GAMBA Scientific Project Results[1]

Final information for the participants of the GAMBA panels in Germany, Ireland and Switzerland

Osteoarthritis (OA) is a painful degenerative disease of the joints that comprises degeneration of cartilage and underlying bone as well as inflammation. Causes are generally unknown. So far, OA can only be treated by pain reducing and anti-inflammatory drugs combined with lifestyle changes (i.e. weight reduction, healthy diet, gentle exercise), and ultimately by a joint replacement.

GAMBA's main objective was the development of a biomimetic implant system using stem cells, gene vectors, nanoparticles and biomaterials such as hyaluronan gel (hyaluronic acid is the main component of the joint liquid) and calcium phosphate (the main component of the bones) granules. This system was aimed at controlling inflammation and inducing cartilage and bone repair in osteoarthritic tissue by virtue of the controlled action of therapeutic genes.

The following workpackages (WP) have been part of GAMBA:

Gene Vectors (WP1): The first set of gene vectors did not work well enough in mesenchymal stem cells (MSC) from bone marrow, although regulation was working as anticipated in other cell lines. As an alternative, researchers tried to enhance the productivity of the vectors through the introduction of the antibiotic doxycycline gene switch system to enforce the production of the anti-inflammatory (IL-10) and the bone (BMP2) proteins. Work is ongoing.

Temporal control of the healing proteins was successful as production of the anti-inflammatory and bone inducing proteins only started after the pharmacological start signal with doxycycline.

Hyaluronan Gel (WP2): The gel was further optimized and combined with the calcium phosphate granules and the magnetic nanoparticles resulting in a composition suitable for testing with cultured cells. Magnetic nanoparticles do not influence the gel negatively. Under an AC magnetic field at 43 degrees Celsius, the gel "swelled" as planned which is necessary for starting the work of the gene vectors. Cells produced the therapeutic proteins within the gel with the help of adenoviral vectors; this also worked with biodegradable nonviral vectors, but to a lesser extent. In addition, the gel also shows good biocompatibility (no negative influence on the living system) in animal experiments.

Calcium phosphate granules (WP 3): the granules were tested and optimised as carriers for the gene vectors; the goal was a drug formulation. For regulatory reasons it is easier to have the components (in this case: the vectors and the granules)

[1] Source: Annual project report 3: http://gamba-project.eu/project/annual-reports

separately so that the physician can combine the two (or more) components prior to injecting the system into the joint. In addition, a biomimetic scaffold (mimicking biological structures) was developed composed of fibers (mimicking collagen, the white fibers of tissue like bone) and the granules (mimicking bone crystals).

Fighting inflammation in the joint (WP4): Scientists believe that changes in the synovium (joint membrane) may lead to the development of OA. On the one hand, the stem cells in the synovium contribute to repairing damaged tissue like cartilage or bone, but this is prevented by inflammation. The protein Interleukin 10 (IL-10) can be anti-inflammatory but can also stimulate negative immune effects and therefore increase inflammation. Viral IL-10 (produced by human mesenchymal stem cells containing vectors) seems to have only the anti-inflammatory effect. In addition, it had a positive influence on the development of a cartilage cell from mesenchymal stem cells.

In synovium donated by patients undergoing knee replacement there were varying effects of viral Il-10: some donors' synovium reacted well and produced more of the anti-inflammatory protein tested; others' synovium reacted not so well: the more macrophages were present, the smaller was the positive effect so researchers now try to hinder the macrophages from producing inflammatory factors. Some positive effects of viral IL-10 on levels of immune cells could also be shown in animal tests but the effect was not strong enough to prevent OA from developing.

As mentioned above, one of the switches (tet-on) tested to start the production of viral IL-10 worked well in the mesenchymal stem cells, while another (inflammation-induced) did not. The inflammation-induced gene switch however worked in chondrocytes (cartilage cells).

Cartilage regeneration (WP5): Researchers in GAMBA were able to develop a new model system that is more representative for the situation in the joint than conventional laboratory models. In this model, TGFbeta (a cartilage protein) which was originally necessary for cartilage formation is no longer required; instead, an artificial defect is made in a piece of bone with cartilage on top (from cow hoofs) and filled with stem cells. Proteins secreted by the bone stimulate stem cells to become cartilage cells. The effect could only be shown with the "right" hydrogel: the Swiss gel from WP2 worked well, while another substance tested (fibrin, the "blood glue") did not work without the addition of TGFbeta. In addition, with the Swiss hydrogel, there was no unwanted bone formation in the cartilage (which would turn cartilage stiff). No gene therapy was applied here, since on the one hand the non-viral gene transfer was not working well enough and on the other hand in the developed new model, cartilage regeneration occurred even in the absence of added TGFbeta. Obviously, the surrounding tissue in this particular cartilage defect model provided sufficient

growth factors (therapeutic proteins) to stimulate cartilage regeneration. Hence this model was not suitable to test any additional benefit provided by the gene vectors.

Bone regeneration (WP6): Researchers tested untreated human mesenchymal stem cells (without vector) that had been loaded on granules from WP3 in mice. Already after 4 weeks, there was a large amount of bone tissue present, after 8 weeks the granules were completely covered by mature bone. In a rabbit trial (36 animals) there was very efficient bone formation while testing the same granules. The additional presence of a nonviral vector (plasmid) showed slightly better results, but did not increase bone formation in a statistically significant way.

Testing results in a large animal model (WP7): It was originally planned to test the positive effects in previous work packages in goats. But as the planned therapeutic solutions could not be fully proven in small animal studies, the GAMBA team refrained from the goat trial for scientific and ethical reasons.

Summary

Altogether, GAMBA researchers were able to test and further develop some components to better osteoarthritic conditions in vitro and through animal experiments, but not a whole system which was the original vision of GAMBA. For regulatory reasons, a multi-component system is less likely to be approved than single components because of complexity and therefore higher risks.

In general, **nonviral gene vectors did not work sufficiently well** to encourage stem cells to release significant amounts of the healing proteins. Due to strict safety regulations adenoviral vectors could not be tested by all partners. So the researchers will now concentrate on enhancing the productivity of the vectors.

The **best results** were achieved in **bone formation** with stem cells and ceramic granules; an additional gene therapy showed only slightly better results. It was also shown that the **temporal control of the genetic production of healing proteins** (growth factors) through gene vectors is working well. The **hydrogel** showed **good results** on biocompatibility; the gel was also **successfully combined with stem cells**.

To a lesser extent, **inflammation was reduced** through the healing protein IL-10 produced by viral gene vectors in the stem cells. In addition, IL-10 positively influenced stem cells to develop into cartilage cells.

Cartilage was best produced by introducing a mixture of the hydrogel and stem cells into a bone defect (no gene therapy).

GAMBA as a consortium will not proceed while of course **all partners will continue their research** on unresolved challenges in bone and cartilage regeneration. Several partners have emphasized their interest in individual collaborations with 2 or 3 partners on specific topics of the GAMBA project.