used

As described above, the GAMBA approach minimises the spatial risk by introducing the substances directly into the joint. This is complemented by GAMBA's special approach which also relies on a time limit for the activity of the therapeutics. However, the fact that GAMBA is relying on a mix of several new procedures which have potential interactions, could increase the risk and also poses difficulties for the determination of dosage. In the following sections we outline the specific risk factors of the different approaches.

4.2.1 Risk factors of gene therapeutics

As shown in the Chronicle of gene therapies (see Compendium Chapter 4.7) there is a small margin between hope and tragedy. One weakness of gene therapy is the vector technology, i.e. the packaging and transport medium for the therapeutic gene sequences: Which carrier transports the DNA sequences into the cells? Will these gene vectors themselves leave traces in the cells? Do they penetrate the nucleus und will the new DNA sequences be integrated into the genome and, if yes, in which location (see Gene vectors, Compendium 4.2)?

"What distinguishes the risks of somatic gene therapy trials from those for conventional drugs is not so much the level of risk … but rather their level of complexity and of uncertainty.", said the bio-ethicist Jonathan Kimmelman (Kimmelman 2008, p. 239).

Fig. 11: Possible side effects of gene therapy

	Side effect	Definition	Possible result
1	”Insertional mutagenesis”	Integration of the therapeutic gene in unfavourable locations + malignant cell degeneration	Risk of cancer
2	Pathological cell mutation of the target cells	Defence reaction of the cells against the therapeutic gene	Risk of cancer
3	Unwanted integration of the therapeutic gene into the genome	The therapeutic DNA sequence is passed on to all daughter cells	Permanent production of the proteins
4	Overproduction of the gene product	Too many proteins at once (overdose)	Overload of the immune system, cancer
5	Undesired immune response	Body reacts defensively to the foreign matter	Inflammation, failure of the immune system
6	Infections through viral gene vectors	Not all disease causing parts of the virus were removed	Disease that the virus “normally”, transmits
7	Reactivation of existing viruses	Through contact with other viruses gene vectors turn into disease triggers	Disease that the virus ”normally”, transmits
8	Dispersion of the gene vectors in the body	Undesired distribution of the therapeutic DNA sequences in the body	Could disrupt cell communication; undesired generation of the gene product in other locations
9	Integration into the genetic makeup of egg and sperm cells	Gene vector is integrated into reproductive cells	Possible transmission to offspring
10	”Interferences”	Drug interactions	Interactions with other substances
11	Disruption of the protein balance	Increased production of a protein that has several functions within the cell	Disruption of cell communication
12	Therapeutic gene is incomplete	Segments with unknown functions are missing	Disruption of cell communication
13	Virus attacks immune cells	Scavenger cells are infected	Weakening / Failure of the immune system (Gelsinger death)

Table: own compilation

As listed in the table above the following undesirable side effects are conceivable when gene therapeutics are used (based on Klug et al. 2010, Table 1 p. 63):

1. The cells can degenerate due to the incorporation of the therapeutic DNA sequence at an unfavourable location in the genome (**Insertional mutagenesis**). This can cause cancer. For example this undesirable effect was the reason that some children suffering from the immunodeficiency disease SCID-X1, who were treated with gene therapy, later developed leukaemia (Fehse 2008). However, in the case of the hereditary disease SCID an insertion of the therapeutic DNA sequence into the target genome is desirable to achieve a long-term therapeutic success. Unfortunately, in some cases, this insertion has caused a malignant change of the cells. Up to now, there is no universally recognised system which could be used to exactly assess the risk for an insertional mutagenesis (Kimmelman 2008).
2. It is also possible that the therapeutic gene used could cause negative effects such as **pathological cell changes** or even cancer, no matter where it might be incorporated into the genome, or at all. It is suspected that this negative effect was an additional factor in the case of the SCID children (DFG 2006).
3. An incorporation of the therapeutic gene can also lead to an **undesirable long-term production** of the gene product.
4. A **potential overproduction** of the gene product can also not be ruled out. This could cause undesirable side effects such as autoimmune reactions (i.e. the immune system attacks the own body) or cancer.
5. Usually the body reacts to a foreign substance with a defence reaction. Consequently, the gene therapeutics have the potential to cause an **unwanted immune response** to the newly introduced biological substances. Negative interactions with other substances are also possible. The widely publicised fatality during a gene therapy trial, the death of Jesse Gelsinger in 1999, was due to a very strong immune reaction to an excessively high dose of adenoviruses used as gene vector (see Chronicle of Gene Therapy, Chapter 4.6).
6. **Infections with viral vectors** are also a potential risk. This risk should be eliminated as far as possible by removing the virus genome before it is used as a gene vector.
7. Also a virus could be **reactivated by another virus** and could then proliferate. This could lead to an infection and/or an unintended proliferation of the therapeutic product or even an undesirable spread in the body.
8. A potential **spreading of the gene vector** in the body can also not be ruled out – as was shown in investigations with some gene vectors in animal models (Gonin & Gaillard 2004). This means that an undesirable production of the gene product in tissues and organs, which are not the target of the therapy, is possible in principle. However, the choice of gene vector plays a very important role.

9. A **possible integration** of the therapy genome into the **genotype** of egg or sperm cells cannot be completely ruled out. During a clinical trial in which haemophiliacs were given gene therapeutic treatment with an adeno-associated virus, traces of the DNA were temporarily found in the sperm cells (Manno et al. 2006). In animal experiments, some adeno-associated viruses that were administered to the prostate could be detected in the gonads and the epididymis, a tightly coiled tube in the testis where sperm may be stored and mature, but not in the sperm cells as such (Gonin & Gaillard 2004).
10. **"Interferences"**: So-called drug interactions occur with almost all drugs, but so far in the case of gene therapeutics there has been little research into these interactions. When several vectors are used successively there is a possibility of an immune reaction to the proteins or to other substances (see also point 5, above) (BBAW 2008, p. 71).
11. **Disruption of the protein balance**: When an additional gene is incorporated into the cell as done with GAMBA, "the defective as well as the healthy gene are active in the cell. This leads to the production of the defective and of the healthy protein" (Simon 2004, p. 8). This can lead to a disruption of cell communication.
12. **The therapeutic gene is incomplete**: A gene is made up of several DNA sequences, the functions of which are not all known. When incomplete genes are introduced (in the belief that "superfluous" sections can be omitted) this can have an impact on the functionality of the gene (Simon 2004 p. 9).
13. **Virus attacks immune cells**: Viruses are specialised in certain cell types. If the virus finds its special cell type in animal trials, but it is missing in subsequent human trials, the virus can end up attacking the immune system instead – as has happened in the death of Jesse Gelsinger in 1999 (see History of Gene therapy research, Compendium Chapter 4.6) (Simon 2004, p.10).

All of these and further risks of therapeutics have to be investigated in pharmacological and toxicological studies, in the test tube with cells and in animal experiments, before they can be tested in clinical trials with patients. Between 1989 and June 2010 more than 1640 clinical gene therapy trials have been conducted worldwide (Wiley 2010). More than 6000 patients were treated up to the end of 2009 (VfA 2009).

Risk minimisation strategies in GAMBA

To eliminate known risks from the very beginnining, the GAMBA project uses only non-viral and adenoviral gene vectors which do not integrate their gene freight into the genome of the target cells (see gene vectors, Compendium Chapter 4.2ff). That means that they penetrate the cell and are present in the nucleus, but their genome is not integrated into the chromosomes of the cells. Therefore, due to constant cell division, the number of cells with the gene vector halves each time. This is a kind of timer which prevents a long-term production of the gene product, which means a significant reduction of the cancer risk mentioned above.

However, it cannot be ruled out completely that the therapeutic DNA sequences might be integrated into the chromosomes of the cells. This also applies to the therapeutic genes used in the GAMBA project, which are transported into the nucleus with the help of non-viral and adenoviral gene vectors. For example genes from non-viral vectors, such as plasmids (small circular DNA molecules that can replicate independently) could integrate into one of 150,000 cells (Ledwith et al. 2000). However, in the case of adenoviral vectors scientists assume that

the integration rate will be lower (Stephen et al. 2010). Such an integration of a foreign genome normally has no consequences, but under certain circumstances it may lead to changes that turn the cells into cancer cells.

With regard to possible immune reactions of the body, the choice of gene vector also plays a decisive role. The adenoviral vectors used in GAMBA may well cause an immune reaction, as most people have built up a good immune defence against these classical cold viruses, with antibodies and macrophages that destroy these viruses. Therefore, the adenoviral vector will be protected with a kind of magic cloak. Alternatively a non-viral vector, a so-called plasmid, which is also surrounded by a protective layer, will be used. This vector causes very few immune reactions.

Furthermore, the number of affected target cells is limited within the relatively isolated joint, which decreases the risk of a concentration in other organs in principle. Preliminary trials conducted at the Technical University of Munich with non-viral gene vectors which are supposed to stimulate the bone to produce more growth factor BMP-2, have shown that neither the vectors nor the proteins produced could be detected outside the implant.

4.2.2 Risks of proteins as growth factors

With the help of so-called growth factors – proteins that function as signal molecules and stimulate the cells to act in a certain way – the stem cells used are meant to specialise further. Bone proteins are supposed to stimulate the production of bone cells, cartilage proteins the production of cartilage cells.

In this context, the determination of the right dose is important to ensure that the proteins take effect in the desired target area (bone/cartilage) and do not stray from the target area within the joint as this could lead to harmful side effects.

It has been shown that high doses of the bone protein (BMP-2) could potentially lead to **undesirable ossification** of the cartilage and can even be involved in the formation of painful lateral ossification of the joints of osteoarthritis patients (Williams 2008). Also studies of animals with a high level of the cartilage protein (TGF-ß) in their bloodstream, show that they have **less elastic** and less hard **bones** with a low calcium phosphate content (O'Brien 2005).

Bearing in mind that growth factors are naturally involved in many different signal processes in the body, it is of special importance to ensure that they spread as little as possible within the body (biodistribution). The cartilage and bone proteins used in GAMBA appear to be involved in many different growth, tissue healing and development processes (Johnsen 2009 and Koesters et al. 2010).

4.2.3 Risk factors of stem cells

Great hope is attached to the research of so-called adult (i.e. non-embryonic) stem cells, which can be taken from the body and which can have a healing effect in other parts of the body. GAMBA relies on mesenchymal stem cells (see p. 17f) taken from the bone marrow. Unfortunately, there has been little research into the risks attached to these cells. This means GAMBA operates on the edge of current knowledge.

Damage to health through side effects can't be ruled out was the warning of diabetes experts concerning a therapy with adult stem cells. Among other things, there are certain **cancer risks**. The developmental biologist Lewis Wolpert wrote: "Verifiably stem cells are the driving force in a tumor and stem cells are often the original source of a tumor" (Wolpert 2009, p. 193). A possible reason: tumor forming cells have certain characteristics in common with stem cells, such as an unlimited life span and the ability to specialise into various different cell types. They are therefore regarded as cancer stem cells. They are most likely derived from disregulated damaged stem cells or from their direct descendants (Clarke & Becker 2007).

During a study at the Autonomous University of Madrid, stem cells were cultured for eight months before they were injected into mice. During this time they divided up to 140 times. It was shown that the oldest cells did in fact cause cancer (o.V. 2008). At the same time stem cells which were only outside of the body for a short time and therefore had only undergone a limited number of divisions in the lab seemed to be safe.

A fast and effective processing of the stem cells is also advisable because they might specialize much too quickly into cartilage and bone cells (Stoddart et al. 2009) and thus become unusable for the planned therapy. Researchers are already looking into solutions for this problem, e.g. slowing the specialisation with the help of a protein (University of Rochester Medical Center 2010) or speeding up the extraction and isolation of stem cells after taking them from the bone marrow (Hebrew University of Jerusalem 2009).

Some potential risks are by and large limited due to the localised administration within the relatively closed "joint system" (see gene therapy risks above). Furthermore, the mesenchymal stem cells used in GAMBA are meant to initially stay within their matrix and to change into the desired target cells – cartilage and bone cells – within the matrix. This means they are then no longer stem cells. This could eliminate one of the greatest risks, i.e. spreading stem cells that change into cancer cells and damage neighbouring organs or even the brain (Yoffe 2010).

However, the so-called homing of stem cells – after microfracturing of bone, precursor cells automatically migrate to the injury location to start the healing process (Mao 2010 and Zittlau 2010) – shows that an exchange between bone marrow and the inside of the joint is possible. This highlights a further potential risk of mesenchymal stem cells: studies have shown that they interact with the blood forming stem cells (hematopoetic stem cells) in the bone marrow (Mendez-Ferrer et al. 2010, Miyoshi & Stappenbeck 2009).

4.3. Risk factors of nanoparticles

Nanoparticles are not a modern invention of the last 10 or 20 years. Miniscule particles with a diameter of several hundred nanometres or less are released in all combustion processes and also develop naturally through various processes (Krug & Wick 2011). Most knowledge of nanoparticles is derived from studies on particulate matter (air particles which are smaller than 100 nanometres) and technically produced nanoparticles, such as nano-impregnation sprays, and nanoparticles used in sun screens and tooth pastes. Biologists and toxicologists call those particles "nano" which can take various, not always defined ways, into organisms. This means that, for an assessment of potential risks, nanoparticles smaller than 250 nanometers are relevant (Krug & Wick 2011). We only speak of nanotechnology when these particles

are specifically produced.

Because nanoparticles are so tiny they can easily enter the body through the skin, lungs or the gastro-intestinal tract and can then spread through the blood stream or the lymphatic system. The consequences of a possible absorption in one or the other way are all controversial (Thorbrietz et al. 2008). It is known, for example, that nanoparticles in biological liquids surround themselves with a whole range of biopolymers, specifically proteins. This protein corona with the incorporated nanoparticles then influences the exchange with other proteins in the surrounding area. For a toxicological assessment the size and the surface characteristics and the properties of the material of the nanoparticles play an important role (Krug & Wick 2011). However, nanotoxicologists criticise "the fact, that there are no sufficiently standardised methods, which are suitable to understand the biological effects of nanomaterials" (Krug & Wick 2011). This must be changed and nanomaterials should also be tested on a case by case basis, like chemicals.

Previous studies on the health implications of nanoparticles or materials were mainly focused on inflammatory reactions in the lungs or the crossing of tissue barriers (such as the blood-brain barrier), and also on the possible toxic effects of the metals, organic substances or carbon tubes. In this context, GAMBA staff in the lab could be potentially contaminated with nanoparticles through the skin or the lungs. However, this could only occur with improper handling. The laboratories have to be equipped with an appropriate air filtration system.

In the therapy GAMBA strives for, synthetic iron oxide nanoparticles would be injected into the affected joint only. Similar iron oxide particles are routinely used for MRI scans. Furthermore, the particles used will be biodegradable.

Nevertheless, there is still a possibility that the nanoparticles will spread in the body and could accumulate in certain organs, such as the liver. The mobility of nanoparticles was shown in a study which aimed to keep as many magnetic nanoparticles within a tumor, to kill the tumor through heat. In some tumors almost all particles were still present after a period of 24 hours, in others only three quarters of the injected nanoparticles could still be detected. (Richter et al. 2010). That means they spread in the body or were excreted.

Research in gene therapy needs more justification than other areas, because this research centres on man himself and this makes profound changes in the idea of man and the nature of man seem possible.

Voß 2010, p. 42

5. Ethical aspects of the GAMBA topics

5.1 Ethics, what's this? What role does it play in this project?

Ethics strives to find out what "good life" is (according to Socrates). Ethics will ask questions about a topic and show the full spectrum of all aspects that need to be considered. Furthermore, applied ethics tries to give guidance after considering all arguments. Ethics is also looking for principles and criteria that have the potential to evaluate the legitimacy of a decision (Rehmann-Sutter 2003, p. 16) or at least help to come to a better evaluation.

In practice there is a differentiation between ethics and morals. Morals are a collection of norms and rules which guide actions. Ethics is the (scientific) reflection on these morals. There are always morals; however they are not always useful for the solution of moral problems. The task of ethics is to illuminate the different aspects (Manzeschke 2011).

The main problem of all ethical considerations is the pressure of the „force of the factual"[5]: How can a reasonable handling of the development of gene therapy and stem cell research be achieved, when all pondering on the topic has already been overtaken by real developments? It would be desirable to have members of society agree to a development before it becomes a fact. One solution would be, to start the assessment as early as the basic research. Starting the social discussion process early is part of this project.

The aim of the GAMBA dialogues (Patient and Citizen Panels) is that the participants, representatives of the general public, read this manual and listen to various experts in the panels to gather information. Then, after intensive discussions with each other and the experts, they will collect the main arguments, discuss and evaluate them. Finally they will draft recommendations. The information contained in this Ethics chapter is also part of this process.

5.2. The Medical Principles[6]

The principles of biomedical ethics were put up in 1994 by Tom L. Beauchamp and James F. Childress and have been widely used in medical ethics ever since. However, the application of these principles in specific individual cases can result in contradictions (see below).

Self-determination/autonomy: This means individual freedom from external constraints and manipulative exertion of influence, but also the promotion of the decision-making ability of the patient. Therefore patients must have been given extensive information about the opportunities and risks, must have understood the information and must be in a position to make a competent decision. Any therapeutic measure must be legitimised by express consent of the

[5] Mieth (2003) even calls it the „force of the fictitious": Even though there are only three licensed gene therapeutic medical devices so far, there has been talk of gene THERAPY for the last 30 years, even though this „only applies to a collection of research projects and genetic experiments" (Mieth 2003, S. 36).

[6] comp. Marckmann 2000.

patient ("informed consent"). The critical factor is the measure of information: Who defines what level of patient information is adequate? In the field of gene therapy, where even doctors are not aware of all consequences and risks, this is a particularly difficult question (for more on "informed consent" see Chapter 5.5).

Damage prevention (nonmaleficence): At first sight, this means the obvious principle that a doctor should not harm his patients. Injuries, strains and risks caused by medical attention should be avoided. However, the risks of new therapy approaches are often not yet fully understood and consequently there are many uncertainties here. This principle must be weighed against the third principle, the principle of benevolence.

Benevolence: On the one hand this is about a fundamentally benevolent attitude of the doctor towards his patient, but also about the individual gain of the patient. The therapy should be suited to the natural course of the disease and potentially beneficial alternative therapies have to be considered (see Fuchs 2011). Because medication and therapies can cause undesirable side effects, the benefit for the patient should always outweigh any possible damage.

Justice: The distribution of benefit and burden between different persons is the important principle here; in the case of gene therapy we are also talking about the balance between the insights gained, which will benefit future patients (but not the trial participant), and the risk that the patient is taking.

This **gene therapy example** will try to clarify the weighting of benevolence and damage prevention: The immunodeficiency disease X-SCID (ADA-SCID) forces patients to live in a completely sterile environment, like in a bubble. 20 children living with this disease were gene therapeutically treated; in 16 the therapy showed very good results. However, some years later four of the children developed leukaemia as a result of the therapy. Three of these were successfully treated, one child died. 10 years later 18 of the little patients are still alive (Sheridan 2011, p. 121). Should we now treat all SCID children because this means a chance of a normal life or is this morally unacceptable, because the risk of cancer is too high (see also Chronicle of gene therapy, Compendium Chapter 4.7)?

5.3 Societal assumptions of new therapies

For the evaluation of the GAMBA topics it is helpful to recall the assumptions which form the bases of molecular and cell therapies. On the one hand there is the "central genetic dogma" (Francis Crick, 1966). It is based on the assumptions that

- Genes are sophisticated chemical programmes that control all life processes;
- The entire information of a gene is coded in the base sequence; and
- There is a hierarchical relationship between genes and the organism, i.e. information always flows in one direction, from the DNA to the RNA to the protein (Graumann 2000, p. 45f., see also Compendium, Chapter 2 "The basic modules of life").

Many biologists and biochemists have adopted this "genetic dogma" as the basis of their work. This concept is also called "**Programme Genomics**" (Rehmann-Sutter 2010, p.33) or "**biomedical model**": Symptoms (or a disease) could be clearly treated with a causal therapy. The treatment success justifies the intervention. Critics accuse this view of Reductionism: "Gene therapy continues the problematic trend of reducing man to his biological components and therefore must be refused" (cited in Schmidt 1995, p.227).

In contrast the "**System Genomics**" (Rehmann-Sutter 2010, p.33) are based on an interaction between the DNA and the cellular components. Instead of: a DNA sequenced (gene) ALWAYS results in a specific protein, it is: a DNA sequence develops into different proteins, depending on need and the conditions in the cellular environment. Thus "an individual gene can be the template for the production of several proteins" (Müller 2003, p. 42). Traits of people, but also of diseases are therefore "not controlled by individual genes but by a network of hundreds of genes. Even subtle changes of an individual gene can lead to a highly sensitive reaction of the gene systems" (Bahnsen 2008, see also "Epigenetics", Compendium Chapter 2.2).

According to Systems Genomics the case history of a patient shouldn't be reduced to a single factor, i.e. the biochemical defect. Also quite often results could not be explained by a single cause, but were the result of a complex fabric of interactions and several causes. Gene therapy, in the view of the critics, would be a further step on the way to a "technology-driven" health care system. The 'blindness' towards psychosomatic, social and environmental factors for the development of disease would be continued (cited in Schmidt 1995, p. 228).

5.4 Conflicting views of mankind

As seen already in the case of societal assumptions, the **scientific view of mankind** stands in contrast to the **humanistic view**. The first sees the human body as a "biochemical large-scale reactor" and has the aim to perfect the imperfect human, to shape humans in a perfect manner. The second sees man as an imperfect being; it is part of being human to acknowledge this imperfection, including ones fragility and unfinished state (Manzeschke 2011). "The sensitivity of man is inconceivable without his ability to endure suffering" (Mieth 2004, p. 41).

According to the critics of the scientific view of mankind, the experimental research process reduces man to a sum of his components; there is a tendency to concentrate on increasingly smaller, seemingly fundamental processes and units. However, it would be important to see how the separation into individual processes, made for good reasons, is taken into consideration in the intellectual reconstruction of the disease (see Kollek 2004, p. 32). That means that certain aspects of a disease can be meaningfully explained by limiting it to a few components; but if one tries to reverse the principle and tries to "reassemble" man "in his entirety" from these components, this automatically results in the picture of a complicated machine, that consists of billions of tiny "machines" (the cells). But is the whole not more than the sum of its parts?

Viewed historically, from antiquity to the age of enlightenment (beginning with Kant in the 18th century) the term "bios" (Greek for "life") was synonymous with the art of living your life. The focus on the biological processes only started later and aims at an improvement of the conditions of living. This raises the question of the impact assessment: do the "improvements" not also entail changes that are no real improvements (see Mieth 2003, p. 35f.)?

The arguments for both views of mankind are demonstrated by the examples "Disease" and "Ageing /Death":

Fig. 12: Ethics: Arguments for different views of mankind

Example	Scientific view of mankind	Humanistic view of mankind
Diseases	**Man in need of repair** There are already artificial limbs There is intensive research into artificial inner organs Defective gene: Exchange / supplementation to overcome the malfunction	**Natural Regeneration** Disease is a part of life Man as a system, everything is connected Making some adjustment is not beneficial enough and could have negative effects Psycho-social factors play an important role
Age/Death	**Prolongation of life** A longer life is generally desirable Hope "Fountain of Youth": Aging is delayed through technical and medical support	**Natural Aging** Aging and death are part of the circle of life and are intrinsic to humanity When we accept ageing and death our life becomes worth living

Source: own compilation

5.5. Patient safety: Informed consent

The informed consent of patients is supposed to protect the patient and serves the autonomy of the patient (see Ethics, Chapter 5.2). It is required for the donation of human biological material from the human body as well as for the participation in clinical trials. Consent may only be given after prior information in writing and in a personal conversation with the doctor.

5.5.1 Donations of human biological material

GAMBA uses different so-called human biological materials, as listed in table 9 in Chapter 2.5. Mesenchymal **stem cells** were donated by patients in Ireland and the Netherlands or are sourced from animals; the genes and promoters which are incorporated into the vectors, are also derived from donated cells. The adenoviruses (cold viruses) and the DNA structures for the non-viral vectors are commercially available.

When researchers want to use human biological material, e.g. bone marrow (which can be used to isolate stem cells), the donors have to give their express consent. They are given a leaflet that explains to them that the donated material will be used for experiments in the lab. The information leaflet used by NUI Galway, contains the following information (Murphy & Barry, undated):

- Why the human biological material is needed (e.g. to better understand the causes of a disease and to develop suitable therapies)
- What is taken exactly (e.g. bone marrow, blood, parts of a replaced joint)
- The risks and opportunities as well as what complications are possible

- How the material is to be stored and whether genetic data will be stored or whether the donation will be anonymous
- How many donors are needed, how long the sampling will take and how it proceeds
- Whether the donor is paid
- That participation is completely voluntary at all time
- Who the contact person is for questions or in the case of complications.

Ethical aspects of the use of human biological material are down to data protection which is in contrast to the need of the researchers to know as much as possible about the donor (for example to be able to establish why some cells or tissues react well to the new therapies and others don't. Donors are differentiated into "good" and "bad" donors). From the point of view of the donor, the data should not be traceable to avoid discrimination. Also, at the time of the donation, the eventual purpose for which the donation will be used is not always clear. It is possible that perhaps the individual donor would later no longer be ready to give their consent for a certain purpose.

5.5.2. Informed consent to clinical trials

Before any medical product is licensed so-called clinical trials have to be conducted (see Compendium Chapter 4.5). This also applies to so-called gene transfer drugs. In Germany 79 gene transfer trials have been conducted up to now; one trial is ongoing in Ireland (Wiley, undated). Clinical trials have to be approved by ethics committees (see Chapter 7 on Ethics in the Compendium).

In contrast to the sampling of human biological samples, the requirements for the **informed consent** to be given for the participation in clinical trials of gene therapeutics are considerably more comprehensive and there is clear legal regulation (see Chapter 6 of the Compendium). In Ireland the Irish Medicines Boards stipulates that the patient has to be informed about the nature (it is a test programme), significance (patient is either treated with the new drug or a placebo) and impact (risks, but also possible benefits) of the clinical trial in full detail. In this context it is of utmost importance that the risks are not treated as statistical probabilities, but are made clear to the patient with all their possible consequences. He also needs to be informed about the alternatives (Deutsch & Spickhoff 2008, p.757).

An effective consent requires that the patient has been informed comprehensively and promptly, and also that the patient is capable of forming an opinion on the nature, extent and impact of the measures. He needs to be able to assess the inherent health risk without feeling pressurised and needs to be able to come to a valid decision (BMJ, undated). Critics state however, that even with well planned trials, the patient information is often insufficient. The leaflets are overloaded with legal terms, the wording is not geared towards lay people and quite often they are too long. Especially with Phase-I-Trials where possible toxicity and dose ranges are determined, there would be very little prospect of a cure and a cure is not the aim of the trial, critics argue. This would have to be made sufficiently clear to the test person (Druml 2003, p. 1353).

From an ethical point of view the protection of patients is of the utmost importance, especially concerning new therapies which carry a great deal of uncertainty. Sick people are frequently very insecure and grasp at every ray of hope, especially if their illness is life threaten-

ing (Strassmann 2010) or where their quality of life is severely limited (e.g. through chronic pain or permanently restricted mobility).

Ethicists stress that patients are usually not in a position to take responsibility for the choice of therapy; the capabilities and abilities of patients are quite often overestimated and therefore lead to an empty ritual: "Would you please sign here". In most cases a test person can't be expected to fully comprehend the scientific and medical background of a trial. Nevertheless a test person should be able to understand the effects of the trial (opportunities, risks and alternatives) and then give his/her consent to participate because of the firm belief that the trial will make an important contribution to future medical progress (Rehmann-Sutter 2006, p. 700f).

5.6. Distinction between somatic gene therapy/germ line therapy

In somatic gene therapy (gene therapy with the body's own cells; somatic – relating to the body) the genome of individual cells is manipulated (as in the GAMBA project, see p. 17f). In contrast a germ line therapy interferes directly with the genome of the egg or sperm cells or of the fertilised ovum and thus changes the genome of the treated person or the embryo. This change will be passed down to potential offspring. Especially with hereditary diseases there is a theoretical possibility to repair a defective gene in the cells of the germ line and this would ensure health for several generations. But such germ line therapies are extremely controversial and carry a lot of risk. They are prohibited in Europe.

In Europe there is a broad and unequivocal consensus to reject germ line therapy, not least because a) an effective control of serious side effects is impossible as future children could be affected; b) a limitation to "serious illnesses" cannot be defined clearly; and c) because the responsibility for one's own decision also affects other people (all descendants) and one person does not have the right to make these decisions (Rehmann-Sutter 2003, 228ff.).

GAMBA employs somatic gene therapy (s. p. 17f). Therefore we only discuss the pros and cons of this form of gene therapy.

Further ethical aspects, such as ethics committees, unrealistic promises of cure, conflicts of interest, enhancement of humans, animal ethics, research politics as well as patents on building blocks of life will be discussed in the **Compendium** in **Chapter 7**.

5.7. Pros and Cons of somatic gene therapy

Ethical aspects of gene therapy have been discussed in great detail for more than 30 years. This discussion was partly fuelled by the "Martin Cline affair" in 1980, when the ambitious doctor conducted a human gene therapeutic experiment in Italy and Israel that had already been turned down by the ethics committees and the National Institute of Health (NIH) in America (Rehmann-Sutter 2003, p.15 ff). In the meantime, abou 1700 (January 2012) clinical trials have been officially approved – most of them in the US – and many of those have concluded. Nevertheless, a possible breakthrough of gene therapy is still pending. Only three gene therapeutics have been licensed so far, however, none of these were licensed in the US or in Europe.

The following table lists the various ethical aspects discussed in the relevant literature in the form of a list of pros and cons; the focus is on somatic gene therapy. This list is not exhaustive.

Fig. 13: Somatic gene therapy: Pro and Con

Topic		Pro	Con
A: Ethical principle arguments ("deontological arguments")			
A1	Mans' mandate	There is an obligation to utilise gene therapy	Gene therapy is a transgression
A2	Image of nature	Gene therapy is based on nature	Gene therapy is artificial
A3	Human dignity	Man is primarily an individual being	Man is primarily a social being
B: Medico-ethical pragmatic arguments			
B1	Level of innovation	Gene therapy is similar to other therapies	Gene therapy is fundamentally new
B2	Duty of care	There is an obligation to help / heal	There is a risk of causing damage
B3	Efficacy	Gene therapy is a causal therapy	Alternative therapies are being neglected
C: Socio-political arguments			
C1	Public opinion on benefits/ risks	Benefits predominate and should be stressed	Risks predominate, but are neglected in the debate
C2	Regulation	A legal limitation is possible	Danger of "opening the floodgates"
C3	Distributional justice	Investment in the future	Unjust distribution
C4	Social effects	Gene therapy contributes to a harmonisation of society	Gene therapy leads to stigmatisation / discrimination
C5	Commercialisation	Has many advantages	Has many disadvantages
C6	Quality of target and media	Is high	Is low
C7	Varied interests	Have a positive effect	Have a negative effect

Source: own compilation (according to Schmidt 1995)

Further information on the arguments can be found in the **Compendium Chapter 7.1**

Bibliography

Akademie für Technikfolgenabschätzung (2003): Die Zukunft des Menschen - Kongress-dokumentation. 2003

Arthritis Ireland: www.arthritis.ie

Bahnsen, U. (2008): Erbgut in Auflösung. In: Die Zeit, 12.06.2008

Barmer GEK (2010): BARMER GEK Report Krankenhaus 2010

BBAW (2008): Gentherapie in Deutschland. Eine interdisziplinäre Bestandsaufnahme. http://www.gentechnologiebericht.de/gen/publikationen/gentherapie-in-deutschland-2008

Beauchamp T.L., Childress J.F. (1994): Principles of Biomedical Ethics. New/York, Oxford

Beer, W.; Droste, E. (2006): Biopolitik im Diskurs. In: Bundeszentrale für Politische Bildung (Hg.), 2006

BMJ (Hg.) (undated): Patientenrechte in Deutschland. Leitfaden für Patientinnen/Patienten und Ärztinnen/Ärzte

Brandt, K. (2010): Defining Osteoarthritis: What it is, and what it is not. In: The Journal of Musculoskeletal Medicine, 09.09.2010, p. 338.350

Charbord, P. (2010): Bone Marrow Mesenchymal Stem Cells: Historical Overview and Concepts. In: Human Gene Therapy, Sept. 2010, p. 1045–1056

Chen F. H. et al. (2008): Mesenchymal Stem Cells in Arthritic Diseases. In: Arthritis Research & Therapy, 10.10.2008, p. 223

Clarke, M.F.; Becker, M.W. (2007): Krebs – sind Stammzellen schuld? In: Spektrum der Wissenschaft, Januar 2007, p. 56-63

Coleman, C.M. et al. (2010): Mesenchymal Stem Cells and Ostteoarthritis: Remedy or Accomplice? In: Human Gene Therapy 22.07.2010, p.1239-1250

Cordonnier T. et al. (2010): 3D environment on human mesenchymal stem cells differentiation for bone tissue engineering. In: J. Mater. Sci: Mater Med, Vol. 21 (3), 981-987 2010

Deckers R. et al. (2009): Image-guided, noninvasive, spatiotemporal control of gene expression. In: PNAS, 27.01.2009, p. 1175–1180

Deutsch, E.; Spickhoff, A. (2008): Medizinrecht. Berlin et al.

DFG (2006): Entwicklung der Gentherapie. In: http://www.dfg.de/download/pdf/dfg_im_profil/geschaeftsstelle/publikationen/entwicklung_gentherapie_0612_dt.pdf

DGRh (2010): Rheumatologen fordern, Arthrose stärker zu beforschen. http://idw-online.de/pages/de/news385462, 08.09.2010

Druml. C. (2003): Arbeit und Effizienz von Ethikkommissionen. In: Der Onkologe, Sep.03, p. 1349-1354

Evans, C.H. et al. (2009): Orthopedic Gene Therapy in 2008. In: Molecular Therapy, Feb. 2009, p. 231–244

Evans, C.H. et al. (2004): Osteoarthritis Gene Therapy. In: Gene Therapy, Nov.04, p. 379-389

Evans, C.H. et al. (1996): Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. In: Human Gene Therapy, Jul.96, p.1261–1280

Evans. C.H. et al. (2008): Arthritis gene therapy's first death. In: Arthritis Research & Therapy, 27.5.2008, p. 110

Fehse, B. (2008): Insertionsmutagenese – Implikationen & Möglichkeiten der Vermeidung. www.dg-gt.de/docs/InsMutFehse.pdf, July 2008

Fuchs M. (2011): Ethische Aspekte der Gentherapie. In: Sturma, D. et al. (Hg.): Gentherapie. Ethik in den Biowissenschaften - Sachstandsberichte des DRZE. Freiburg i.B. (to be published in the autumn of Herbst 2011), DRZE-Blickpunkt

German Federal Statistics Office (StBA) (Hg.) (2010): Krankheitskosten. 11.08.2010

Gonin, P; Gaillard, C. (2004): Gene transfer vector biodistribution: pivotal safety studies in clinical gene therapy development. In: gene therapy, Nov.04, p. 98–108

Goyenvalle, E. et al. (2009): Biofunctionality of MBCP ceramic granules (TricOsTM) plus fibrin sealant (Tisseel) versus MBCP ceramic granules as a filler of large periprosthetic bone defects: an investigative ovine study. In: J Mater Sci Mater Med., 13.10.2009, p. 1949-1958

Graumann, S. (2000): Die Somatische Gentherapie. Tübingen/Basel

Grolle, J. (2010): Konkurrenz für Gott. In: Der Spiegel, 1/2010

Groß, J. (2010): ACT - Wir bauen ein Trägermaterial und säen Knorpelzellen (Interview mit Gaissmaier). In: Spektrum der Wissenschaft, Juli 2010

Hebrew University of Jerusalem (2009): New stem cell technology developed at Hebrew University leads to better treatment for complicated bone fractures. http://www.alphagalileo.org/ViewItem.aspx?ItemId=63285&CultureCode=en, 30.11.2009

Irish Medicines Board www.imb.ie

idw (2010): Arthrose-Forschung: Hilfe für kaputte Gelenke. In: idw, 2.7.2010

Johnsen, I.K. (2009): Einfluss von BMP-2 und BMP-5 auf Funktion und Tumorgenese der Nebenniere. http://www.freidok.uni-freiburg.de/volltexte/7116/index.html, 11.12.2009

Katsnelson A. (2010): Physiology: The bones of contention. In: Nature, 19.8.2010, p. 914-915

Khademhosseini, A. et al. (2010): Organersatz aus der Retorte. In: Spektrum der Wissenschaft, März 2010, p. 88-95

Kimmelman, J. (2008): The ethics of human gene transfer. In: Nature Review Genetics, 2008, p. 239-244

King, N.M.P.; Cohen-Haguenauer, O. (2008): En Route to Ethical Recommendations for Gene Transfer Clinical Trials. In: Molecular Therapy, 2008/3, p. 432-429

Klug, B. et al. (2010): Regelungen zur Überwachung von Wirksamkeit und Sicherheit nach der Zulassung. Risikomanagement und Rückverfolgbarkeit von Arzneimitteln für Neuartige Therapien. In: Bundesgesundheitsblatt, 2010

Koesters R. et al. (2010): Tubular Overexpression of Transforming Growth Factor-β1 Induces Autophagy and Fibrosis but Not Mesenchymal Transition of Renal Epithelial Cells. In: American Journal of Pathology, 2010, p. 632-643

Kollek, R. (2003): Die Maschinisierung des Menschen. Gen- und Nanotechnologie und die Würde des Individuums. Ein Kommentar. In: Akademie für Technikfolgen-abschätzung: Die Zukunft des Menschen - Kongressdokumentation, 2003, p. 31-34

Krug, H. F.; Wick, P. (2011): Nanotoxikologie – eine interdisziplinre Herausforderung. In: Angewandte Chemie, Jan. 2011, p. 2–23

Layrolle, P. et al. (2009): In vivo performance of an injectable biphasic calcium phosphate bone filler. In: Key engineering Materials Vols, 2009, p. 583-586

Ledwith, B.J. et al. (2000): Plasmid DNA Vaccines: Investigation of Integration into Host Cellular DNA following Intramuscular Injection in Mice. In: Intervirology, 2000, p. 273–281

Manno, C.S. et al. (2006): Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. In: Nature Medicine, 12.02.2006, p. 342-347

Manzeschke, A. (2011): Anmerkungen zur Ethik der Gen- und Stammzelltherapie. persönl. Mitteilung, 10.2.2011

Mao, J. (2010): Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. In: The Lancet, 29.7.2010, p. 440-448

Marckmann, G. (2000): Was ist eigentlich prinzipienorientierte Medizinethik?. In: Ärzteblatt Baden-Württemberg, 12/2000, p. 74-77

Mendez-Ferrer, S. et al. (2010): Mesenchymal and haematopoietic stemcells form a unique bone marrow niche. In: Nature, 12.08.2010, p. 829-836

Michael, J.W.-P. et al. (2010): Epidemiologie, Ätiologie, Diagnostik und Therapie der Gonarthrose. In: Dtsch Arztebl Int 2010; 107(9), p. 152-62

Mieth, D. (2003): Die Maschinisierung des Menschen. Gen- und Nanotechnologie und die Würde des Individuums. Ein Kommentar. In: Akademie für Technikfolgen-abschätzung: Die Zukunft des Menschen - Kongressdokumentation., 2003, p. 35-42

Miyoshi H. und Stappenbeck T.S. (2009): The Role of Stromal Stem Cells in Tissue Regeneration and Wound Repair. In: Science, 26.06.2009, p. 1666-1669

Müller, H. (2003): Gentheraphie: Unter besonderer Berücksichtigung der Behandlung von Erbkrankheiten. In: Rehmann-Sutter (2003), p. 41-58

Murphy, M. (2003): Stem Cell Therapy in a Caprine Model of Osteoarthritis. In: Arthritis & Rheumatism, 12.12.2003, p. 3464-3474

Murphy, M.; Barry, F. (undated): Osteoarthritis Patient Information Leaflet. National University of Galway, Irland, undated

o.V. (2008): Stammzellen bilden Tumore. In: Die Zeit, 12.2.2008

O'Brien, J. (2005): Bone quality regulator identified, suggests drug target. In: eurekalert, 13.12.2005

Osch, van G. et al. (2010): Articular cartilage repair and the evolving role of regenerative medicine. In: Open Access Surgery, 09.09.2010, p. 268-277

Osch, van G. et al. (2009): Cartilage repair: past and future – lessons for regenerative medicine. In: J. Cell. Mol. Med., Mai.09, p. 792-810

Pereira R.C. et al. (2009): Novel injectable gel (system) as a vehicle for human articular chondrocytes in cartilage tissue regeneration. In: J Tissue Eng Regen Med, 26.01.2009, p. 97–106

Pitzalis C. et al. (2008): Identification of the Molecular Response of Articular Cartilage to Injury by Microarray Screening. In: Arthritis & Rheumatism, 25.04.2008, p. 1410-1421

Pschyrembel, W. (2007): Pschyrembel Klinisches Wörterbuch. Ausgabe 2007

Rehmann-Sutter, C. (2010): Ethik des molekularen Blicks. http://www.imgwf.uni-luebeck.de/_data/Ethik_des_molekularen_Blicks_Rehmann-Sutter.pdf

Rehmann-Sutter, C. (2006): Ist ein informed consent wirklich möglich?. In: Therapeutische Umschau, Nov.06, p. 699-702

Rehmann-Sutter, C. (2003): «Titel». Tübingen/Basel

Richter, H. et al. (2010): Magnetorelaxometry for localization and quantification of magnetic nanoparticles for thermal ablation studies. In: Phys Med Biol, 2010, p. 623-633

Robert-Koch-Institute RKI (Hg.) (2007): Gesundheit in Deutschland. In: Gesundheits-berichterstattung des Bundes, 20.02.2007

Salzmann G. M. (2005): Tissue Engineering und Gentransfer zur Therapie osteochondraler Läsionen am Kniegelenk. Dissertation

Schlingensiepen, I. (2006): In Deutschland gibt es viel mehr Arthrose-Kranke als vermutet. In: Ärztezeitung, 10.11.2006

Schmidt, K.W. (1995): Systhematische Übersicht zu den in der Debatte um den somatischen Gentransfer verwendeten Argumenten und Problemanzeigen. In: Bayertz, K. et al.: Somatische Gentherapie – medizinische, ethische und juristische Aspekte, 1995, p. 169-231

Sheridan, C. (2011): Gene Therapy finds its niche. Gene therapy is finally poised to make a constibution of debilitating, highly penetrant genetic diseases that have proved intractable to other regimes. In: Nature Biotechnology, Feb.11, p. 121-128

Simon, P. (2004): Entwicklung, Risiken und therapeutischer Nutzen der Gentherapie. In: Analysen der Friedrich-Ebert-Stiftung zur Zukunft der Biotechnologie

Sohier, J. et al. (2009): Porous beta tricalcium phosphate scaffolds used as a BMP-2 delivery system for bone tissue engineering. In: Journal of Biomedical Materials Research, 19.03.2009, p. 1105-14

Stephen S.L. et al. (2010): Chromosomal Integration of Adenoviral Vector DNA In Vivo. In: Journal of Virology, 10/2010, p. 9987-9994

Stoddart M.J. et al. (2009): Cells and biomaterials in cartilage tissue engineering. In: Regenerative Medicine, 2009, p. 81-98

Strassmann, B. (2010): Überlebt, aber wie?. In: SZ, 30.09.2010, p. 43

Techniker Health Insurance (Techniker Krankenkasse (Hg.) (2002): Arthrose. Eine Information für Patienten und Angehörige. www.akdae.de/Arzneimitteltherapie/Patientenratgeber/Arthrose.pdf, 21.04.2010

Thorbrietz P. et al. (2008): Nanomedizin: Anwendungsfelder, mögliche Risiken und ethische Fragen. http://www.nano-jugend-dialog.de/daten/MPSNanomedVorstudieEnd.pdf, 2008

Ueblacker, P. et al. (2004): Inducible nonviral gene expression in the treatment of osteochondral defects. In: Osteoarthritis Cartilage, Dez.04, p. 711-719

University of Rochester Medical Center (2010): Promoting Healing by Keeping Skeletal Stem Cells 'Young'. In: http://www.sciencedaily.com/releases/2010/03/100330142429.htm, 31.03.2010

VfA (2009): VfA-Positionspapier Somatische Gentherapie. www.vfa.de, 11/2009

Voluntary Health Insurance Ireland, www.vhi.ie

Wakitani S. et al. (2002): Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. In: Osteoarthritis and Cartilage, März 2002, p. 199-206

Wehling, P. et al. (2009): Clinical Responses to Gene Therapy in Joints of Two Subjects with Rheumatoid Arthritis. In: Human Gene Therapy, Feb. 2009, p. 97–10

WHO (Hg.) (2003): The Burden of musculoskeletal conditions at the start of the new millenium. In: WHO Technical Report Series 919, 2003

Wiley (2010): Gene Therapy Clinical Trials Worldwide. In: http://www.wiley.com/legacy/wileychi/genmed/clinical/, as of July 2011

Williams, G. (2008): Researchers offer first direct proof of how osteoarthritis destroys cartilage. In: eurekalert, 02.09.2008

Witte F. et al. (2010): ROR2 and Indian Hedgehog regulate digit outgrowth mediated by the phalanx-forming region. In: PNAS, 26.7.2010

Wolpert, L. (2009): Wie wir leben und warum wir sterben. Das geheime Leben der Zellen

Yuanyuan W. (2009): Relationship between body adiposity measures and risk of primary knee and hip replacement for osteoarthritis: a prospective cohort study. In: Arthritis Research & Therapy, 05.03.2009, doi:10.1186/ar2636

Zittlau, J. (2010): Ein Lächeln, das nachwächst. In: Die Welt, 29.05.2010

List of sources of figures

Titel: S. Siebert/clkerdesign

P. 10: S. Siebert/ScienceDialogue

P. 12: C.H. Evans; courtesy of

P. 13: C.H. Evans; courtesy of

P. 16: L. Geissler/ScienceDialogue

P. 18: L. Geissler/ScienceDialogue

P. 19: Chr. Plank; courtesy of

P. 23: L. Geissler/ScienceDialogue

P. 25: B. Lugger/SciendeDialogue

P. 28: K. Zöller/ScienceDialogue

P. 32: S. Siebert/ScienceDialogue

P. 36: K. Zöller/ScienceDialogue

P. 45: K. Zöller/ScienceDialogue

P. 49: K. Zöller/ScienceDialogue