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Gene therapy of cancer

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Gene therapy was initially thought of as a means to correct single gene defects in hereditary disease. In the meantime, cancer has become by far the most important indication for gene therapy in clinical trials. In the foreseeable future, the best way to achieve reasonable intratumoral concentrations of a transgene with available vectors is direct intratumoral injection with or without the aid of various techniques such as endoscopy or CT-guidance.

At present, viral and non-viral methods of gene transfer are used either in vivo or ex vivo/in vitro. The most important viral vectors currently in use in clinical trials comprise retroviruses, adenoviruses, adeno-associated viruses, and herpes viruses. None of the available vectors satisfies all the criteria of an ideal gene therapeutic system, and vectors with only minimal residues of their parent viruses ("gutless vectors") as well as completely "synthetic viral vectors" will gain more and more importance in the future. Non-viral gene therapy methods include liposomes, injection of vector-free DNA ("naked DNA"), protein-DNA complexes, delivery by "gene gun," calcium-phosphate precipitation, electroporation, and intracellular microinjection of DNA.

The first clinical trial of gene therapy for cancer was performed in 1991 in patients with melanoma, and since then more than 5000 patients have been treated worldwide in more than 400 clinical protocols. With the exception of a case of fatal toxicity in a young man with hereditary liver disease treated intrahepatically with high doses of adenovirus, side effects have been rare and usually mild in all these studies and expression of the transgene could be demonstrated in patients in vivo. However, despite anecdotal reports of therapeutic responses in some patients, unequivocal proof of clinical efficacy is still lacking for most of the varied approaches to gene therapy in humans. As well as our only fragmentary understanding of the molecular pathophysiology of many diseases, the principal reason for the present lack of clinical success of gene therapy is the very low transduction and expression efficiency in vivo of available vectors.

Despite the complexities of gene therapy for cancer, the numerous different approaches can be subdivided into three basic concepts: (1) strengthening of the immune response against a tumour, (2) repair of cell cycle defects caused by losses of tumour suppressor genes or inappropriate activation of oncogenes, and (3) suicide gene strategies. In addition, the importance of gene marker studies and gene therapeutic protection of normal tissue are briefly covered in this review.

Keywords: gene therapy; cancer; vectors

Introduction

The first clinical trial of gene therapy was performed in 1990 in children with adenosine deaminase deficiency, a severe combined immunodeficiency syndrome [1]. Since then, more than 5000 patients have been treated worldwide in more than 400 clinical protocols. A recent summary of 425 published trials shows the following distribution of "indications" for gene therapy: 65.6% cancer, 12.9% monogenic diseases, 7.8% infectious diseases, 3.8% other diseases, 9.4% gene marking, and 0.5% healthy volunteers [2]. Except for the ill-fated attempt at gene therapy which led to the September 1999 death of a young man [3], side effects were rare and usually mild, and expression of the transgene could be demonstrated in patients in vivo. A first breakthrough of gene therapy has been reported by Alain Fischer's group, who apparently cured two boys suffering from severe combined immunodeficiency disorder (SCID) [4].

However, despite anecdotal reports of therapeutic responses in many patients, unequivocal proof of the clinical efficacy of any of the varied approaches to gene therapy of human cancer is still lacking [5]. One of the main impediments to the

Abbreviations:
IL: interleukin
INF: interferon
TNF: tumor necrosis factor
GM-CSF: granulocyte/macrophage-colony stimulating factor

potential success of gene therapy is the fact that cancer is a disease of many sequentially acquired mutations, which may or may not show a particular hierarchy in causing and maintaining malignant transformation. It is therefore unlikely that the correction of a single gene defect will be sufficient to reverse this process in the majority of cancer cells within a given tumour. Furthermore, our only fragmentary understanding of the molecular pathophysiology of cancer, and the very low transduction and expression efficiency in vivo of available vectors, help to explain the present lack of clinical success with gene therapy of cancer.

Methods of gene transfer

To eliminate potential risks of gene transfer in vivo, such as induction of malignant transformation or evolution of new viral diseases in humans, the development of vectors with the highest possible safety profile is mandatory. At the same time, the key to eventual future success for gene therapy is the availability of gene vehicles with much better transduction efficiency in vivo than those currently used in clinical trials. At present, viral and non-viral methods of gene transfer are used either in vivo or ex vivo/in vitro.

Viral vectors

The majority of clinical gene therapy studies carried out so far have used *retroviruses* as vectors to transfer the foreign gene [5, 6], and a May 2000 Internet update of 425 published gene therapy trials reports that 42.3% of these studies had used a retrovirus or retrovirus-transduced cells as therapeutic vector [2].

Advantages of retroviruses are:
- their relatively high transfection efficiency
- stable integration of the transferred genetic material into the genome of a target cell, potentially leading to long-term expression of the transgene
- the absence of immunogenic viral proteins in the target cells

Disadvantages are:
- the fact that only dividing cells are transducable
- the relatively small amount of genetic information (approximately 7.5 kb) that can be packaged into a retrovirus
- the uncontrolled integration of the virus into the genome, leading to a theoretical risk of malignant transformation of the affected cells
- the possibility of homologous recombination of therapeutic vectors with endogenous retroviruses, resulting in replication-competent new viruses

As of today, in more than 1000 patients treated with retroviral vectors neither the induction of a tumour nor the development of replication-competent retroviruses in vivo has been reported.

Adenoviruses have been used in approximately 20% of trials published to date [2]. Their *advantages* are:
- the comparably high viral titres achievable
- experience with more than ten million vaccinations with unmodified adenoviruses without severe side effects
- higher packaging capacity compared with retroviruses
- transfectability of non-replicating cells

Disadvantages are:
- immunogenicity of adenoviruses, making repeated application problematic
- no integration into the cellular genome, leading to loss of genetic information after only a few divisions of transfected cells

Other viral vectors currently used in clinical trials are *herpes-* and *adeno-associated viruses.* None of the currently available vectors satisfies all the conditions for an ideal gene therapeutic system. It is likely that vectors with only minimal residues of their parent-viruses *("gutless vectors")* and completely *"synthetic viral vectors"* will assume increasing importance in the future [5].

Non-viral vectors

Non-viral gene therapy methods are attractive mainly because they avoid the potential risks inherent in all viral transfer vectors. *Liposomes* have been used in numerous in vitro and animal studies of gene transfer and are also found increasingly in the clinical setting (18% of published trials [2]). Short term expression of genes, e.g. during vaccination studies, is achievable with intramuscular injection of vector-free DNA *("naked DNA")*, by plasmid DNA coated onto microscopic gold beads which are then delivered using a hand-held, helium-driven *"gene gun,"* and by the use of *protein-DNA complexes*. Several physico-chemical methods of gene transfer are used exclusively ex vivo/in vitro, e.g. *calcium-phosphate-precipitation*, *electroporation* or intracellular *microinjection* of DNA [5].

Gene transfer and gene therapeutic concepts in oncology

Since at present there are relatively few published clinical studies of gene therapy, this review will need to focus on currently open protocols and possible gene therapy strategies in tumour patients. Despite the complexity of the field, the many varied approaches can be subdivided into three basic concepts: strengthening of the immune response against a tumour, repair of cell cycle defects caused by losses of tumour suppressor genes or inappropriate activation of oncogenes, and suicide gene strategies. In addition, the importance of gene marker studies and gene therapeutic protection of normal tissue will be briefly discussed.

Marker studies

The era of clinical gene transfer began in May 1989 with the introduction of an antibiotic drug resistance gene into tumour-infiltrating lymphocytes (TIL) of patients with melanoma. The procedure proved to be safe and expression of the transgene in vivo could be demonstrated for more than two months [7].

Marker studies became clinically relevant when several groups showed that in patients undergoing high-dose chemotherapy peripheral blood and bone marrow cells marked in vitro before retransfusion later contributed to relapse in leukaemias and neuroblastoma. Similar studies have been initiated in almost all tumours treatable by high-dose chemotherapy [2, 5, 6].

Normal tissue protection

So far, most experimental and all clinical studies attempting to protect normal tissue have targeted precursors of myelopoiesis. In a manner similar to the concept of high-dose chemotherapy with stem cell retransfusion, the goal of these attempts is to increase dose intensity and simultaneously reduce the side effects of conventional cytotoxic drugs. In a recent phase I trial, the MDR1 (multiple drug resistance) gene was retrovirally transferred into CD34+ cells of five patients (two with ovarian cancer, two with breast cancer, and one with glioma) undergoing high-dose chemotherapy and autologous stem-cell transplantation as marrow chemoprotection. Only two patients showed a low level of MDR1 expression, at 3 and 10 weeks after transfer respectively [8]. Numerous clinical trials using the concept of MDR1-transfer into myelopoietic stem cells have been initiated [2, 5].

Immunopotentiation strategies

The concept of immunological tumour therapy has been studied for more than a hundred years. Recently, gene therapy approaches have brought new optimism to the field of tumour immunology, and both immune effector cells and tumour cells have been identified as possible targets of gene transfer.

Improving efficacy of immune effector cells

The first gene therapy study in oncology transferred the gene coding for tumour necrosis factor (TNF) into tumour-infiltrating lymphocytes (TIL) to increase their antitumoral activity. With the same aim a multitude of different cytokines, cytokine receptors, adhesion molecules and "chimeric antibody/T-cell-receptor molecules" have been introduced into different immune effector cells in animal models and clinical trials in humans [5, 6]. Given the complexity of the antitumoral immune response it is unlikely that the restriction to one transferred cytokine gene and one type of effector cell could turn out to be an effective means of destroying a malignant tumour in vivo.

Increasing immunogenicity of tumour cells

Conceptually more convincing are attempts to increase the immunogenicity of a tumour by the introduction of foreign genes directly into tumour cells. This approach may be effective even if neither the immune effector cells involved in tumour cell killing nor tumour-specific antigens (TSA) potentially recognised by these effector cells are clearly defined.

The most frequently used approach consists of the introduction of cytokine genes such as IL-2, IL-4, IL-7, IL-12, INFγ, TNFα, or GM-CSF into tumour cells. When injected intratumorally, subcutaneously or by other routes, these manipulated tumour cells are capable of producing high local amounts of the respective protein without causing the usual systemic side effects of cytokines. Since transfection of autologous tumour cells of individual patients is cumbersome and prone to technical problems in clinical trials, and since efficient vectors for in situ transfection are not currently available, many groups have chosen other ways to overcome these restrictions. Allogeneic tumour cells potentially carrying the same specific antigens as the patients' tumours can be transfected with a cytokine gene and then injected subcutaneously or into the lymphatic system. Alternatively, other autologous, allogeneic or xenogeneic cells such as fibroblasts can be transfected with cytokines, mixed with irradiated tumour cells of the patient and then reinjected into the patient. The common principle of all these studies is the creation of an immunostimulatory paracrine milieu in close proximity to tumour-specific antigens.

In Basel, we have performed a phase I study in different solid tumours and a multicentric phase II study in 28 patients with melanoma, using intratumoral injection of cytokine-transfected monkey fibroblasts (Vero-IL-2 cells), thus demonstrating the limited efficacy of this approach [9] (fig. 1).

In addition to cytokine genes, foreign MHC (major histocompatibility complex) molecules, viral antigens, known TSAs such as the MAGE (melanoma antigen) genes in melanoma or co-

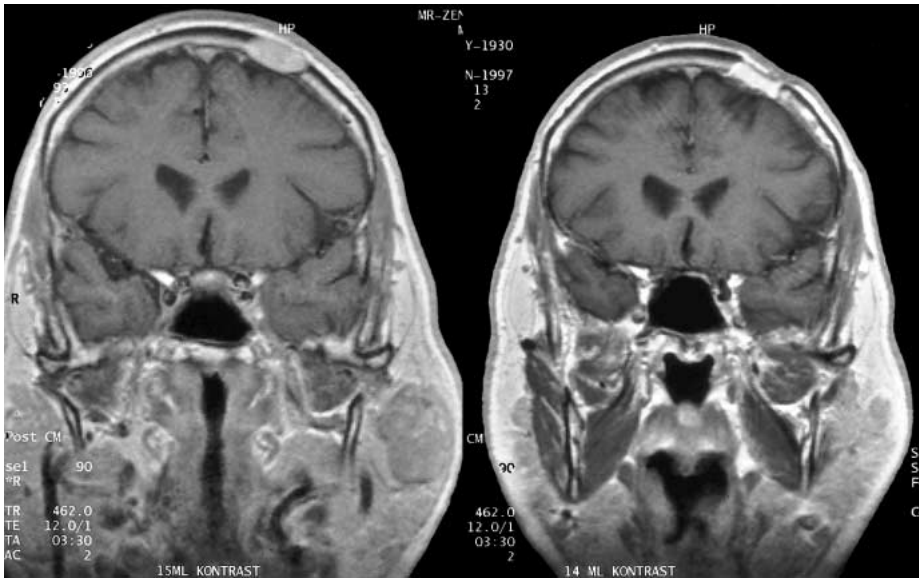


Figure 1

MRI scans of a patient with a leiomyosarcoma with metastases to the elbow, the parotid gland, and the skull. Two months after repeated injection of the elbow metastasis with $5x10^5$ Vero-IL-2 cells, the metastasis in the parotid gland started to decrease in size, and 12 months after therapy, reduction of its volume by more than 90% was seen. The second non-injected metastasis to the skull also decreased in volume by approximately 50%. The scans shown were done immediately before (left) and 12 months after (right) therapy. (Courtesy of G. Bongartz, Division of Radiology, Kantonsspital, Basel)

stimulatory molecules such as B7-1 or B7-2, have been used to increase the immunogenicity of tumour cells in animal models and clinical studies. The different approaches to genetic modification of tumour immunogenicity account for approximately two thirds of all gene therapy studies performed in patients with malignancies, and anecdotal evidence of therapeutic success in individual patients has been reported [6].

Suicide gene therapy

The common principle of suicide gene strategies is selective intratumoral activation of otherwise non-toxic drugs by specific transfer of the activating transgene into tumour cells. The best known example of this type of prodrug activator is the gene coding for herpes simplex virus thymidine kinase (HSV-TK). As opposed to human thymidine kinase, HSV-TK phosphorylates the antiherpes drug ganciclovir to toxic triphosphates with very high affinity. A clinically interesting observation in different models of suicide gene therapy was that transfection of only a minority of tumour cells with the prodrug activator gene could be sufficient to bestow clear benefit on treated animals. Although it was anticipated that a similar "bystander effect" might help to solve the problem of inefficient gene transfer in humans, a study of HSV-TK suicide gene therapy in 15 patients with glioma showed only limited success in smaller tumours (<1.4 ml) and limited gene transfer of only a couple of cell layers around the needle tracks [10]. However, other intratumoral suicide gene therapy trials in prostate and breast cancer have shown tumour regression in a substantial number of patients [11], and improvements in suicide gene therapy may be forthcoming with other potentially more effective enzyme-prodrug systems, or with an increase in tumour specificity by the use of tumour specific promotors such as the PSA (prostate specific antigen) promotor in prostate cancer or a CEA (carcinoembryonic antigen) promotor in tumours expressing CEA.

Repair of damaged cell cycle

The development of malignant tumours is caused by, and closely linked to, alterations in two groups of genes which are involved in the regulation of the normal cell cycle, namely oncogenes and tumour suppressor genes. Oncogenes are generally activated by amplification, overexpression, mutation, or translocation, and alteration in one allele of an oncogene is usually sufficient to cause cell cycle dysregulation. Deactivation of tumour suppressor genes, on the other hand, occurs by deletion or mutational inactivation, and these molecular alterations are frequently recessive, i.e. only the inactivation of both copies of the gene provides the basis for failure of the particular protein function.

Inactivation of oncogenes

Among the most important oncogenes alterated in human tumours are the genes *ras*, *c-myc*, *c-erbB-2*, *abl*, and *bcl*-2. Several phase I studies targeting these genes have been carried out or are in progress, and a trial using daily subcutaneous infusion of an antisense oligonucleotide against *bcl*-2 in nine patients who had relapsed non-Hodgkin lymphoma led to negligible toxicity and several clinical responses [12].

Reactivation of tumour suppressor genes

Tumour suppressor genes lost or mutated in human cancer include the retinoblastoma gene, the gene for the cyclin-dependent kinase inhibitor p16 and the p53 gene. Mutations/deletions of p53, with frequencies of 40–80% in colorectal, bronchial, bladder, and oesophageal carcinoma, can lead to loss of tumour-suppressor function, increased drug resistance, loss of mutational repair, increased tumour angiogenesis, cellular proliferation, and inhibition of apoptosis. Gene transfer of wild-type p53 was shown to reverse these deficiencies and to induce apoptosis in vitro and in preclinical in vivo tumour models. A pilot study in 9 patients with non-small cell lung cancer supports this view [13].

Figure 2

CT scans of a patient treated with 10^9 PFU of adenovirus-p53 before (left) and two months after (right) therapy. The reduction in the size of the injected lesion fulfills the criteria of partial remission. The decrease in the volume of the pleural effusion is due to pleurodesis performed immediately before gene therapy. (Courtesy of G. Bongartz, Division of Radiology, Kantonsspital, Basel)

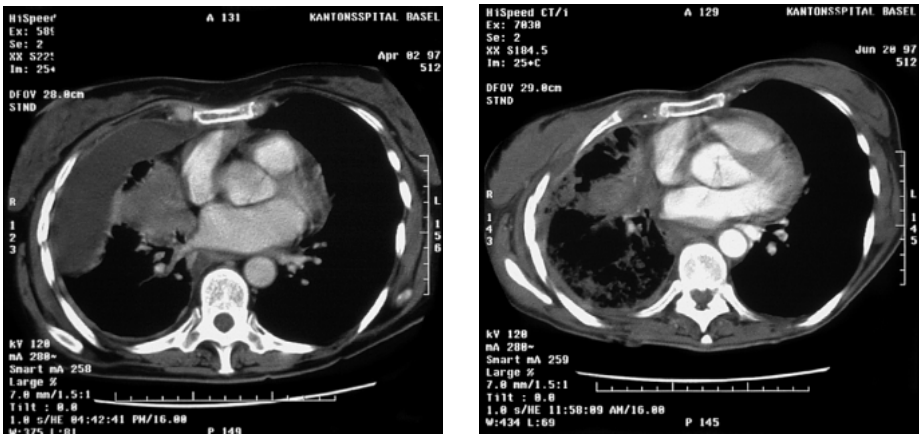
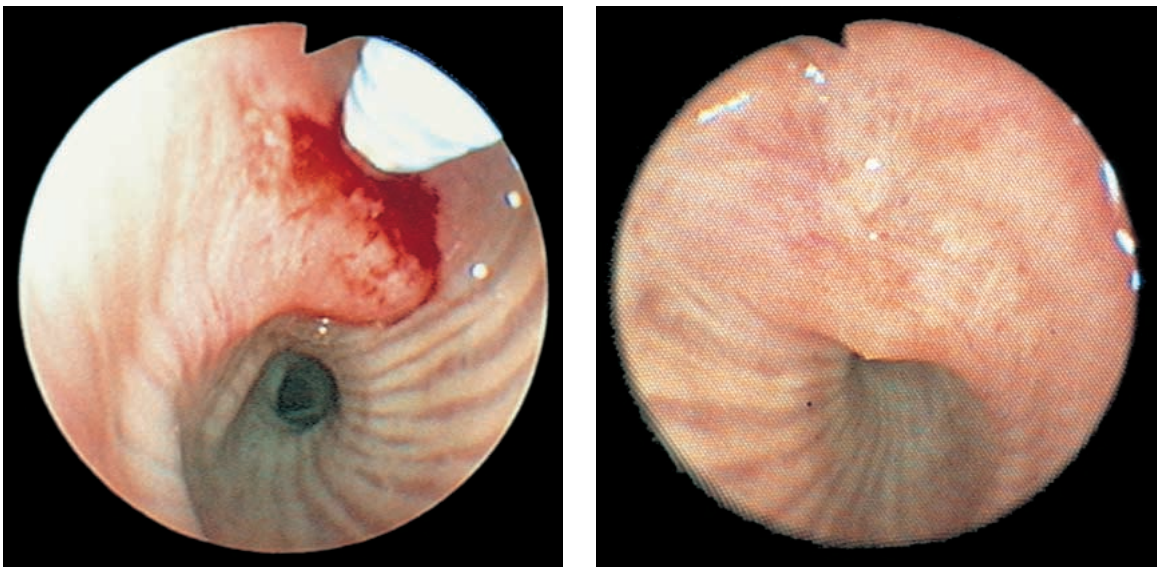


Figure 3

Bronchoscopy in a patient with an adenocarcinoma of the right upper lobe. Figure 3a shows the injection of adenoviral solution using a 22-G-Millrose-catheter. Figure 3b shows the same lesion after three cycles of combined chemo-gene therapy, demonstrating a very good remission of the injected tumour with chiefly scar tissue remaining in situ. (Courtesy of A.P. Perruchoud, Division of Pneumology, Kantonsspital, Basel)



Based on these results we initiated a phase I dose escalation study of a single intratumoral injection of a replication-defective adenoviral expression vector encoding wild-type p53 [14]. Fifteen patients with incurable non-small cell lung cancer were treated in two centres (Mainz/Basel). Treatment was performed either by bronchoscopic intratumoral injection or by CT-guided percutaneous intratumoral injection of the vector solution. No clinically significant toxicity was observed and successful transfer of wild-type p53 was achieved with higher vector doses. Transient local disease control by a single intratumoral injection of the vector solution was observed in several patients, and in one patient a clinical response (partial remission = >50% reduction) of the injected lesion was observed two months after injection (fig. 2).

We next tested the combination of repeated injections of adenovirus-p53 with concurrent platinum containing standard chemotherapy in some 30 patients with non-small cell lung cancer in a multicentre international study, demonstrating the absence of unexpected toxicities and frequent responses to combined therapy (submitted; fig. 3).

At last year's ASCO (American Society of Clinical Oncology) meeting, Yver et al. presented their experience of six different trials involving 226 patients with head and neck cancer and 83 patients with non-small cell lung cancer treated with an adenoviral vector containing the wildtype p53 gene [15]. In all the trials several patients responded to p53 gene therapy, but although no formal evaluation of response rates was given it is clear that fewer than 10% of treated patients benefit from this form of therapy.

Conclusion

A 1996 evaluation of the first 7 years of gene therapy concluded that gene therapy was safe and feasible, but that none of the more than one hundred clinical studies performed so far had formally proven the efficacy of the approach in any human disease. Although anecdotal reports of tumour responses are becoming more frequent in several human malignancies, the situation has not changed dramatically. Main problems are still the lack of vectors with high transduction efficiency in

vivo, the low tumour specificity of available systems, the progressive down-regulation of transcription elements in vivo even after successful transfer of a transgene, and our incomplete knowledge of molecular tumour pathology.

However, the results of some of the published phase I and II studies of cancer gene therapy are encouraging, and important progress in vector technology is expected in the near future. It is likely that tumour-specific vectors and tissue-specific promotors will be in routine use within the next 5–10 years, and that completion of the human genome project will lead to major advances in our understanding of the molecular aspects of carcinogenesis, tumour progression and metastasis. The first intravenously administrable vectors should be available at the end of this period and expression of a transgene in a sufficient percentage of targeted tumour cells should be achieved. The most likely role of gene targeting approaches to the treatment of cancer during the next two decades will be a modest contribution within a multimodality treatment concept consisting of surgery, radiotherapy, chemotherapy, immunotherapy, and endoscopic modalities. At present it would be wholly speculative to predict which of the different approaches to gene therapy of cancer discussed above will become the field's first truly effective contribution to the oncologist's arsenal.

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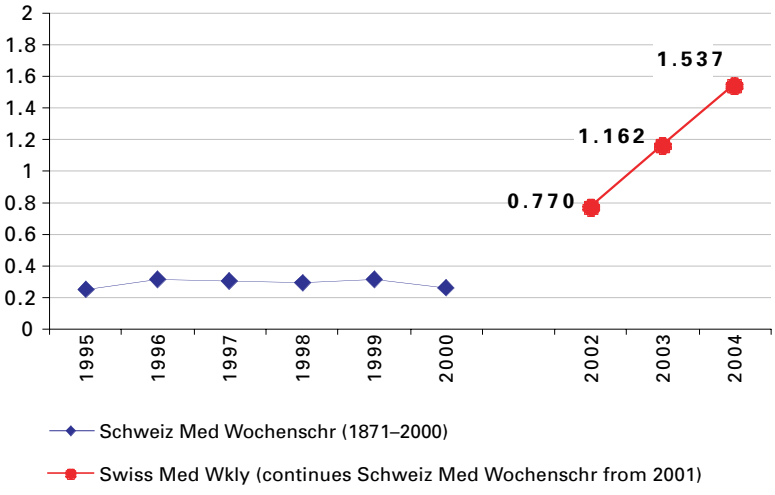

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