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Gene and Cell Therapy in Germany and the EU

K. Cichutek

Paul-Ehrlich-Institut, Division of Medical Biotechnology, Langen, Germany

Correspondence to: Prof. Dr. Klaus Cichutek, Paul-Ehrlich-Institut, Division of Medical Biotechnology, Paul-Ehrlich-Str. 51 – 59, D-63225 Langen, Germany; Phone: 0049-6103-77-2000; Fax: 0049-6103-771252; E-Mail: cickl@pei.de

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Abstract: Gene and somatic cell therapy medicinal products as well as tissue engineered products are summarized under the term advanced therapy medicinal products in the EU. The Paul-Ehrlich-Institut has established a specialized division with various sections providing scientific advice and clinical trial authorization to applicants, thereby protecting patients' health and supporting product development. Gene therapy medicinal products include products suitable for *in vivo* diagnostics, for the prevention of infectious diseases (such as vaccines) and the treatment of common diseases such as cancer, cardiovascular and infectious diseases, and of rare or orphan diseases such as inherited monogeneic diseases. Clinical trial authorization in Germany is provided by the Paul-Ehrlich-Institut and, if genetically modified organism-containing medicinal products are used, includes deliberate release authorization under consultation with the Federal Office for Consumer Protection and Food Safety (BVL). First marketing authorization applications of gene therapy medicinal products developed for the treatment of cancer have been submitted to the European Medicines Agency (EMEA) and have been or are being reviewed by experts from national medicines agencies. Beneficial gene therapy treatment in clinical trials has been reported for infants suffering from adenosine deaminase deficiency-based severe combined immunodeficiency disease. Human somatic cell therapy medicinal products are mainly being developed for cancer immunotherapy; tissue engineered products contain viable cells able to replace, repair or substitute for human tissues or cells, e.g., after myocardial infarction. Chondrocytes are being used clinically for cartilage repair and an application for EU-wide marketing has been submitted to the EMEA. The main issues for cell-containing medicinal products are cell selection, differentiation, manipulation to obtain specific characteristics and avoiding pre-neoplastic alterations. In summary, special expertise is needed to develop advanced therapy medicinal products and has to be met by scientists specialized in regulatory aspects to support patient safety and product development. Consequently, the EMEA will establish the Committee for Advanced Therapies to review future marketing authorization applications.

1. Gene therapy

The initial idea of gene therapy was elaborated following the identification of a number of genes underlying inherited monogeneic diseases (Somia and Verma, 2000; Smith, 2003; Blaese et al., 1995). Gene therapy is the treatment of diseases by transferring new specific, functional genetic material (DNA or RNA) into pre-determined target cells of a patient and the expression of the introduced sequences. For the transfer of functional genetic material, a variety of vectors has been used (Tab. 1). Following genetic modification, cells containing the transferred gene (transgene) form a reservoir for the stable production of the respective gene product in order to restore normal cellular functions, to eliminate abnormal gene functions or to add new biological properties for therapeutic, preventive or diagnostic use (Balicki and Beutler, 2002).

The first human gene therapy phase I studies were initiated in 1989 and 2000 involving children suffering from severe combined immunodeficiency disease due to a defect of the adenosine-deaminase (ADA) gene. Later, SCID due to a defect of the common gamma chain (γC) gene was also treated. Both deficiencies are due to autosomal recessive gene defects, leading to severe combined immunodeficiency diseases (ADA-SCID, SCID-X1) (Blaese et al., 1995; Cavazzana-Calvo et al., 2000; Fischer et al., 2002). In the recent ADA-SCID study carried out in Italy (Auti et al., 2002), patients treated with gene-modified autologous haemaotopoietic stem cells gained clinical benefit. Gene therapy was also shown to be safe within the observation time of several years. In the SCID-X1 study of Alain Fischer and colleagues about ten patients were treated successfully and immune function could be restored. Unfortunately, by now four of these patients developed an acute leukemia-like lymphoproliferative disease, probably caused by

Tab. 1 Gene transfer methods (vectors/delivery systems).

delivery system	description	chromosomal integration machinery
naked nucleic acid	plasmid DNA, in absence of transfection reagents	no
non-viral vector	plasmid DNA/transfection reagent mixture	no
gamma-retroviral vector	derived from murine leukaemia virus (MLV)	yes
lentiviral vector	derived from HIV-1	yes
adenoviral vector	deletions in the virus genes E1, E3 or E4, E2ts, combinations thereof or "gutted" (gene-depleted)	no
conditionally replication- competent adenovirus	therapeutic gene is the virus genome or an additional gene improving tumour cell killing	no
adeno-associated virus (AAV) vector	wildtype AAV-derived	no
smallpox virus vector	MVA ("Modified Vaccinia Ancara")	no
	ALVAC ("Avian Vaccinia")	no
	Vaccinia	no
alphavirus vector	Semliki Forest virus (SFV)	no
herpes-viral vector	Herpes simplex virus	no

gene insertion and activation of proto-oncogenes such as *LMO2*. In a SCID-X1 trial carried out in London by Adrian Thrasher and colleagues (Gaspar et al., 2004), one of the beneficially treated patients also recently developed leukemia. It is currently believed that, apart from the main factor of insertional mutagenesis, the therapeutic gene and the disease may have contributed to the leukemias. Recently, scientists have developed new lentiviral and gammaretroviral vectors with reduced potential to activate genes adjacent to the vector integration site, which will be used in subsequent clinical trials.

Following the initial studies, gene therapy approaches have been undertaken for a wide and diverse range of disease indications beside monogeneic inherited diseases[1]. Today's main targets in clinical gene therapy trials include cancer, cardio-vascular, neurologic, autoimmune and infectious diseases, mainly AIDS (HIV infection). Gene therapy includes preventive vector vaccines and naked DNA vaccines (Scherer et al., 2007). So far, two thirds of those studies have been conducted in the US and one third has been performed in Europe. In China, two gene therapy medicinal products have been marketed for two clinical indications, both being severe types of cancer, based on pivotal clinical trials involving a few hundred patients. However, several ten thousands of patients suffering from a large variety of cancers have been treated in China, apparently representing a high number of off-label uses (Peng, 2007). Marketing authorization approval applications for cancer gene therapy products to the European Commission have been or are being evaluated by the Committee for Medicinal Products for Human Use (CHMP) at the European Medicines Agency (EMEA).

Gene therapy medicinal products are defined in Part IV of Annex I to Directive 2001/83/EC as amended by Directive 2003/63/EC. The European »Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)« provides general guidance with a view to marketing authorization. Due to ethical considerations, human clinical gene therapy is limited exclusively to somatic cells and germ-line manipulation is legally prohibited in the EU by Directive 2001/20/EC. Tests to assess the risk of inadvertent germline integration of vectors have to be carried out in non-clinical and clinical development of a new gene therapy medicinal product in order to reduce this risk to a minimum. The risk of inadvertent germline integration is in general limited only to vectors which are administered *in vivo* and which mediate vector transfer into the nucleus of cells.

General challenges of clinical gene therapy strategies have not only to account for the selection of appropriate diseases and of suitable transgene(s), but also for the identification of the appropriate target cell population, including the accessibility of a sufficient number of target cells for a beneficial *in vivo* or *ex vivo* treatment and the design and development of efficient gene transfer technologies. Some gene therapy medicinal products consist of or contain genetically modified organisms (GMOs) in the sense of Directive 2001/18/EC and require contained use and/or deliberate release notifications/authorizations during clinical trials. The main GMOs in gene therapy medicinal products are genetically modified cells, replication-incompetent viral vectors, replication-competent oncolytic viruses or microbes mediating transfer of plasmid DNA into human somatic cells. All the GMOs listed above may be shed into excreta of a treated patient. Such excreta include urine, faeces, sperm and excreta on mucosal surfaces of the body. If these excreta would contain infectious material, a risk of transfer into the environment at large and the particular risk of transmission to other humans in close contact with the patients administered with the respective gene therapy medicinal products may exist. No adverse effect due to release of a GMO-containing gene therapy medicinal product has been published so far. However, shedding studies to assess the presence of vector nucleic acids in excreta are usually performed during non-clinical developments in suitable animals and during one or more clinical trials. If vector nucleic acids are detected, presence of vector-containing infectious material is tested for. If none of these data are available, data known from

[1] Clinical trial statistics for gene therapy are available at http://www.wiley.com.

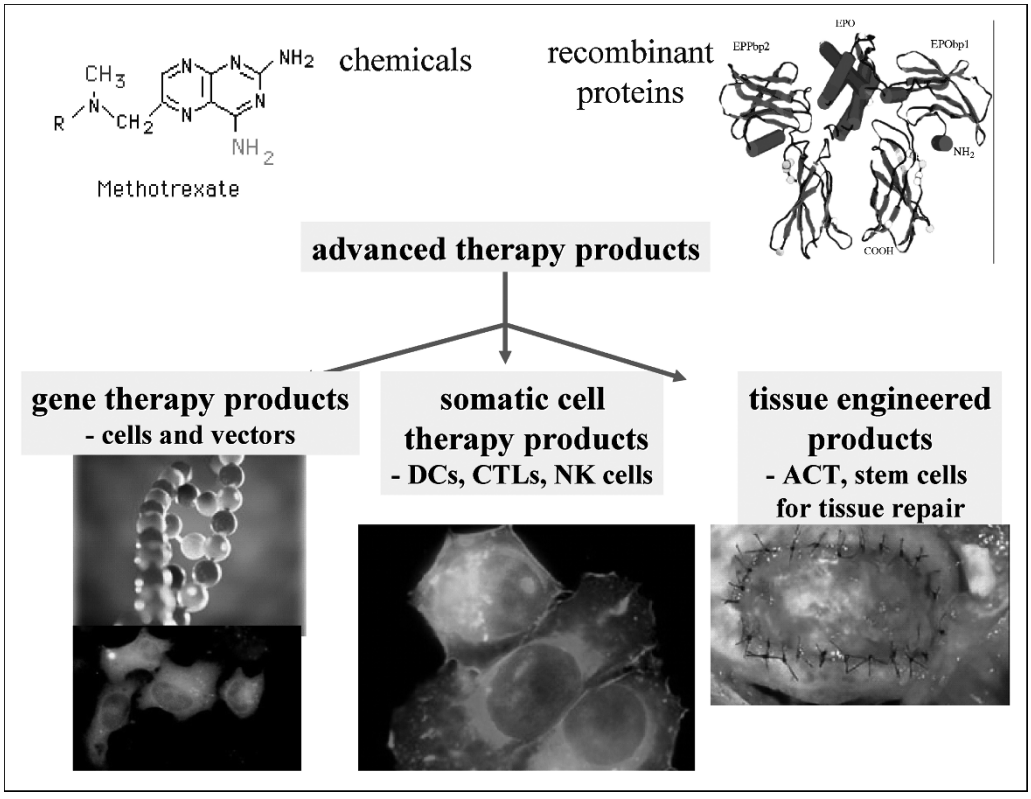

Fig. 1 A change in paradigm: genes and manipulated cells as medicinal products.

a similar product can be taken to assess a possible risk of transmission or a theoretical risk assessment can be made. In all cases, the following steps of the environmental risk assessment are taken:

Step 1: Identification of GMO characteristics which may cause adverse effects.
Step 2: Evaluation of the potential consequences of each adverse effect, if it occurs.
Step 3: Evaluation of the likelihood of the occurrence of each identified potential adverse effect.
Step 4: Estimation of the risk posed by each identified characteristic of the GMO(s).
Step 5: Application of management strategies for risks arising from the deliberate release or marketing of the GMO(s).
Step 6: Determination of the overall risk of the GMO(s).

More detailed guidance for the environmental risk assessment will be provided by the CHMP Guideline on scientific requirements for the environmental risk assessment of medicinal products for human use containing or consisting of genetically modified organisms (GMOs), which has recently been published for comments by the EMEA. In Germany, the contained use of GMO-containing products is subject to a notification or authorization by the authority competent for the Gene Law in the respective member state where the clinical trial site is located. The clinical trial authorization is provided by the Paul-Ehrlich-Institut and encompasses the deliberate release authorization, for which the Paul-Ehrlich-Institut has consulted the Federal Office of Consumer Protection and Food Safety (BVL).

2. Human Somatic Cell therapy and tissue engineered products

Human somatic cell therapy products and tissue engineering products, together with gene therapy medicinal products have been termed advanced therapy products in the EU (Fig. 1; Sanzenbacher et al., 2007). Human somatic cell therapy products and tissue engineering products are being developed for a number of clinical applications. They contain viable human cells engineered, substantially altered or manipulated to change the cell characteristics to suit their respective clinical application. Tissue engineering products are developed to substitute, repair or replace human tissue or cells. Somatic cell therapy products, which currently also encompass TEPs until the latter ones will form a new group of medicinal products after December 2009 (Regulation of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004), are mainly cell-based vaccines or immunotherapy products such as cytotoxic T lymphocytes or natural killer cells (Hinz et al., 2006). In Germany, autologous chondrocytes used for cartilage repair and some other autologous cell-containing medicinal products used for tissue engineering are currently marketed based on manufacturing authorization. This is due to § 21 (2) No. 1a of the German Medicinal Product Law. According to legislation, somatic cell therapy medicinal products and tissue engineered products (TEPs) require marketing authorization via the centralized procedure coordinated by the EMEA and provided by the European Commission. Those TEPs currently marketed based on manufacturing authorization

will require marketing authorization after a transition period of up to 5 years.

The Paul-Ehrlich-Institut provides clinical trial authorization and scientific advice for somatic cell therapy and gene transfer products and experts of the Paul-Ehrlich-Institut are involved in the scientific assessment of respective marketing authorization applications. The EMEA has formed a Working Party for Cell-based Products and a Gene Therapy Working Party to support the CHMP in guiding product development for marketing. In 2008, a Committee for Advanced Therapy (CAT) will be established to deal with advanced therapy medicinal products and to draft an opinion on marketing authorization.

Scientifically, the main issues in developing products containing cells are cell selection, differentiation, manipulation to obtain specific characteristics and avoiding pre-neoplastic alterations. Some of these products, presumably mainly the tissue engineered products, will be combined with material providing physical support within the target tissue such as scaffolds or matrices. Assessment of marketing authorization applications of such combination products will also be done by the CAT.

3. Literature

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