This publication is the result of a project commissioned by STOA under Framework Contract IP/A/STOA/FWC/2005-28 on "Future Development of Cancer Therapy". It contains contributions and discussions arising from a workshop that took place at the European Parliament in Brussels in February 2007 under the title "Gene, Vaccine and Immuno-therapies against Cancer: New Approaches to an Old Problem".

Only published in English.

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Manuscript completed in February 2007.

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Executive summary

The search for new cancer therapies is one of the most pressing tasks of biomedical science. Due to cancer's complexity and heterogeneity, we obviously cannot expect to find one therapeutic solution for all the cases and its forms. The tremendous increase in our knowledge of the molecular biology of carcinogenesis (the mechanisms of cancer formation) has led to the development of new therapeutic approaches during the past 10-20 years. The most important can be classified as gene therapy, vaccine therapy (or "active" immunotherapy) and antibody therapy (or "passive" immunotherapy).

All the new approaches to cancer therapy are linked by the scientific finding that cancer is a disease resulting from the accumulation of genetic modifications within a cell. In delineating these new therapeutic strategies, the basic premise is to determine as many properties of cancer cells as possible and outline an effective biomedical action against them. It is very difficult to distinguish consistently between the different therapeutic approaches because they do not appear as clear cut methods, but rather as basic strategies or concepts that often follow the same paths and use the same tools. Almost all approaches focus on several different targets in the patient's body. Altering cancer cells (inside or outside the body, connected with delivery via gene therapy) and/or cancer-specific targets in combination with the activation or support of the patient's own immune system seems to yield a promising treatment. Nevertheless, it is still not fully understood which components of the immune system are best addressed by vaccine or antibody approaches.

Medical results and prospects

Most of the new therapies are at an early stage of development. The ones that currently are medically most relevant either follow the preventive vaccine approach (directed at "transmissible", virus-related cancers) or are "passive" immunotherapies using monoclonal antibodies such as trastuzumab (Herceptin®). An immune response, the goal of "active" immunotherapies, has a potentially long-term clinical impact on the course of the disease by stabilising the patient's condition, and thus prolonging survival, rather than by destroying much or all of the tumour. The patients most likely to benefit are, therefore, those who have a minor tumour burden or who have undergone surgical tumour removal but have a high risk of relapse.

All three fields of innovative cancer therapy hold significant potential for the treatment of tumour patients within the next decade, whereby vaccine and antibody therapies are probably the most promising, while gene therapy will in many cases serve rather as a supporting method. Traditional hormonal and chemotherapies will not lose their relevance in the foreseeable future, but will increasingly be combined with different forms of immunotherapies. Surgery will remain important as well, firstly, because it is necessary for histological diagnosis and, secondly, because surgery will be an integral part of all those innovative treatments delivered directly to the tumour tissue.

Research funding and economic aspects

The new approaches to cancer therapy seem to be well-represented in the EU research programmes and funding in European Framework Programmes FP6 and FP7. FP7 will reinforce the clinical aspects of cancer research. It is important to be aware of the weaknesses of cancer immunotherapies, in principle due to products that are often very complex and include several compounds, to the laborious technical procedures that are necessary or to the use of patient-specific biological material.

Consequently, the commercial potential of many immunotherapeutic products is limited. This field of research thus has an enormous need for the provision of economic resources in order to continue advancing.

Although the question of cost-effectiveness cannot reasonably be addressed in more detail for most of the new therapies due to their early stage of research and development, these rather individual or even personalised treatments will presumably be very cost intensive. Even today, use of the anti-cancer drug Herceptin® represents a substantial financial challenge to the public health insurance systems in many European countries, leading to very heterogeneous administration rates for economic reasons. On the other hand, while the costs of treatment will certainly increase, further developments in cancer therapy will push forward general scientific and medical innovation and improvement, leading to positive economic and societal effects.

Adjusting approval procedures and facilitating access to technology

At the moment, cancer immunotherapies are subject to the same regulations as the production of any other medical product for human use. The standard clinical study designs used for oncology drug development are based on criteria that are suitable for conventional chemotherapy but less appropriate for cancer vaccines, which are much more complex and heterogeneous. With the introduction of common EU regulations, a significant drop in the numbers of clinical cancer vaccine trials has been observed in Europe. Guidelines regarding the manufacture, quality control, and preclinical and clinical testing of cell-based products for human use are being developed by the European Medicines Agency and can hopefully be applied to cancer vaccines as well.

Company ownership rights and protective policies create various barriers to the study of new cancer drugs in combinatory cancer vaccine trials. Improved cooperation between laboratories, industry and clinics is essential for us to tackle remaining challenges and appreciate the full potential of cancer immunotherapy.

Challenges for future technology assessment studies

One topic that might be illuminating for future analysis would be to check which areas of cancer research are systematically under-represented on the current research agenda, e.g. because they promise no or only very limited economic benefits. A study should be scheduled on a medium-term basis to provide useful results for the second part of the European Research Programme 7 (FP7) and, above all, for the preparation of FP8. It is imperative for as many relevant institutions, organisations, and stakeholders as possible to be involved in order to gather and include all the relevant information and viewpoints and because of the possible impact of such an investigation.

Another important focus of future studies could be the possible improvement of the EU regulation on clinical studies and clinical testing with the aim of increasing medical performance and economic competitiveness. In view of the demands from many areas of up-to-date drug and therapy development for current evaluation and approval procedures to be adjusted, the question should be considered whether restricting studies to a single technology or, on the contrary, incorporating different case studies would give more comprehensive results.

1. Introduction

Cancer is one of the main health problems of mankind. Despite extensive research being conducted over many years, effective cures have been developed only for some forms of cancer, so that the menace is not substantially reduced. The search for new and better therapies is a constant challenge to the biological and medical sciences and should undoubtedly be supported by European Research Policy in a very fundamental way.

The following paper is the final result of the technology assessment (TA) pre-study "Future development of cancer therapy". The mission of this pre-study carried out on behalf of the European Parliament's Scientific Technological Options Assessment (STOA) Panel was to give an overview of the state of research on cancer aetiology and therapy on the basis of available literature (especially from TA studies covering aspects of the subject) as well as an analysis of the status of cancer research in the European Framework Programme. During the project, the analysis became focussed on a more detailed picture of the most promising current cancer therapy approaches, i.e. gene therapy, vaccine therapy and immunotherapy.

The overall aim of this pre-study is to provide a basis for the STOA panel to decide on more comprehensive and detailed analyses or TA studies covering the subject of cancer research and therapy.

Methodology and scope of the project

A preliminary literature scan (conducted in the preparation phase of the project) revealed hardly any relevant TA studies or other high-level policy consulting reports dealing with the topic "current cancer therapy approaches". The literature analysis thus had to be expanded to include reviews and original papers in scientific journals. On the basis of a first analysis of relevant web-based information, as well as available reviews and interviews with specialists (from research & development departments of universities, national institutes, pharmaceutical and biotechnology companies as well as health professionals from hospitals), four therapy approaches were identified, which represent the most advanced biomedical research and development and which are regarded as playing an important role for cancer treatment in the near future:

gene therapy,

stem cell therapy,

vaccine therapy and

immunotherapy (also referred to as antibody or "biological" therapy).

Detailed research of the literature (covering publications from 2002 to 2006), using these terms and related key words, revealed a list of some 150 articles. An analysis of these papers was used as the basis for a draft background paper and a questionnaire, mainly orientated towards the relationship of, synergies between and comparison of the different therapeutic approaches. The background paper and questionnaire were used as a starting point for collaboration with six experts, all of them scientists and physicians, covering the four therapy approaches:

Dr. Nedime Serakinci (Stem Cell Therapy), Dr. Inge Marie Svane and Dr. Per thor Straten (Vaccine Therapy), Prof. Dr. Michael Untch (Antibody/Immunotherapy), Prof. Dr. Michael Weller (Neuro-oncology), Prof. Dr. Burghardt Wittig (Gene Therapy).

All six contributed to the project by answering the questionnaire and by writing a short status report (expert opinion) on their special area of expertise, and four of them presented their point of view during the STOA Workshop "New Therapies for Cancer: Prospects, Promises and Problems" on February 7, 2007 in the European Parliament in Brussels.

The results of the literature review, the answers from the questionnaires as well as the results of the expert opinions (cited as "N.N., expert opinion") and additional information from the presentations have been integrated in a condensed form in this paper. Contributions by other participants from the discussions during the workshop were also taken into account, especially with regard to the concluding sections of the report (Sects. 10 & 11).

Structure of the report

The following two sections (2 & 3) provide an introductory overview of cancer as a basic medical problem and of cancer incidence and mortality in the EU, taking Germany as an example. Section 4 then goes into some detail regarding the molecular biology of carcinogenesis – quite a complicated and demanding scientific issue, but unavoidable as a precursor to depicting and discussing the new therapeutic approaches in sections 5 (gene therapy), 6 (immunotherapy) and 7 (vaccine therapy). Since the examples of exploring stem cells as therapeutic tools in the fight against cancer are very rare, we decided not to classify stem-cell therapy as a distinct category, but to regard it as part of the gene therapy approaches. Basically, as our analysis shows, it is very difficult to distinguish between the different therapeutic approaches, both in terms of classification (especially when the terms "biological", "vaccine", "antibody" and/or "immuno" are not used consistently) as well as of conceptual overlaps, since in many cases different therapeutic approaches are combined. Therefore, a comparison of the therapeutic approaches (in section 8) does not lead to any judgment on alternatives, but rather to an examination of their synergies. The coverage of cancer research and therapy by the European research programmes is delineated in section 9. Section 10 then summarises the insights gained by this report, and in the final section 11, some ideas are presented on possible future analyses and studies covering the subject of cancer research and therapy.

The complete list of scientific publications yielded by the literature scan, as well as the completed questionnaires and presentation slides from the workshop are provided separately in a supplement aimed at readers with specialised interests.

2. Cancer as a basic medical problem

Cancer is the second leading cause of death worldwide. Out of a total of 58 million deaths worldwide in 2005, cancer accounted for 7.6 million or 13%. The main types of cancer are: lung (1.3 million deaths/year), stomach (almost 1 million deaths/year), liver (660 000 deaths/year), colon (655 000 deaths/year) and breast (500 000 deaths/year). More than 70% of all cancer deaths in 2005 occurred in low and middle income countries. Cancer mortality is expected to increase further, with an estimated 9 million people dying from cancer in 2015, and 11.4 million in 2030 (WHO 2006).

Although much effort and scientific progress has been made in the last two decades, there is still a lot of work to be done in order to improve the patients' situation. It is important to note that cancer is not a single disease with a single type of treatment. There are more than 100 different kinds of cancer, each with at least one treatment. However, improvements in cancer detection, diagnosis, and treatment have increased the survival rate for many types of cancer (www.cancer.gov).

2.1 Definition and classification

Not all tumours are cancerous; they can be either benign or malignant.

Benign tumours are not cancerous. They can often be removed and, in most cases, they do not reappear. Cells from benign tumours do not spread to other parts of the body. And, most importantly, benign tumours are rarely life-threatening.

Malignant tumours are cancerous. Cells in malignant tumours are abnormal and divide without any control. Cancer cells invade and destroy the tissue surrounding them. Cancer cells can also break away from a malignant tumour and enter the bloodstream or lymphatic system.

Blood vessels include a network of arteries, capillaries, and veins through which the blood circulates in the body. The lymphatic system carries lymph and white blood cells through lymphatic vessels to all the tissues of the body. By moving through the bloodstream or lymphatic system, cancer can spread from the primary (original) cancer site to form new tumours in other organs. The spread of cancer is called metastasis.

Five main categories are used in the classification of cancer:

Carcinoma: A carcinoma is a cancer found in epithelial tissue that covers or lines surfaces of organs, glands, or body structures. Many carcinomas affect organs or glands that are involved in the production of secretion, such as breasts. Carcinomas account for 80-90% of all cancer cases.

Sarcoma: A sarcoma is a malignant tumour growing from connective tissue, such as cartilage, fat, muscle, tendons, and bones. The most common sarcoma, a tumour on the bone, usually occurs in young adults. Examples of sarcoma include osteosarcoma (bone) and chondrosarcoma (cartilage).

Lymphoma: Lymphoma refers to a cancer that originates in the nodes or glands of the lymphatic system (spleen, bone marrow), which produce white blood cells and clean body fluids, and invades organs such as brain and breast. Due to morphological differences, lymphomas are classified into two categories: Hodgkin's lymphoma and non-Hodgkin's lymphoma, which occur more frequently.

Leukaemia: Leukaemia, also known as blood cancer, is a cancer of the bone marrow that keeps the marrow from producing functioning red and white blood cells and platelets. White blood cells are needed to resist infection.

Red blood cells are needed to prevent anaemia. Platelets keep the body from bruising and bleeding easily. Examples of leukaemia include acute myelogenous leukaemia, chronic myelogenous leukaemia, acute lymphocytic leukaemia, and chronic lymphocytic leukaemia. The terms 'myelogenous' and 'lymphocytic' indicate the type of cells involved.

Myeloma: Myeloma grows in the plasma cells of bone marrow. In some cases, the myeloma cells accumulate in one bone and form a single tumour, called a plasmacytoma. However, in other cases, the myeloma cells accumulate in many bones, forming many bone tumours. This is called a multiple myeloma.

2.2 Causes and risk factors of cancer

There is not one single cause of cancer, it is rather the interaction of many factors. The factors involved are age, genetics, environment, chronic infections and lifestyle. As will be shown in section 4 in more detail, cancer occurs because of changes in genes responsible for cell growth and repair. These changes are the result of the interaction between genetic host factors and external agents which can be categorised as (Stewart/Kleihues 2003)

physical carcinogens such as ultraviolet (UV) and ionising radiation

occupational exposure to chemical carcinogens such as asbestos

lifestyle such as heavy alcohol drinking and tobacco smoking

environmental pollution in air, water and soil, e.g. sulphur dioxide (SO_2)

dietary and nutritional factors such as excess salt intake or obesity in combination with lack of physical activity

genetic susceptibility in forms of inherited mutations

biological carcinogens such as

chronic infections by viruses, such as hepatitis B virus (HBV, causing liver cancer) or human papillomavirus (HPV: cervical cancer), bacteria such as *Helicobacter pylori* (causing gastric cancer) or parasites such as *Schistosoma haematobium* (causing bladder cancer)

contamination of food by mycotoxins such as aflatoxins (products of *Aspergillus* fungi) causing liver cancer

Tobacco use is the single most important risk factor for cancer and causes a large variety of cancer types such as lung, larynx, oesophagus, stomach, bladder, oral cavity, accounting for approximately 30% of all cancer cases. Although there are still some open questions, there is sufficient evidence that dietary factors also play an important role in causing cancer. This applies to obesity as a compound risk factor per se as well as to the composition of the diet such as lack of fruit and vegetables and high salt intake. Lack of physical activity has a distinct role as a risk factor for cancer. There is solid evidence that alcohol can cause several cancer types such as oesophagus, pharynx, larynx, liver, and breast (WHO 2006). An estimated 40% of cancer could be prevented by healthy diet, physical activity and non-smoking.

Age has a great impact on survival rates and types of the cancer. The survival rate during childhood is about 75 percent, while the survival rate of adults with cancer is 60 percent. This difference is thought to be caused by genetic alterations, accumulating during time. Cancer in children is more responsive to therapy, because children tolerate more aggressive therapy.

Environmental exposures to, e.g., pesticides, fertilisers, and power lines have been investigated as a direct cause of childhood cancers. There is evidence of cancer occurring among non-related children in certain neighbourhoods and/or cities.

2.3 Therapy options in general

Treatment of cancer can include surgery, radiation therapy, chemotherapy, hormone therapy, and biological therapy. One single method or a combination of methods may be used, depending on various factors such as the type and location of the cancer, whether the disease has spread, and the patient's age and general health. As the treatment of cancer can also damage healthy cells and tissues, it often causes side effects, which are sometimes worse than the disease itself. Physicians generally discuss the treatment options carefully, weighing the likely benefits of killing cancer cells against the risks of possible side effects.

Surgery means an operation to remove cancer. The side effects of surgery depend on many factors, including the size and location of the tumour, the type of operation, and the patient's general health status. In some cases, there is pain after surgery, which can usually be controlled with drugs. It is also common for patients to feel tired or weak for a while after surgery.

Radiation therapy (or radiotherapy) uses high-energy rays to kill cancer cells in a targeted area. Radiation can be administered externally by an instrument that targets radiation at the tumour area. It can also be introduced internally: needles, seeds, wires, or catheters containing a radioactive substance are placed directly in or near the tumour. Radiation treatments are painless. The side effects are usually temporary, and most of them can be treated or controlled. Radiation therapy may cause a decrease in the number of white blood cells, which help to protect the body against infections. With external radiation, it is also common for the patient to suffer temporary hair loss in the treated area and for the skin to become red, dry, tender, and itchy.

External radiation does not cause body radioactivity. With internal radiation (or implant radiation), the patient may need to stay in the hospital, separated from others, while the radiation level is at its peak. Implants may be permanent or temporary. The amount of radiation in a permanent implant decreases to a safe level before the patient leaves the hospital. With a temporary implant, no radioactivity is left in the body after the implant is removed.

Chemotherapy is the use of drugs that kill cancer cells throughout the body. Healthy cells can also be harmed, especially those that divide quickly. One may use one drug or a combination of drugs. The side effects of chemotherapy depend mainly on the drug(s) administered and the dose(s). Hair loss is a common side effect of chemotherapy; however, not all anticancer drugs cause this. Anti-cancer drugs may also cause temporary fatigue, poor appetite, nausea and vomiting, diarrhoea, and mouth and lip sores. Drugs that prevent or reduce nausea and vomiting are used to treat these side effects. Normal cells usually recover when chemotherapy is over, so most of the side effects gradually disappear after the treatment ends.

Hormone therapy is used to treat cancers that depend on hormones for their growth. It works by preventing cancer cells from getting or using the hormones they need to grow. This treatment may include the use of drugs that either stop the production of certain hormones or change the way hormones work. Another type of hormone therapy is surgery to remove organs that produce hormones. For example, the ovaries may be removed to treat breast cancer, or the testicles may be removed to treat prostate cancer.

Hormone therapy can cause a number of side effects: tiredness, fluid retention, weight gain, hot flashes, nausea and vomiting, changes in appetite, and, in some cases, blood clotting. Hormone therapy may also cause loss of bone substance in pre-menopausal women. Depending on the type of hormone therapy used, these side effects may be temporary, long lasting, or permanent.

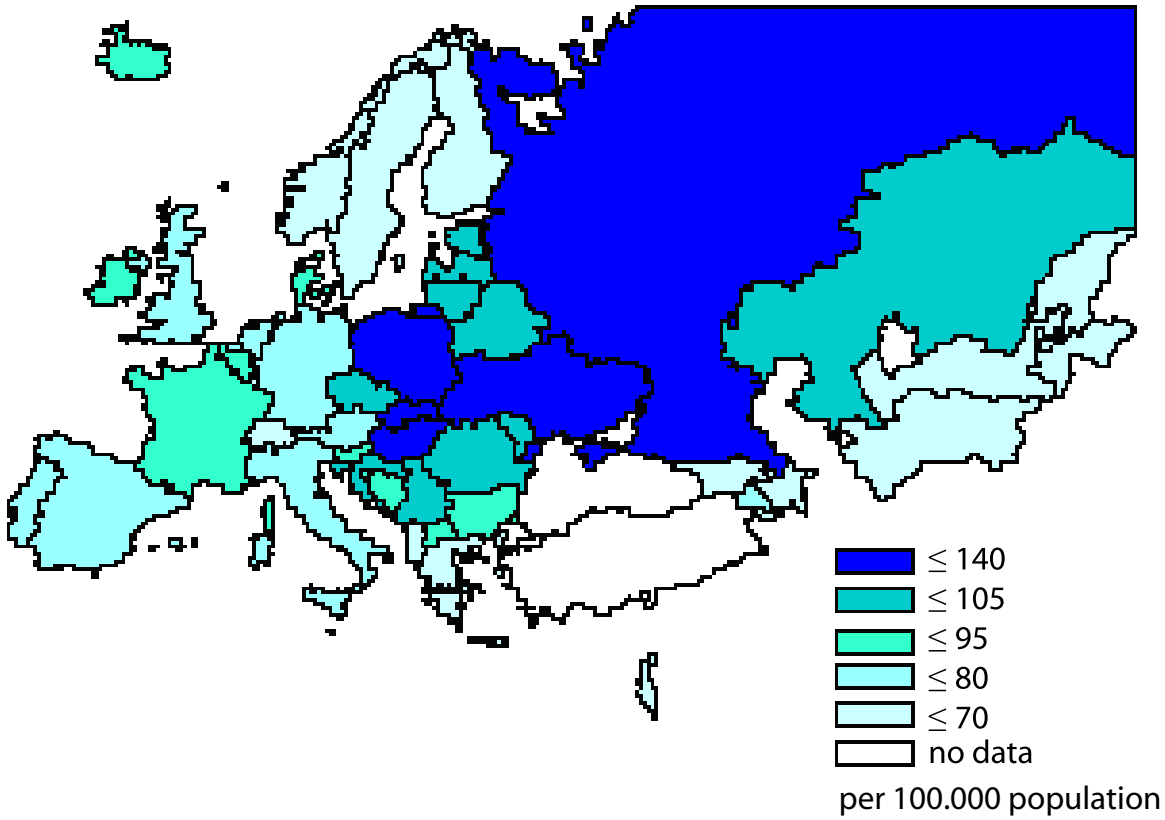
Biological therapies use the body's immune system, either directly or indirectly, to fight the disease and to alleviate some of the side effects of cancer treatment (and are a main focus of this report, see sections 6 & 7). Monoclonal antibodies, interferon, interleukin-2, and colony-stimulating factors are some of the pharmaceutical molecules used here.

The side effects caused by biological therapies vary according to the specific treatment. In general, these treatments tend to cause flu-like symptoms, such as chills, fever, muscle aches, weakness, loss of appetite, nausea, vomiting, and diarrhoea. Patients also may bleed or bruise easily, get a skin rash, or suffer swelling. These problems may be severe, but they usually vanish after the treatment is finished.

3. Cancer incidence and mortality in the EU and Germany

Although there a more than 100 different kinds of cancer worldwide, 13 types of cancer account for 80%-90% of all cancerous diseases. The following figures illustrate mortality and incidence rates for cancer as a whole, as well as for three selected, important types of cancer (prostate in men, cervix in women, and colon in both). The data are taken from the "Atlas of Health in Europe" (WHO 2003) and from the German society of epidemiological cancer registers (GEKID) together with the Robert Koch Institute (GEKID/RKI 2006).

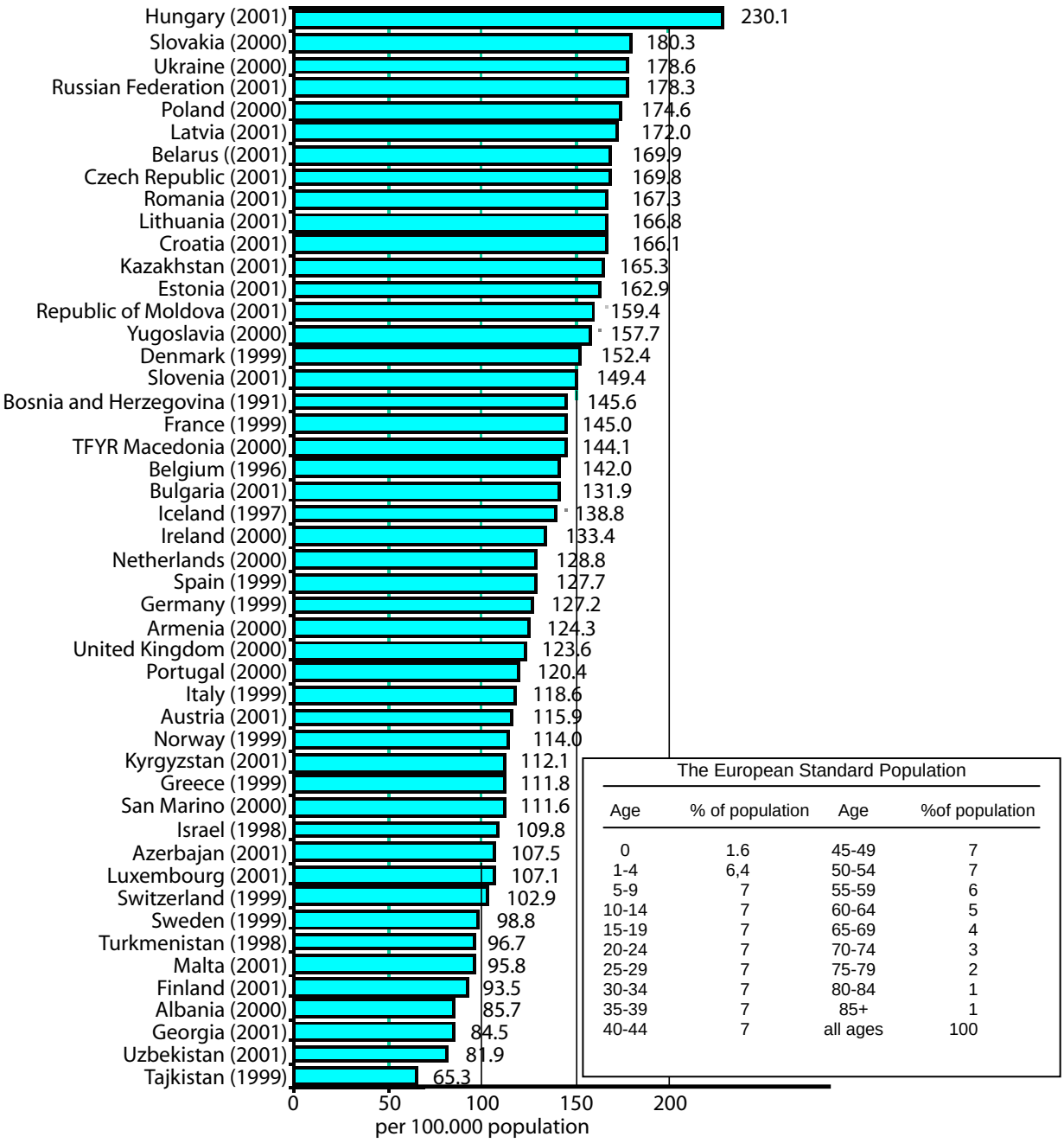
Fig. 1: Deaths from cancer in Europe (ASDR per 100000 population), 0-64 years



Data are given as age-standardised death rates (ASDR), showing the number of deaths in a "standard" population. This facilitates international comparability by removing differences in rates caused by different population age structures between countries. The European standard population was used to calculate the standardised death rate (SDR) (see figure 2).

Source: WHO 2003, p. 43 (http://www.euro.who.int/document/e79876.pdf)

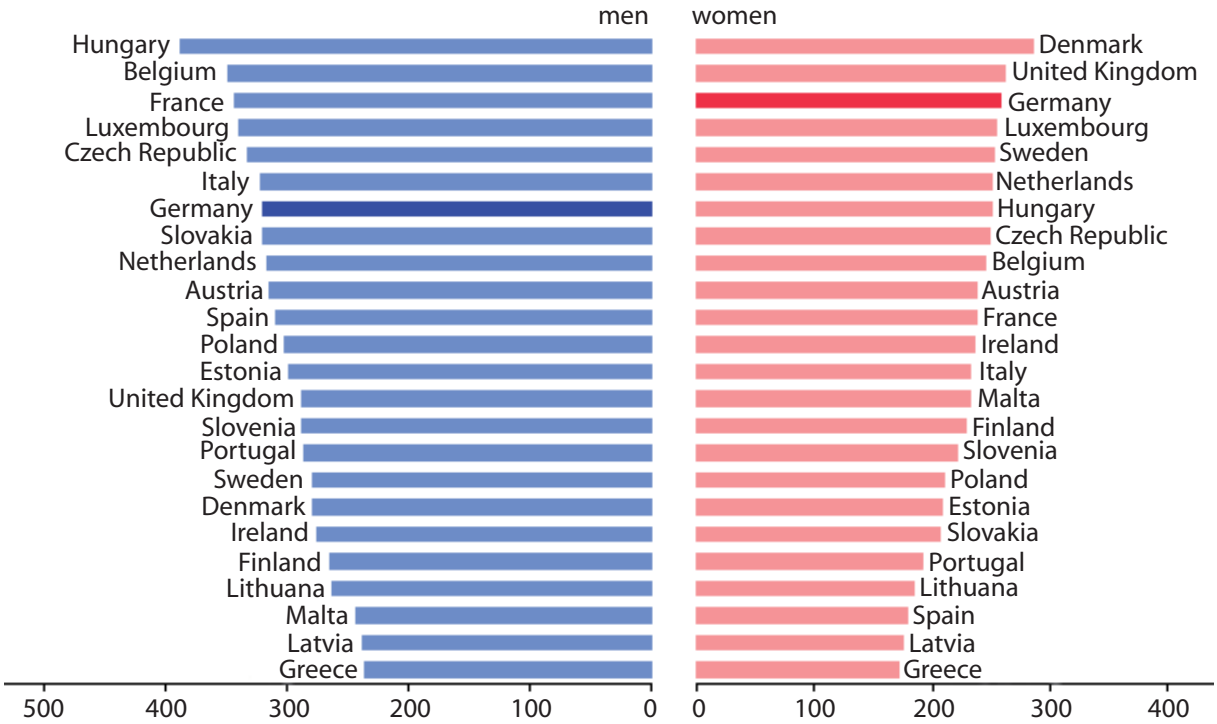
Fig. 2: Deaths from cancer in Europe (ASDR per 100 000 population), 25-64 years



Many factors, such as variations in national definitions, incomplete registration in some countries (e.g. former USSR countries) or other specificities in data recording and processing may influence the comparability of the national statistics. With the introduction of standardised death rates (SDR), better comparison could be achieved.

Source: WHO 2003, p. 42 (http://www.euro.who.int/document/e79876.pdf)

Fig. 3: Age-standardised cancer incidence in the European Community per 100 000 (2002)



Due to demographical development in the European Community, the incidence of cancer increases with the mean age of the population.

Source: GEKID/RKI 2006 (http://www.ekr.med.uni-erlangen.de/GEKID/Doc/kid2006.pdf), based on data from GLOBOCAN (http://www-dep.iarc.fr/globocan/database.htm)

Cancer mortality in Germany 2002 (GEKID/RKI 2006)

Men: lung (26.3%), colon (12.8%), prostate (10.4%), stomach (6.0%), pancreas (5.6%), kidney (3.5%), pharynx (3.4%), leukaemia (3.2%), oesophagus (3.2%), urinary bladder (3.1%), non-Hodgkin's lymphoma (2.5%).

Women: mammary gland (17.8%), colon (14.9%), lung (10,4%), pancreas (6.7%), ovaries (5.9%), stomach (5.8%), leukaemia (3.4%), non-Hodgkin's lymphoma (2.7%), uterus (2.7%), kidney (2.6%), urinary bladder (2.0%), cervix (1.8%).

Cancer incidence Germany 2002 (GEKID/RKI 2006)

Men: prostate (22.3%), colon (16.3%), lung (14.9%), urinary bladder (8.6%), stomach (5.1%), kidney (4.7%), pharynx (3.6%), pancreas (2.8%), malignant melanoma (skin) (2.8%), non-Hodgkin's lymphoma (2.7%), leukaemia (2.5%), testis (2.0%), oesophagus (1.7%).

Women: mammary gland (26.8%), colon (17.4%), lung (6.1%), uterus (4.8%), stomach (4.0%), malignant melanoma (skin) (3.7%), urinary bladder (3.5%), pancreas (3.2%), cervix (3.2%), kidney (3.1%), non-Hodgkin's lymphoma (3.0%), leukaemia (2.3%).

Fig. 4a: Prostate cancer in men (age-specific incidence per 100 000) (Germany 2002)

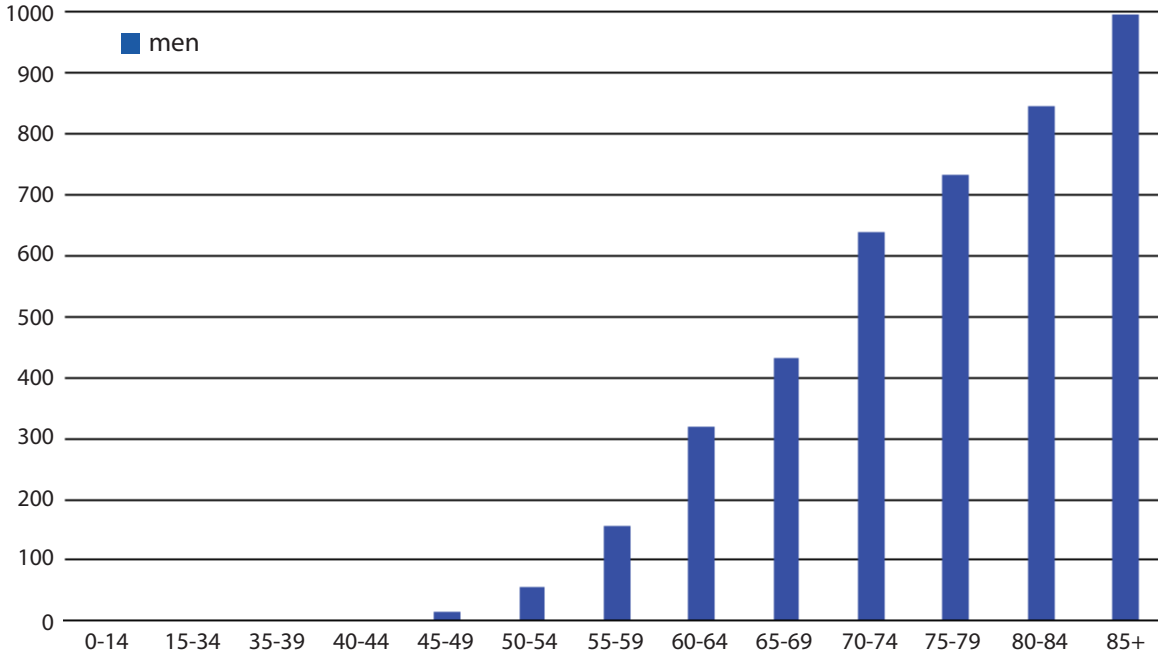
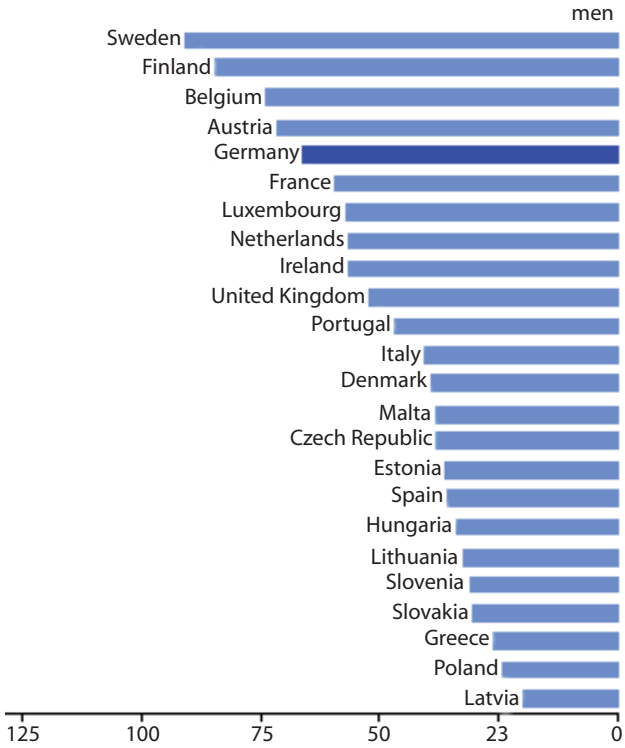


Fig. 4b: Age-standardised prostate cancer cases in Europe per 100 000 (2002)



Since the early 1980s, the national campaign for the early detection of prostate cancer has led to an increase in new incidences. This is due to the implementation of new diagnostic methods, e.g. the prostate-specific antigen (psa) test. The relative 5-year-survival rate increased to approximately 82%.

Source: GEKID/RKI 2006 (http://www.ekr.med.uni-erlangen.de/GEKID/Doc/kid2006.pdf), based on data from GLOBOCAN (http://www-dep.iarc.fr/globocan/database.htm)

Fig. 5a: Cervical cancer in women (age-specific incidence per 100 000) (Germany 2002)

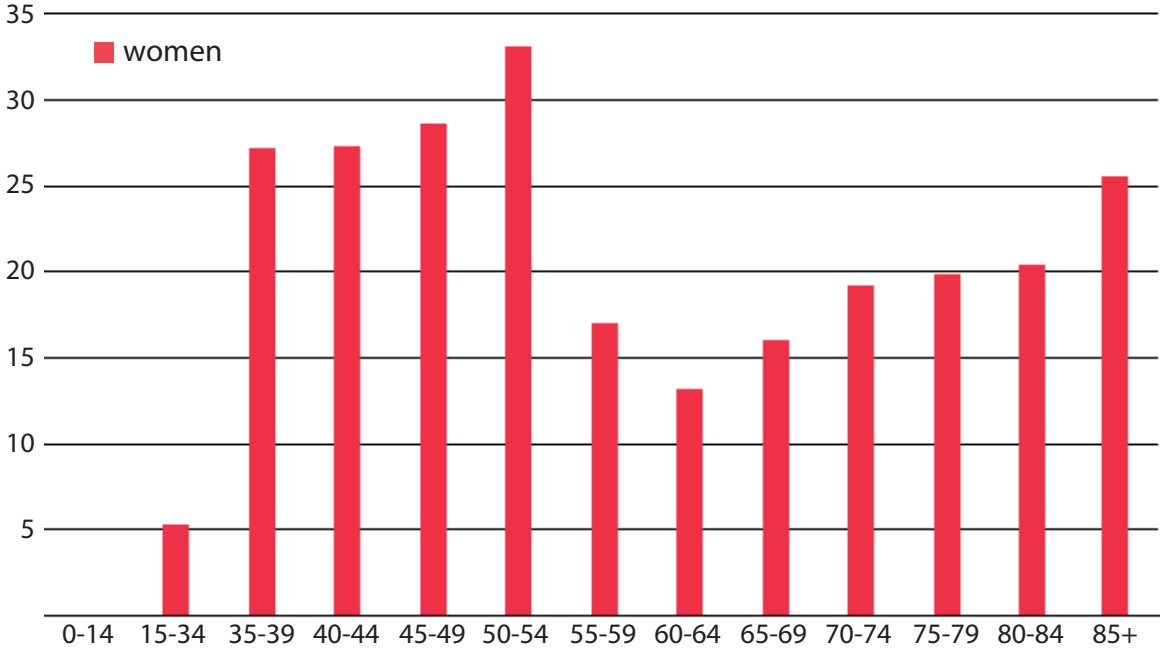
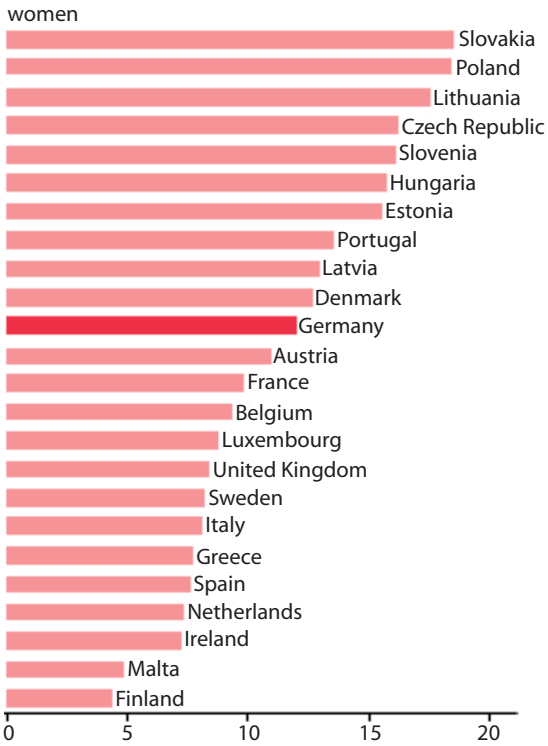


Fig. 5b: Age-standardised cervical cancer cases in Europe per 100 000 (2002)



There are two "age peaks" of incidence in cervical cancer, the first peak between 35 and 55 years and the second between 65 and 80 years. Chronic infections with HPV are the main cause of cancer. Co-infections with herpes simplex virus (HSV) and/or chlamydia are regarded to elicit the disease. The relative 5-year-survival rate increased in the last 20 years to approximately 67%. Strong efforts to develop a HPV vaccine resulted in a vaccine becoming commercially available in 2006.

Source: GEKID/RKI 2006 (http://www.ekr.med.uni-erlangen.de/GEKID/Doc/kid2006.pdf), based on data from GLOBOCAN (http://www-dep.iarc.fr/globocan/database.htm)

Fig. 6a: Colon cancer in men and women (age-specific incidence per 100 000) (Germany 2002)

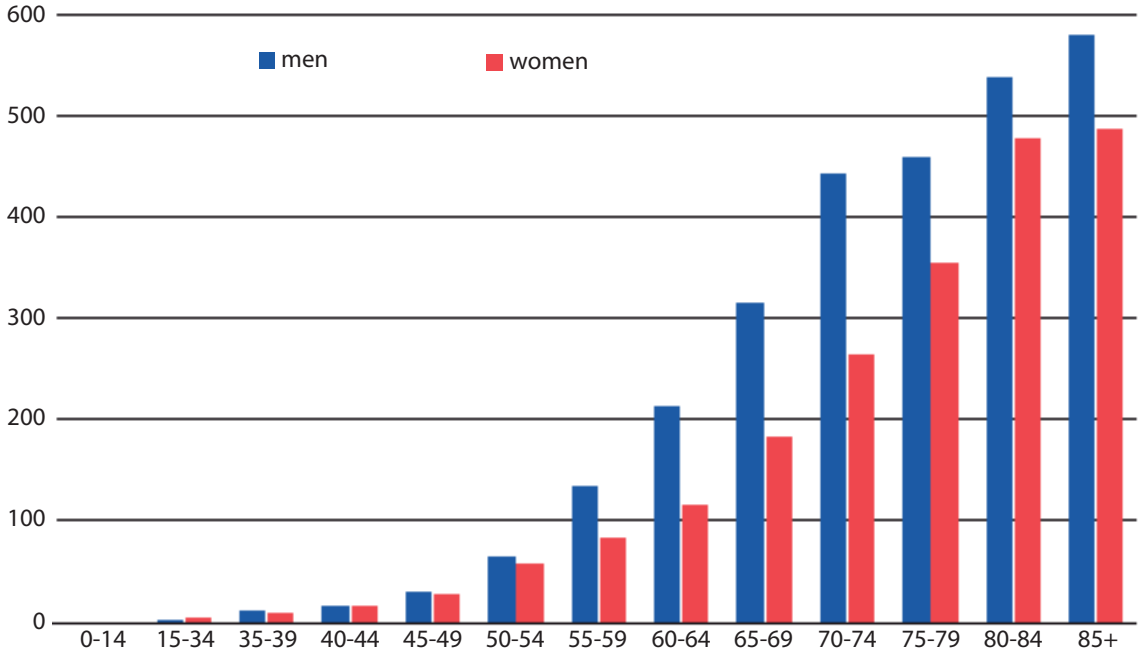
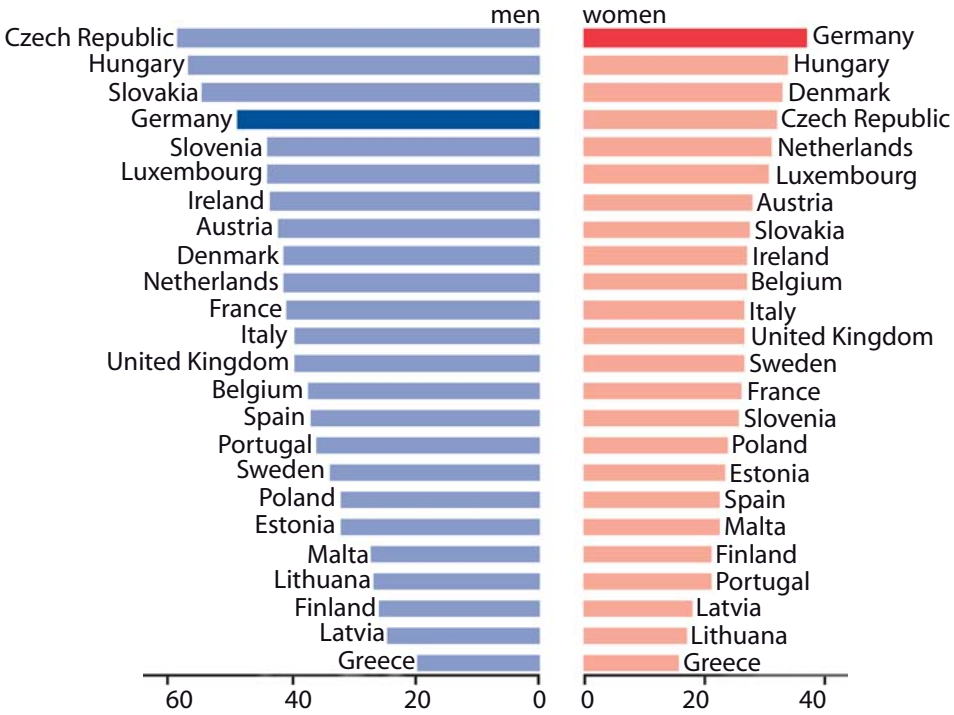


Fig. 6b: Age-standardised colon cancer cases in Europe per 100 000 (2002)



Colon cancer has the second highest incidence for both women and men. The average age for men is 69 years, for women 75 years. Colon cancer is also the second most frequent cause of death from cancer in men and women. The incidence has increased for men and women since 1970 due to national early detection programs. The relative 5-year-survival rate increased to approximately 56%

Source: GEKID/RKI 2006 (http://www.ekr.med.uni-erlangen.de/GEKID/Doc/kid2006.pdf), based on data from GLOBOCAN (http://www-dep.iarc.fr/globocan/database.htm)

4. Carcinogenesis

In order to understand, depict and analyse the recent therapy approaches to cancer, it is necessary to discuss the mechanisms of its formation (carcinogenesis). As the non-specialist reader will soon realise, the topics of this paper, like the project as a whole, are quite complicated and demanding scientific issues.

There are some specific properties which distinguish cancer cells from healthy tissue cells (see en.wikipedia.org)*:*

They are resistant to apoptosis (the main form of programmed cell death).

They have an uncontrolled ability to divide, often at an increased rate.

They are self-sufficient in growth signals.

They exhibit altered differentiation.

They are unsusceptible to anti-growth factors and contact inhibition.

They can invade adjacent cells or tissues (usually by secreting metalloproteinases, enzymes which digest extra-cellular matrices from other cells).

They secrete molecules, e.g. growth factors that stimulate the growth of blood vessels.

4.1. Uncontrolled proliferation

Proliferation or cell division is a physiological process that occurs in almost all tissues and under many circumstances. Normally, the balance between proliferation and programmed cell death (usually in the form of apoptosis) is maintained by tightly regulating both processes to ensure the integrity and functionality of organs and tissues. Carcinogenesis is thought to be provoked by mutations of genetic elements that influence this balance (called homeostasis) of proliferation and cell death. The result is uncontrolled cell division leading to tumour formation. An uncontrolled and rapid proliferation of cells can at first lead to benign tumours which can later develop into malignant tumours (cancer). Benign tumours do not spread to other parts of the body or invade other tissues, and they are rarely life-threatening unless they compress vital structures or are physiologically active (for instance, producing a hormone). Malignant tumours can spread to distant locations (metastasise), invade other organs, and thus become life-threatening.

Probably all cancers originate from a single cell (see also: the stem cell paradigm in section 4.7). This cell does not usually carry all properties for developing cancer at once, but develops step-by-step in a “micro evolution process”. More than one mutation seems to be necessary for the initiation of carcinogenesis. Typically, a series of several mutations of different classes of genes is required before a normal cell will transform into a cancer cell (this is called the “multi-hit model"). These mutations concern genes which play vital roles in cell division, cell death, and DNA repair and can cause a cell to lose control of its proliferation. With every further mutation, the cell develops a selective advantage against adjacent cells. This again leads to an increased chance of the descendants acquiring extra mutations, giving them even more selective advantages until a cell clone develops, which in combination with other mutations endangers the complete organism. Most experts assume that 5-10 mutations are the critical number to really start this process (only few theories say that 2 mutations can be sufficient). Cells that acquire only “some” mutations are thought to become a benign tumour. When cells accumulate “enough” mutations, they will become a malignant tumour.

4.2 Oncogenes

Cancer is quite definitely a gene disease. About 5% of cancer cases are regarded as being caused by inherited gene alterations, whereas more than 90% are results of the multifactorial gene mutations, described above. These mutations are thought to be promoted or provoked by lifestyle or environmental factors (see section 2.2). For example, smoking causes almost 30% of all cancer cases worldwide. Further factors are exposure to certain chemicals, obesity, nutritional factors and being exposed to sunlight for too long (UV radiation), particularly in children who are much more susceptible to environmental threats than adults (WHO 2006).

There are two major differences between cancer and so-called genetic diseases in the narrow sense. Firstly, cancer is caused mainly by mutations in somatic cells, whereas other genetic diseases are caused solely by mutations in the germline (however, some individuals have inherited genetic mutations that predispose them to develop specific types of cancer). Secondly, an individual cancer does not result from a single mutation, but rather from an accumulation of several incidences, depending on the type of cancer. More than 100 different genes (and their products) that play a role in carcinogenesis have already been identified, and it is obvious that there are still many more to be discovered (Fogar et al. 2005).

An oncogene is any gene that encodes for a protein able to transform into cancerous cells. Of the many known oncogenes, all but a few are derived from normal cellular genes whose products (proteins) participate in cellular growth-controlling pathways. E.g. the *ras* gene is a proto-oncogene that encodes for an intracellular signal-transduction protein; the mutant *ras*D gene is an oncogene, whose encoded oncoprotein provides an excessive and uncontrolled growth-promoting signal.

Important genes for regulation of cell growth (and mitosis) are so-called proto-oncogenes and tumour-suppressor genes. Because most proto-oncogenes are vital for basic cellular functions, they have been highly conserved over the course of evolution.

Proto-oncogenes

Proto-oncogenes influence cell growth in a variety of ways. Many of them produce molecules that act as “chemical messengers” between cells and thus control cell division. There are several classes of proto-oncogenes, e.g.:

- growth factors
- receptors for growth factors
- intracellular signal-transduction molecules
- transcription factors (which influence the genetic activity of a cell)
- cell-cycle control molecules

Generally, activation or conversion of a (normal) proto-oncogene into an (mutated, cancer-evoking) oncogene involves a gain-of-function mutation. At present, at least three mechanisms are known that can produce oncogenes from the corresponding proto-oncogenes:

- point mutations in a proto-oncogene that result in an altered protein product
- reduplication of a DNA segment that includes a proto-oncogene, leading to overexpression of the encoded protein
- chromosomal translocation that brings a growth-regulatory gene under the control of a different promotor, causing inappropriate expression of the gene

An oncogene formed by point mutations encodes for an oncoprotein that differs slightly from the normal protein encoded by the corresponding proto-oncogene. Reduplication of DNA segments generates oncogenes whose protein products are identical with the normal proteins. Their oncogenic effect is due to their expression at higher-than-normal level (overexpression) or in cells where they are not normally expressed. The gain-of-function mutations that convert proto-oncogenes to oncogenes act dominantly, meaning that mutation in only one of the two gene copies always present (called alleles) is sufficient for induction of cancer.

4.3 Tumour-suppressor genes

Tumour-suppressor genes generally encode for proteins that inhibit cell proliferation. The loss of one or more of these "brakes" contributes to the development of cancer. Five classes of proteins are generally recognised as being encoded by tumour-suppressor genes:

proteins that promote apoptosis (programmed cell death)

enzymes that participate in DNA repair

checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal

intracellular proteins that regulate or inhibit progression through a specific stage of the cell cycle

receptors for secreted hormones that function to inhibit cell proliferation

In contrast to proto-oncogenes, whose activation involves gain-of-function mutations, the conversion of tumour-suppressor genes into oncogenes is due to loss-of-function mutations.

Since generally one copy of a tumour-suppressor gene suffices to control cell proliferation, both alleles (gene copies) of a tumour-suppressor gene must be lost or inactivated in order to promote tumour development. Thus, oncogenic loss-of-function mutations in tumour-suppressor genes act recessively. Tumour-suppressor genes in many cancers have deletions or point mutations that prevent production of any protein or lead to production of a non-functional protein.

Genetic studies of cancer-prone families led to the initial identification of many tumour-suppressor genes. A classic case is retinoblastoma, which is caused by loss of function of RB, the first tumour-suppressor gene to be identified (see table 1). The protein, which is encoded by RB helps to regulate progression through the cell cycle. Children with hereditary retinoblastoma develop retinal blastoma early in their life and generally in both eyes. Each tumour that develops is derived from a single cell.

Table 1: Some examples of tumour-suppressor genes, their functions and tumour types

Gene	Function	Tumour Types
p53	Cell cycle, regulation, apoptosis	Brain tumours, sarcomas, leukaemia, breast cancer
RB	Cell cycle regulation	Retinoblastoma, osteogenic sarcoma
WT1	Transcriptional regulation	Paediatric kidney
NF1 (neurofibromin 1)	Catalysis of RAS inactivation	Neurofibromas, sarcomas, gliomas
NF2 (neurofibromin 2)	Linkage of cell membrane to cytoskeleton	Schwann cell tumours, astrocytomas, meningiomas, ependynomas
APC	Signalling through adhesion molecules to nucleus	Colon cancer

Source: Technical fact sheet, Indiana State University (http://web.indstate.edu/thcme/mwking/tumor-suppressors.html)

Chromosomal abnormalities in human tumours

It has long been known that chromosomal abnormalities abound in tumour cells. As a rule, the chromosomal abnormalities are not the same in all tumours: each tumour has its own set of anomalies. Human cells ordinarily have 23 pairs of chromosomes (they are diploid), recognised by their well-defined substructure, but tumour cells are usually aneuploid (i.e. they have an abnormal number of chromosomes — generally too many), and they often contain translocations (fused elements from different chromosomes). Cells with abnormal numbers of chromosomes form when the cell-division checkpoints are non-functional. Defects in these checkpoint controls are common in tumour cells; the molecular basis for these defects is increasingly being discovered.

The p53 checkpoint control protein

Although the protein p53, which was described for the first time in 1979, has several functions, its ability to act as a checkpoint-control protein which arrests the cell cycle if DNA is damaged or chromosomes are abnormal is most relevant in respect to its tumour-suppressing function. *p*53 mutations occur in more than 50% of human cancers. All *p*53 mutations seem to abolish its ability to bind to specific DNA sequences respectively to activate gene expression. When, as a result of mutations, the p53 checkpoint control does not operate properly, damaged DNA is replicated, producing novel mutations and further DNA rearrangements that in the end can lead to highly transformed, metastatic cells. The consequences of mutations in the *p*53 gene provide a dramatic example of the fundamental significance of cell-cycle checkpoint control for the health of a multicellular organism.

Defects in DNA-repair systems associated with certain cancers

A link between carcinogenesis and the failure of DNA repair is suggested by the finding that humans with inherited genetic defects in certain repair systems have an enormously increased probability of developing certain cancers. One such disease is xeroderma pigmentosum.

Individuals with this disease suffer from skin cancers called melanomas and squamous cell carcinomas very easily if their skin is exposed to the UV rays in sunlight. Cells of affected patients are unable to repair UV damage or to remove chemical substituents on DNA bases.

Such damage is commonly repaired by an excision-repair mechanism. The complexity of mammalian excision-repair systems is demonstrated by the fact that at least seven different genes lead to xeroderma pigmentosum lesions, all having the same consequences.

Genes involved in apoptosis (telomerase expression and cancer cells)

Telomeres, the physical ends of linear chromosomes, consist of tandem arrays of a short DNA sequence (TTAGGG in vertebrates, and thus also in human cells). While the double-stranded DNA cannot be completely replicated during cell division until the end of the molecule, in the germ line and in rapidly dividing somatic cells (such as stem cells) an enzyme called telomerase adds the TTAGGG repeats to the ends of the chromosome. The absence of telomerase leads to the shortening of the telomeres with each cell cycle. Complete loss of telomeres results in end-to-end chromosome fusions and finally the death of the cells. As most human somatic cells lack telomerase, the shortening of telomeres is regarded as part of the programmed cell-death mechanisms.

Most tumour cells, however, overcome this barrier by expressing telomerase. Many researchers believe that telomerase expression is essential for a tumour cell to become immortal, and specific inhibitors of telomerase have been suggested as therapeutic agents against cancer.

4.4 Angiogenesis

Tumours, whether primary or secondary, require the recruitment of new blood vessels in order to grow to a large mass. In the absence of a blood supply, a tumour can grow into a mass of about 106 cells, roughly a sphere 2 mm in diameter. At this point, division of cells on the outside of the tumour mass is balanced by the death of those in the centre due to an inadequate supply of nutrients. Unless they secrete hormones, such tumours cause few problems. However, most tumours induce the formation of new blood vessels (neovascularisation) that invade the tumour and nourish it. If this process is due to the protrusion of capillary buds and sprouts, which is typical for tumours, it is called angiogenesis. Although this complex process is not understood in detail, it can be divided into several discrete steps: degradation of the basal lamina that surrounds a nearby capillary, migration into the tumour of endothelial cells lining the capillary, division of these endothelial cells, and formation of a new basement membrane around the newly elongated capillary (Cao 2005).

Cancers that lack angiogenesis remain dormant. The angiogenic tumour switch is activated when the balance shifts between angiogenic inhibitors and stimulators (Yance/Sagar 2006). In normal tissues, the process of neovascularisation is subtly controlled by a series of endogenous polypeptides that are secreted during growth, healing and tissue renewal. Many tumours produce growth factors that stimulate angiogenesis directly; other tumours somehow induce surrounding normal cells to synthesise and secrete such factors. Basic fibroblast growth factor (bFGF), transforming growth factor α (TGFα), and vascular endothelial growth factor (VEGF), which are secreted by many tumours, all have angiogenic properties (Yance/Sagar 2006).

One of the most mysterious aspects of angiogenesis is that a primary tumour will often secrete a substance that inhibits angiogenesis around secondary metastases. In this case, surgical removal of the primary tumour may stimulate growth of its metastatic secondary tumours. Several natural proteins that inhibit angiogenesis (e.g., angiogenin and endostatin) or antago-

nists of the VEGF receptor have excited much interest as therapeutic agents since they might be useful against many kinds of tumours. While new blood vessels are constantly forming during embryonic development, only few form normally in adults; thus angiogenesis inhibitors should have few adverse side effects.

4.5 Metastasis

Metastatic cells lose contact with other cells in their tissue of origin and overcome the usual limitations to cell movement provided by basal laminae and other bodily structures. As a result, metastatic cells can invade adjoining tissue or enter the circulation and establish themselves in distant parts of the body.

The basal lamina (or basement membrane) is the physical barrier which keeps cells separated. It underlies layers of epithelial cells (which form the outside coating of organs) and surrounds the endothelial cells of blood vessels (which form the inside coating). Basal laminae also represent the boundaries of different compartments of the body. Tumour cells often produce and secrete molecules (enzymes) that trigger the digestion of components (mostly proteins) of the basal laminae as a prerequisite for penetrating them. An important pathway is the release of so-called plasminogen activator, which cuts off a piece from the serum protein plasminogen, thereby converting it into the active protease (protein-digesting enzyme) plasmin. Secretion of a small amount of plasminogen activator causes a large increase in plasmin activity (a typical biochemical "cascade effect"). A striking analogy to invasion by tumour cells occurs in the extra-embryonic cells of the foetus which also secrete plasminogen activator in order to permit implantation in the uterine wall.

When the basal lamina disintegrates, tumour cells can enter the bloodstream, but fewer than 1 in 10 000 cells which escape the primary tumour survive to colonise another tissue and form a secondary, metastatic tumour. Such a cell must first adhere to an endothelial cell lining a capillary and migrate across or through it into the underlying tissue. To be able to metastasise, a tumour cell must be able to multiply and adhere to distinct types of cells. The wide range of altered behaviours that underlie malignancy may have their basis in new or variant surface proteins made by malignant cells.

4.6 Cancer-causing viruses

In terms of recent therapies for cancer, viruses as causative agents are of special relevance, because the so-called prophylactic vaccine therapies are directed against them (section 7.1). Usually, therapies are used to treat cancer that has developed as a consequence of the patient's exposure to carcinogens such as chemicals or radiation, regardless of their nature, and are in this respect unspecific. However, in the case of viruses, the carcinogen itself is the target of the intervention, and thus the therapy can be highly specific.

Several viruses are known to be carcinogenetic or to enhance the onset of neoplasia, e.g.:

human papillomavirus (HPV): cervical cancer

hepatitis B and C virus (HBV and HCV): hepatocellular carcinoma

Epstein-Barr virus (EBV): Burkitt lymphoma, Hodgkin´s lymphoma

human herpesvirus-8 and human immunodeficiency virus (HHV-8 and HIV): Kaposi´s sarcoma

human T-cell leukaemia virus (HTLV-1): adult T-cell leukaemia

These viruses use different strategies to elicit tumour cells. In humans, DNA viruses are predominant. The known oncogenes of DNA viruses are integral parts of the viral genome required for viral replication.

HPVs infect epithelial cells (the outside coating) and inactivate the central anti-oncogenes *Rb* and *p*53, a process which leads to benign warts. One-third of about 100 types are sexually transmitted. Some serotypes of the HPVs (16, 18, 31, 33 and 35) can cause cervical cancer.

More than 80% of the world's cases of liver cancer (hepatocellular carcinoma) are attributable to infection by the hepatitis B and C viruses, and some 350 million people are chronically infected with one of the seven subtypes of HBV. HBV inactivates host anti-oncogenes by integrating parts of its genome into the DNA of the liver cells.

The co-infection of HHV-8 and HIV leads in immune-suppressed individuals to Kaposi´s sarcoma. HHV-8 infection alone does not cause cancer. But the combination with HIV that suppresses the immune system leads to the oncogenicity of endothelial cells. There are probably several genes involved. Two of them encode for homologues of the human *Bcl*-2 and *cyclin* D genes that control the progression of cells through the cell-cycle.

HTLV-1, a retrovirus, infects T cells and can cause leukaemia and lymphoma. It is spread by sharing syringes or needles used to inject drugs, through sexual contact, and from mother to child at birth or through breast-feeding.

Helicobacter pylori, not a virus, but a bacterium which leads to gastric ulcer and sometimes to stomach cancer, shares some mechanisms with cancer-causing viruses. Its major cancer-causing protein CagA leads to the depolarisation and mobilisation of epithelial cells in the inner coating (mucosa) of the stomach and gives them the ability to move throughout the tissue. These modifications are regarded to be one origin of gastric cancer.

New findings suggest that key-molecules could play an important role in the common molecular mechanisms of cancer, infections and inflammatory diseases of epithelial cells or tissues (http://www.dkfz-heidelberg.de/en/presse/pressemitteilungen/2006/dkfz_pm_06_76_e.php). These so-called meta-proteins (e.g., NOD2 and DMBT1) could turn out to be a central point in the treatment of cancer and other severe diseases.

4.7 The stem cell paradigm (or hypothesis)

The stem cell paradigm originates from developmental biology and oncology. It suggests that cancer develops as a result of mutations of adult stem cells (Serakinci, expert opinion). The cells are the origin of other subcomponent cells of the tumour and retain key properties of stem cells. Furthermore, cancer relapse and metastasis are also caused by these cells.

The stem cell paradigm does not interfere with earlier concepts of carcinogenesis, e.g. the "multi-hit model". It just points to adult stem cells as the site where the process starts (Olivera et al. 2002).

Cancerous stem cells were first identified in 1997 when John Dick's research group from the University of Toronto transferred a few blood stem cells from human leukaemia patients into mice and observed leukaemia develop in them.

In 2003, Michael Clarke succeeded in finding cancer stem cells in breast tumours. The majority of the cells were incapable of further growth, but a handful were able to seed new cancer cells and maintained their ability to proliferate and to generate mature cells. In 2004, cancer stem cells were additionally detected in human brain tumours and bone cancer (Serakinci, expert opinion).

Like normal stem cells, tumour stem cells exist in very low numbers, but they can replicate to give a multitude of cells. Unlike normal stem cells, however, cancerous stem cells lack the controls which tell them when to stop dividing. Traditional chemotherapy kills off the majority of the tumour cells, but if any of the cancerous stem cells survive the treatment, the cancer may return. The stem cell paradigm (or hypothesis) has fundamental implications for cancer risk assessment, early detection, prognosis, and prevention (Serakinci, expert opinion). Furthermore, the cancer therapies currently used aim at killing differentiated tumour cells while sparing the rare cancer stem cells population.

Research into the differences in gene expression between normal and tumour stem cells may lead to the development of new treatments targeting the root of the problem — the cancer stem cell itself.

If the stem cell paradigm (hypothesis) is right, it is evident that a cancer treatment which fails to eliminate the cancer stem cells will probably allow the reappearance of the tumour. Even if 99.9 % of the tumour cells are destroyed by surgery plus chemo- and/or radiotherapy, the remaining "silent" cancer stem cells can lead to a relapse after some time. To prevent this, specific strategies for targeting those cancer stem cells have to be developed (Li et al. 2007). The new biomedical approaches like gene, vaccine, and immunotherapy probably represent the most rational and perhaps the most promising attempts.

4.8 A remark on the distinction between gene, vaccine and immunotherapy

It is very difficult to distinguish between *gene, vaccine and antibody or immunotherapy* with regard to their therapeutic mechanisms. All of the new cancer therapy approaches are linked by the scientific finding that cancer is a disease resulting from the accumulation of genetic modifications within a cell. The basic attempt in delineating new therapy strategies is to determine as many properties of cancer (stem) cells as possible and outline an effective biomedical action against them. In many cases, gene transfer is used to deliver pharmaceutically active compounds such as (monoclonal) antibodies or signalling molecules to the tumour site in order to activate the immune system, which can then fight the cancerous cells.

The different terms must be considered not as clear-cut methods, but as basic strategies or concepts that often follow the same paths and use the same tools. Most approaches aim to fight cancer from more than one direction. On the one hand, they alter the genes of targeted cells, thus, e.g., causing apoptosis, while at the same time healthy immune cells are trained to fight against potential remissions by identifying new developing cancer cells and killing them.

Depending on the definition of "gene therapy" used, one may include very different therapeutic approaches such as the insertion of suicide genes into tumour cells or using anti-sense technology to shut down genes or parts of genes in cancer cells (see the following section).

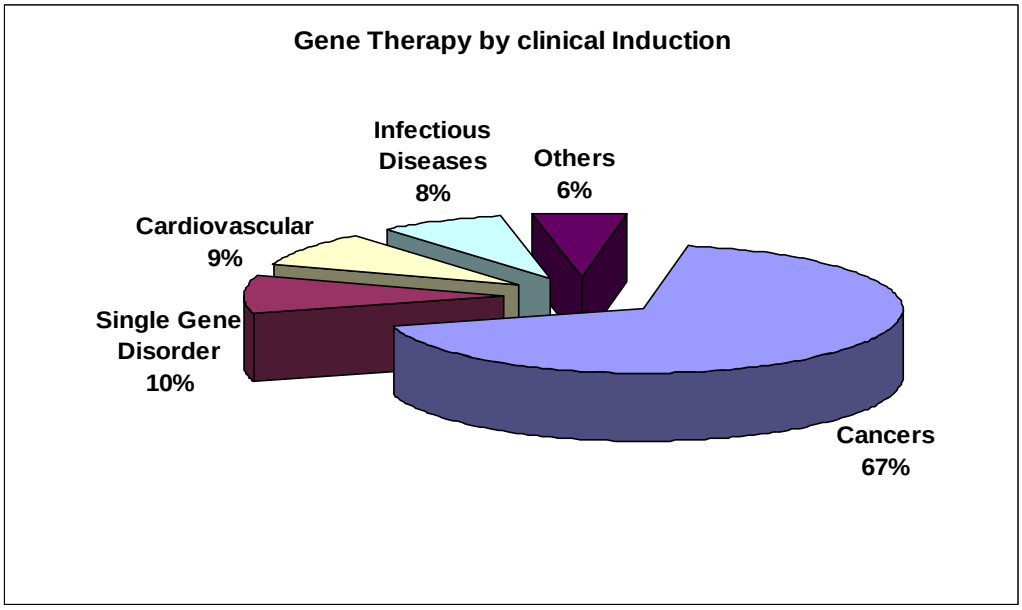
Particularly with regard to the term "stem cell therapy", one must take into account that it is used in different contexts: on the one hand to denote the use of stem cells as specially suited and in some cases highly specific vectors (as is shown in the following section), on the other hand to indicate the need to target stem cells during therapy due to their potentially lethal effects in terms of the stem cell paradigm (described above).

As already mentioned in the introduction, due to the very rare examples of exploring stem cells as therapeutic tools against cancer, we decided not to classify stem cell therapy as a distinct category but as part of gene therapy approaches.

5. Gene therapy

In general, gene therapy can be defined as a set of approaches involving the transfer of genetic information to cells, tissues or organs of a patient in order to overcome a genetic defect or to provide a protective function.

Fig. 7: Distribution of gene therapy approaches by clinical indication (worldwide) in 2000



Source: NIH RAC database (National Institutes of Health Recombinant DNA Advisory Committee, 2000) (http://www4.od.nih.gov/oba/RAC/GeMCRIS/GeMCRIS_public.htm)

Data from the National Institutes of Health Recombinant DNA Advisory Committee database (NIH RAC) for the year 2000 show that cancer was at that time the disease most frequently targeted by gene therapy (figure 7; more recent data were not found). Of the 350 gene therapy clinical trials, 67 % were directed towards cancer.

5.1 Approaches/Protocols

There are a number of different approaches and protocols. Some approaches target cancer cells in order to destroy them or prevent their growth, other approaches target healthy cells to enhance their ability to fight cancer.

A “normal”, functional gene can be inserted into the genome to replace a non-functional, cancer-causing gene. This is the most common approach at present.

Other gene therapies attempt to stimulate the body’s natural ability to attack cancer cells; some involve the development of enzymes that destroy viral or cancerous genetic material within cells.

In some studies, genes were injected into cancer cells to make them more sensitive to chemotherapy, radiation therapy, or other treatments. In other protocols, genes are incorporated into healthy blood-forming stem cells to make these more resistant to the side effects of high doses of anti-cancer drugs.

In a further approach, cancer cells are injected with genes that can be used to destroy them. In this protocol, “suicide genes” are introduced into cancer cells. Later, a pro-drug (an inactive form of a toxic drug) is given to the patient. The pro-drug is then activated only in the cancer cells containing the “suicide genes,” which leads to the destruction of the cancer cells.

Additional research focuses on the use of gene therapy to prevent carcinoma from developing new blood vessels (angiogenesis).

Epidermal growth factor receptor (EGFR) and insulin growth factor 1 receptor (IGF1R) are widely recognised as validated cancer therapy targets, although perturbation of the activity of the protein's function through traditional chemical techniques appears to be quite challenging (see also section 2.1). With reference to the cancer gene therapies in figure 7, 31 % used in vitro immunotherapies, 32 % used in vivo immunotherapies and 15 % were based on pro-drug suicide therapies.

5.2 Vectors for gene therapies: properties and problems

A gene cannot be inserted directly into a person's cell, instead the therapeutic genes have to be delivered into the cells, tissues, or organs by biochemical delivery systems ("vectors" or "shuttles") such as viruses, liposomes or (receptor) proteins, which display different characteristics (table 2). A further differentiation is made concerning the place/site of the genetic alteration: in vivo or ex vivo. In some clinical trials, cells from the patient’s blood or bone marrow are removed and grown in the laboratory. The cells are exposed to a virus that is carrying the desired gene. The virus enters the cells and inserts the desired gene into the cells’ DNA. The cells are grown in the laboratory and are then reintroduced into the patient’s body by injection into a vein. This type of gene therapy is called ex vivo because the gene is transferred into specimens of the patient’s cells grown outside his body. In other approaches, vectors (often viruses) or liposomes (fatty particles) are used to deliver the desired gene directly to cells in the patient’s body. This form of gene therapy is called in vivo.

Many gene therapy clinical trials rely on retroviruses to deliver the desired gene. Other viruses used as vectors include adenoviruses, adeno-associated viruses, lentiviruses, poxviruses, and herpes viruses. These viruses differ in how well they transfer the genes to cells, which cells they can recognise and infect, and whether they alter the cell’s DNA temporarily or permanently. Thus, different vectors have to be tested, depending on the specific requirements of the study.

Table 2: Characteristics of the most common gene delivery systems for gene therapy

	Viral systems			Non-viral systems	
System	Adenovirus	Adeno-associated virus	Retrovirus	Receptor-mediated endocytosis	Liposomes
Gene construct	Double-stranded DNA	Single-stranded DNA	RNA	DNA	DNA
Target cells	Replicating and non-replicating cells	Replicating and non-replicating cells	Replicating cells	Cells expressing specific receptors	Replicating and non-replicating cells
Chromosomal DNA integration	No	Yes, usually at a specific site on chromosome 19	Yes, random	Rare event	Rare event
Efficiency of transduction	> 90%	> 90%	< 30%	Low	Low
Induction of host immunity towards the vector	Yes	Yes	Yes	No	No
Gene expression	Transient transfection	Long term expression	Stable transfection	Transient	Transient
Safety for the host	Possibility of recombination with ubiquitous wild-type virus	May cause deletions or changes in chromosome19	Possibility of insertion mutagenesis/oncogenesis	Safe	Safe

Source: Fogar et al. 2005

The different characteristics of the vector systems can be advantages or disadvantages, strengths or weaknesses, depending on their use. In many cases, the efficiency of transduction is not sufficient for therapeutic measures, or an activation of the immune system cannot be achieved. One important parameter is whether the genetic alteration has to be lasting or temporary (stable or transient transfection).

Of overall importance is the question of biological safety, which means that the vector itself does not create a novel threat to the patient's health. Because viruses can usually infect more than one type of cell, gene therapy is always accompanied by substantial risks. Especially in the case of modified retroviruses, which are partly derived from highly infectious pathogens, it is absolutely imperative to avoid disintegration and spreading of the viral DNA.

Other concerns include the possibility that transferred genes can be “overexpressed”, producing so much of the missing protein as to be harmful, or that the viral vector could cause inflammation or an immune reaction, or also that the virus could be transmitted from the patient to other individuals or into the environment.

Another danger arises if the new gene is inserted at the wrong location in the DNA, eventually causing cancer by itself or other harmful mutations to the DNA.

In the last 10-15 years tremendous progress has been made concerning the understanding of gene therapy approaches. Scientists have learned which genes or combinations of genes are of interest for their research, they have found and altered different kinds of delivery systems to fit different tissues and cell types, but they have also realised that it is very difficult to cure cancer using this approach. The choice of the vector system to target the desired cells or tissues has particularly proved to be one of the main challenges in planning effective (cancer) gene therapy.

5.3 Examples of approaches with adenovirus-based vector systems

In the past few years, evidence has grown that adenoviral-mediated gene transfer has particularly great potential, although the high immunogenicity of adenoviral vectors is still one of the obstacles. The properties of adenoviral transfer systems enable researchers to use the virus as a promising transfer tool for cancer gene therapy in order to target cancer cells and, additionally, for genetic immunisation to control the host immune response (Romano 2006).

One approach that uses adenoviral vectors destroys the replication program of tumour cells by using the oncolytic capacities of the virus (O'Shea 2005). It seems that early viral proteins which are necessary for the development of adenoviruses elicit growth deregulation in infected cells similar to that engendered by mutations in tumour cells. Researchers believe that this knowledge could be of importance both for the discovery of novel tumour targets as well as for the design of oncolytic viruses and combination therapies.

A relatively new approach using the advantages of viral therapy in combination with gene therapy is the so-called "targeting gene-virotherapy" of cancer (Liu/Gu 2006): The new therapy was tested in mice and had a stronger anti-tumour effect than either gene or viral therapy alone. Two genes with compensative or synergistic effects were inserted into a virus system (ZD55) and administered alone or in combination. As a result, xenograft tumour masses (transplanted from human tumours) in mice were completely eliminated. The chosen genes were a tumour suppressor gene and granulocyte-macrophage colony-stimulating factor (GM-CSF), a gene that enhances the immunological reactions of cancer patients.

Pancreatic cancer, which possesses the potential for strong growth and metastasis, shows only limited response to conventional therapies such as chemotherapy and/or radiation. At present a chemotherapeutic agent called gemcitabine (GEM) is used as first-line treatment. It interacts directly with DNA and inhibits the proliferation of pancreatic cancer cells. Nevertheless, the survival benefit of GEM for advanced pancreatic cancer remains limited. Studies in nude mice showed that the combination of Ad-NK4 (adenovirus and a synthetic hepatocyte growth factor antagonist) and GEM inhibit three steps of tumour growth, namely, metastasis, angiogenesis, proliferation and invasion. As a result, this combination therapy suppressed both peritoneal dissemination and liver metastasis and resulted in prolonged survival (Ogura et al. 2006).

5.4 Antisense protocols

Antisense oligonucleotides (ODNs) and small interfering RNAs (siRNAs) are capable of inducing sequence-specific silencing of the expression and activity of various target genes. The current limitation to effective ODN and siRNA therapy is the efficient and specific delivery in vivo.

Companies around the world are developing specific delivery vehicles and mechanisms including liposomes, viruses and cell-penetrating peptides for siRNA and ODN, because it is believed that, if efficient delivery is achieved, a whole range of new gene-silencing therapies will become available for any number of diseases (Hiroi et al. 2006; Orr/Dorr 2005; Zhang et al. 2006).

Antisense therapy is strictly speaking not a form of gene therapy, but is often connected to a "true" gene therapy. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesise DNA that will bind to the messenger RNA produced by that gene, effectively switching the gene "off". This synthesised nucleic acid is called an "anti-sense" ODN, because its base sequence is complementary to the gene's messenger RNA, which is called the "sense" sequence. A sense segment of mRNA is thus blocked by the antisense mRNA segment (Zhang et al. 2006).

There are a lot of genes that are well-characterised (e.g. tumour suppressor genes, proto-oncogenes and regulatory genes) and are now targets of antisense therapy approaches. Some of them have reached the status of clinical trials, for example (Orr/Dorr 2005):

*p*53, a tumour suppressor gene (see section 2.2) which is responsive to elements of cellular stress and involved in DNA repair and apoptosis. In a phase I clinical study with patients with acute myelogenous leukaemia (a fast-growing cancer of the blood and the bone marrow), no significant toxicity was observed, and a favourable pharmacokinetic profile of P-ODNs (phosphorathioate ODNs) administered by systemic infusion could be demonstrated.

The *c*-Myb proto-oncogene encodes a transcription factor that is downregulated during differentiation of hematopoietic cells and commonly upregulated in leukaemias. Antisense ODNs were administered by continuous infusion in a clinical phase I study. As a result, no drug-related toxicity was observed, and some disease stabilisation could be demonstrated.

The Bcl-2 protein is a member of a family of proteins that reside within the mitochondrial membrane and are key regulators of programmed cell death, morphologically characterised as apoptosis (see section 2.1). Bcl-2 is an antiapoptotic protein, and overexpression may be linked to tumourigenesis and chemoresistance. In a prostate cancer phase II study an antisense drug (Genasense™) is used alone and in combination with first-line therapeutic agents like paclitaxel, mitoxantrone and docetaxel.

5.5 Stem cell therapy

Quite frequently, stem cell therapy is referred to as a distinct and fully introduced therapy approach on its own. A closer look at respective publications does not support this view. It rather seems that the use of altered stem cells as an anticancer therapy is at a very early stage (Serakinci, expert opinion). Subsequently, only very few publications dealing with these approaches are available.

In one very recent approach, stem cells are used as shuttles for delivering drugs to cancers which have already spread throughout the body (metastasis). The researchers use modified neural stem cells to activate and concentrate chemotherapeutic drugs predominantly at the tumour sites, so that tissue both surrounding the tumour and throughout the rest of the body remains unharmed. This two-part system has been developed to infiltrate metastatic tumour sites first and to activate a chemotherapeutic drug later, thereby localising the drug´s effect to the tumour cells. This technique takes advantage of the tendency of invasive tumours to attract neural stem cells.

Modified neural stem cells were injected into immunosuppressed mice with artificially induced neuroblastoma tumours. After waiting a few days to allow the stem cells to migrate to the tumours, a precursor drug was administered which interacted with an enzyme expressed by the stem cells once it reached them, converting it into an active drug able to kill surrounding tumour cells. As a result, all of the neuroblastoma mice appeared to be healthy and were tumour-free after 6 months. Without treatment all of the neuroblastoma mice died within 2.5 months.

These were the first research results to demonstrate the efficacy of simultaneously targeting multiple solid tumour sites spread throughout the body in the metastatic stage of cancer. It can be speculated that the technique could also be used for other malignant solid tumours, including colon, brain, prostate and breast cancer. Future pre-clinical trials (animal studies) are planned (Aboody et al. 2006).

5.6 Vanguard approaches

Besides those listed in table 2, there are further and novel delivery systems for the introduction of genetic material into cells or tissues. The role of nanotechnology in gene therapy of cancer increases from year to year. Nanoparticles have been used e.g. for *p*53 the gene therapy of cancer, and an intravenous nanoparticle formulation of the tumour suppressor gene FUS1 has been tested in experimental animals. Examples of other techniques are integrin-targeted nanoparticles for site-specific delivery and so-called immunolipoplex formulations which are combinations of (organic) lipid vesicles and biological molecules such as antibodies or receptors (Fukumori/Ichikawa 2006; Jain 2005). New developments of inorganic nanoparticles that interact with biological systems show the entry of new technologies in anticancer treatment. Among inorganic materials, magnetite has been investigated most widely for anticancer therapy. It was earlier used in the hyperthermia treatment of cancer (Ito et al. 2003; Zhang et al. 2002).

6. "Passive" immunotherapy: antibody therapy

In many solid tumours, the response to chemotherapy, radiotherapy and/or surgery is limited, which means that additional strategies used in combination with these "conventional" therapies play an important role in achieving success. Since researchers found that antigens on the surface of tumour cells are cell-specific (tumour-associated antigens, TAAs) and differ from those of "normal" cells, immunotherapies have increasingly become the focus of interest. The term "immunotherapy" usually covers (a) approaches which use activation of the patient's own immune cells (e.g. T cells or dendritic cells) to eliminate cancer cells and which are often referred to as vaccine therapies (or "active" immunotherapy, see section 7), and (b) all kinds of strategies to specifically adjust *(extra-corporally)* individual types of immune molecules or cells in order to use them as a cancer drug or treatment ("passive immunotherapy).

Researchers manipulate T cells, for instance, and try to make them tumour-specific. In so doing, selection of the targeted antigen is of major importance. The majority of TAAs are antigens which are overexpressed on tumour cells and are also present on "normal" cells. Engineered T-cell therapy is still in its infancy, although the approach is being tested in early-phase clinical trials (Mansoor et al. 2005). In the following section, we focus on antibody therapy as a prominent example of biological therapy because it can be used in different approaches and for different purposes.

Antibodies for targeted cancer therapy

The detrimental disadvantage of traditional anti-cancer agents is their toxicity to normal cells which limits the success of therapy. Growing understanding of the differences between malignant and normal cells leads to new anti-cancer agents targeted directly at cells associated with malignant alterations, such as increased proliferation, impaired apoptosis or angiogenesis. Engineered antibodies can address TAAs and can be used to inhibit cell growth, induce apoptosis or to serve as target-reagent of drug-delivery systems (Zangemeister-Wittke 2005).

Antibodies are a potent tool for use as therapeutic agents in various pathological conditions, with a specific focus on cancer. Recently developed technologies (e.g. recombinant technology, humanisation of antibodies, the production and selection of monoclonal antibodies) have achieved two important goals, namely, to overcome most host anti-antibody responses and to extend the half-life of the reagent.

These new findings and techniques now permit antibodies to be set up in broad spectra as anticancer tools. Monoclonal antibodies are utilised as blocking agents against, e.g. angiogenic growth factors and their receptors (Sanz/Alvarez-Vallina 2005). The main strategies for inhibiting the neovascularisation of tumours include: blocking growth-factor activity, inhibiting matrix proteases, directly targeting endothelial cells, targeting and/or blocking extracellular membrane activity sites, and up-regulating endogenous inhibitors.

The best-known example of successful immunotherapy against early breast cancer is a mAb called trastuzumab (Herceptin®). This antibody is considered to be one of the first biological therapies that cures (a specific kind of breast) cancer (Untch, expert opinion). Herceptin® acts as an anti-angiogenic cocktail and addresses not only the human cell surface receptor HER2, but also other anti-angiogenic molecules (Rückert 2005).

Another emerging alternative using antibodies in anticancer therapy is the construction of dual-targeting bispecific (monoclonal) antibodies (BsAbs). BsAbs are derived from the recombination of variable domains of two different antibodies with different specificities. They are thus capable of binding to both antigens of their parental antibodies and targeting two TAAs at once.

In cell culture experiments, antibodies which interfere with both EGFR and HER2 (receptor tyrosine kinases) showed an additive result and inhibited cancer cell growth from 35% and 55% (as single agents) to 80%.

These data suggest that combination therapy could be useful in cancers caused by the mis-regulation of receptor tyrosine kinases (Marvin/Zhu 2006).

An approach using antibodies in combination with radiotherapy is the (boron) neutron capture therapy. This therapy approach requires the introduction of relatively high doses of boron (^{10}B, a chemical element) into tumour cells or tumour vasculature. The effect of this stable isotope is the breakage of DNA strands, causing (tumour) cell growth to stop. A therapy approach targeted at cancer cells could reduce unwanted side effects as well as toxicity. Researchers have composed liposomal vesicles into which lipophilic drugs can be incorporated. They modified the surface with polymers (e.g. to shield them from recognition by the immune system) and coupled monoclonal antibodies to the liposome to target EGFR. This formulation was applied to human ovarian carcinoma cells and endothelial cells, leading to a significant quantity of ^{10}B within the cells. The combination of monoclonal antibodies to target cancer cell receptors and direct treatment with a stable isotope could become an attractive approach for cancer therapy (Krijger et al. 2005).

Another molecular target in tumour therapy is midkine. Midkine is a heparin-binding growth factor which promotes the growth, survival, differentiation and migration of various cells. It also enhances the fibrinolytic activity of endothelial cells, exhibits angiogenic activity through cellular interactions, and is frequently overexpressed in human carcinomas. Researchers produced an antibody (against the midkine receptor) conjugated with doxorubicin (DOX), a compound used in chemotherapy which stops the process of replicating DNA) and were successful in inhibiting the growth of liver cancer cell cultures (HepG2) by internalising conjugated antibodies. This raises the possibility of using anti-midkine antibody conjugated with DOX for cancer therapy (Inoh et al. 2006).

These examples show that antibodies are potent tools, either alone or in combination with other therapies as potential (and already existing, e.g. Herceptin®) approaches to cancer.

7. "Active" immunotherapy: vaccine therapy

There are many different targets that could be addressed either with prophylactic or with therapeutic cancer vaccines. Preventive or prophylactic cancer vaccines are designed to target cancer-causing viruses and prevent viral- or bacterial-borne infections. They are administered to healthy individuals. The intention of therapeutic cancer vaccine approaches, on the other hand, is to alter or support elements of the immune system to fight the disease (also called "active immunotherapy", see section 6).

7.1 Preventive or prophylactic vaccines

One-fifth of cancer cases worldwide (WHO 2006) are due to chronic infections, mainly HBV (causing liver cancer), HPV (causing cervical cancer), HIV (causing Kaposi's sarcoma and lymphomas) and *Helicobacter pylori* (causing stomach cancer) (see section 2.7).

In 2006, the FDA approved two preventive vaccines directed towards infection with HPV, which causes almost all cervical cancers in women. The HPV vaccines from the pharmaceutical companies Sanofi and GlaxoSmithKline are genetically engineered vaccines and use very similar approaches. The so-called “virus-like-particle” vaccines are mainly constructed of envelope and viral surface proteins and are unable to proliferate, because all essential genes for virus replication have been removed. The two vaccines are effective against about 70% of cases of cervical cancer worldwide, Gardasil® from Sanofi against the subtypes HPV-6, 11, 16 and HPV-18, Cervarix® from GlaxoSmithKline against the subtypes HPV-16 and HPV-18 (U.S. National Cancer Institute, Cancer Vaccine Fact Sheet). The FDA approval of Cervarix is still pending and is expected for the end of 2007. Both vaccines are aimed at young girls aged between 9 and 16 years before they have their first sexual contacts. Young boys should also be vaccinated, so they will not spread the viruses.

As HBV causes liver cancer, anti-HBV vaccines have a prophylactic effect against this kind of cancer. However, since HBV itself is a severe disease, cancer prevention here represents rather a side effect of HBV vaccination.

Prophylactic vaccination against human cancer provides a unique opportunity to prevent human suffering for individuals at risk for tumour development. Appropriate vaccines may pose slightly different requirements than vaccines intended for therapeutic use. Prophylactic vaccines will need to prevent tumours far in the future, emphasising the need to establish solid tumour-specific immunological memory (Riemer et al. 2005).

7.2 Therapeutic vaccines ("active" immunotherapy)

A broad variety of molecules in the human immune system are considered to be effective targets for therapeutic anti-cancer vaccines, from single molecules up to whole cells. In most trials therapeutic vaccines are used to activate the immune system of cancer patients to prevent reappearance. Vaccines used to treat cancers take advantage of the fact that certain molecules on the surface of cancer cells are either unique or more abundant than those found on normal, non-cancerous cells. These molecules, either proteins or carbohydrates, act as antigens, meaning that they can stimulate the immune system to make a specific immune response. Researchers hope that, when a vaccine containing cancer-specific antigens is injected into a patient, these antigens will stimulate the immune system to attack cancer cells without harming normal cells (U.S. National Cancer Institute, Cancer Vaccine Fact Sheet; http://www.cancer.gov/cancertopics/factsheet/cancervaccine).

Two schools of thought dominate the research into tumour antigens and tumour vaccines. The first believes that tumour rejection can be obtained only by immunisation with unique tumour-associated antigens (TAAs) (Olivera et al. 2002), supporting the use of undefined tumour-derived material that would contain such antigens as tumour vaccines. This theory assumes that every tumour shares all the criteria from all other tumours, independent of their origin. Other researchers believe that every tumour has its own TAA configuration, depending on the combination of tumour cell subtypes.

Vaccination with purified TAAs was reported to suppress chemically induced mammary tumours in rats (van der Most et al. 2006), while vaccination with p53 protein prevented chemically induced skin cancer in mice. In humans, shared antigens appear to be the predominant targets of tumour-specific immunity (Wang 2006).

Animal studies have shown that cancer vaccines are most effective in preventing tumour occurrence in genetically predisposed individuals or after solid tumour removal. Therapeutic vaccination aims to activate the patient's own immune system via cancer cell recognition by T cells, which are capable of killing cancer cells and leaving normal cells unharmed. The molecular targets recognised, which derive from proteins present in the cancer cell, encompass structures that are potentially applicable in anti-cancer vaccination strategies. In contrast to this, vaccines have until now shown only very limited potential in curing established cancer.

7.3 Adjuvants

Typically, preventive as well as therapeutic vaccines will be injected into the patients in combination with immunostimulating agents, called adjuvants. Adjuvants stimulate the immune response, enhance the vaccine's effect and induce either antibodies or memory cells that are activated in case of a second infection.

There are several adjuvants which use different strategies to boost the vaccine. Adjuvants are an important issue for vaccine research in order to improve the benefit that can be gained from vaccines. Examples of commonly used adjuvants are (U.S. National Cancer Institute, Cancer Vaccine Fact Sheet; http://www.cancer.gov/cancertopics/factsheet/cancervaccine):

> Keyhole limpet hemocyanin (KLH) is a protein made by a shelled marine animal found along the coast of California and Mexico known as a keyhole limpet. KLH is a large protein that both causes an immune response and acts as a carrier for cancer cell antigens. Cancer antigens often are relatively small proteins that may be invisible to the immune system. KLH provides additional recognition sites for immune cells known as T-helper cells and may increase activation of other immune cells known as cytotoxic T-lymphocytes.
>
> Bacillus Calmette Guerin (BCG) is an inactivated form of the tuberculosis bacterium. BCG is added to some cancer vaccines in the hope that it will boost the immune response to the vaccine antigen. It is not well understood why BCG may be especially effective for eliciting an immune response. However, BCG has been used for decades with other vaccines, including the vaccine for tuberculosis.
>
> Interleukin- 2 (IL-2) is a protein made by the body's immune system that may boost the cancer-killing abilities of certain specialised immune system cells called natural killer cells. Although it can activate the immune system, many researchers believe IL-2 alone is not potent enough to prevent cancer relapse. Several cancer vaccines use IL-2 to boost immune response to specific cancer antigens.

Bacterial DNA is recognised by the mammalian organism as foreign due to its different methylation pattern; this leads to activation of the immune system. Nucleotide sequences with non-methylated CG motifs (CpG-ODNs) and thus high similarity to bacterial DNA possess an immuno-modulatory potency and can serve as a "danger signal" in the immune system. CpG stands for cytosine and guanine separated by a phosphate. In vertebrates (e.g. humans), the CpG content of DNA is much lower than in invertebrates (less than 1000 fold). Modified CpG-ODNs are able to induce either a humoral or a cellular immune response (Wittig, expert opinion).

7.4 Clinical trials

Hundreds of clinical trials of cancer vaccines are currently being carried out in patients who already have cancer. The International Committee of Medical Journal Editors has decided that all clinical trials should be entered in a public registry before the start of patient enrolment as a prerequisite for later publication. As a result, clinical trials are registered on a website (http://clinicaltrials.gov) established by the U.S. National Institutes of Health (Svane/Straten, expert opinion). In January 2007, 211 ongoing clinical cancer vaccine trials (phase I-III) were registered at the NIH database, 42 of these in phase III.

Almost all trials (90%) use combinations of immune-stimulating agents or molecules (e.g. cytokines, synthetic peptides) and modified cells of the patients (e.g. antigen-presenting cells, APCs, or dendritic cells). Some vaccine trials are using modified viruses such as vaccinia viruses, adenoviruses or fowlpox virus (6%). The combination of either chemo- or radiation therapy with immuno-stimulating agents was found in 25% of the trials. Monoclonal antibodies and chemotherapeutic agents or transfected cells were used in another 25% of all listed trials. GM-CSF (granulocyte-macrophage colony-stimulating factor) is considered as a very promising immuno-stimulating agent and was used in 32% of all vaccine trials.

During the past decades, a large number of phase II cancer vaccine studies on a variety of cancer types have been published, several of them with encouraging results. The number of following phase III studies is significantly lower, and only a few lived up to expectations. However, some either therapeutic, adjuvant or prophylactic cancer vaccines have actually achieved significant positive clinical results (Svane/Straten, expert opinion).

7.5 Therapeutic versus prophylactic cancer vaccines

Prophylactic vaccines which protect against infectious diseases are a well-established, successful feature of modern medicine and have also proved successful in cancer prevention; two vaccines – that protect against liver cancer and cervical cancer – have been approved by the FDA and are available on the market. In contrast, the efficacy of therapeutic vaccines against established cancers still has to be proved. The two crucial differences between prophylactic microbial vaccines and therapeutic cancer vaccines are (Svane/Straten, expert opinion):

1. Prophylactic microbial vaccines are given *prior* to infection in order to prevent the infectious disease, while therapeutic cancer vaccines are given after emergence of the cancer in order to combat tumours already present.

2. Although cancer cells are indeed recognised by the immune system, the response is weak compared to anti-infectious responses. In part this is due to the fact that cancer cells are of "self" origin, i.e. are the result of a stepwise transformation of normal cells.

Thus, the HPV and HBV vaccines against cervical cancer and liver cancer are in fact not directed against the cancer cells themselves, but rather against the causative agent of the development of the cancer. The significant differences between prophylactic *versus* therapeutic and anti-viral *versus* anti-cancer vaccinations imply that not all of the knowledge and experience with prophylactic microbial vaccines can be converted directly into the development of therapeutic cancer vaccines.

Consequently, this specialised vaccine concept necessitates a substantial and persisting research effort to become an effective new treatment modality in cancer therapy (Svane/Straten, expert opinion).

Considering therapeutic vaccination against spontaneous cancers, several important findings over the past decade point to an implementation of vaccination regiments in clinical oncology. Firstly, the immune system possesses the unique capacity of distinguishing between cancer and normal cells. Although still in its infancy, data from numerous phase I and II trials – in addition to a limited number of phase III trials – strongly suggest that biological responses are inducible and that vaccination, in particular in an adjuvant setting or in patients with limited disease, will have a clinical impact. With regard to potential synergies with conventional therapies, the lack of specific tumour cell targeting remains to be one of the major drawbacks of chemotherapy. In particular since the dogma of the incompatibility between vaccination and more conventional treatments such as chemotherapy has been challenged, we expect considerable progress to be made in the development and efficacy of therapeutic vaccination against cancer, either alone or in combination with conventional therapies. It is worth noting that the main big pharmaceutical companies are engaged in one or more clinical immunotherapy trials in cancer; a trend that has increased over the past few years in particular. The time frame for the FDA's/EMEA's approval of the first product for the treatment of spontaneous cancer based on therapeutic vaccinations is estimated to be in the range of 3–5 years (Svane/Straten, expert opinion).

8. Therapeutic approaches in comparison: alternatives or synergies?

At this stage of research, it seems difficult to estimate the success of the different gene therapies and of the "passive" and "active" anti-cancer immunotherapies. An immune response has a potentially long-term clinical impact on the course of the disease by stabilising the condition and thus prolonging survival rather than by performing massive tumour elimination. The most likely patients to benefit are, therefore, those with minor tumour burden or patients who have had their tumour surgically removed but who have a high risk of relapse. In these categories of patients, disease stabilisation, frequency of relapse, time-span to relapse and length of survival are the most rational parameters for evaluating cancer vaccine effectiveness (Svane/Straten, expert opinion).

The most unambiguous results, or the greatest actual medical relevance, are achieved by the preventive vaccine approach (directed towards "transmissible", virus-related cancers), and by immunotherapies using monoclonal antibodies such as trastuzumab (Herceptin®), which is a very good example of research results being directly applied to patients (Untch, expert opinion).

Almost all approaches focus on aiming at several and different targets in the patient's body. The altering of cancer cells (inside or outside the body, connected with delivery via gene therapy) and of cancer-specific targets in combination with activation or support of the patient's own immune system seems to yield a promising treatment. Nevertheless, it is still not fully understood which components (T cells, APCs, oncogenes, tumour suppressor genes, etc.) of the immune system are best addressed in vaccine and immuno-approaches.

Radiation therapy and chemotherapy are two essential cancer treatment options; it would, therefore, seem obvious to assess cancer vaccines in combination with these interventions. It was previously thought that chemotherapy would be disadvantageous when applied together with a cancer vaccine due to its immunosuppressive properties. However, it is becoming apparent that chemotherapy may actually have some beneficial properties as it can reduce regulatory immune (T cell) activity that otherwise inhibits the anti-cancer immune response activated by the cancer vaccine. It is possible that an optimised chemotherapy regimen may be able to suppress this undesired inhibitory activity without incurring pronounced adverse effects. Furthermore, cancer cell killing performed by chemotherapeutic agents may also enhance vaccination strategies by releasing new molecular immune targets from the tumour cells (Svane/Straten, expert opinion). Radiotherapy-induced tumour cell death is a potent method of treating localised cancers, but has no curative potential when more disseminated disease is present. The radiation-elicited tumour cell death could, however, be used as an additional immune activator during vaccination therapy, due to the release of large amounts of tumour antigens and the induction of inflammation involving the so-called "danger signals" which attract the immune system to the tumour.

Thus it is becoming evident that immunotherapy is potentially synergistic with other cancer treatment modalities, such as chemotherapy and radiation therapy. This potential for synergy should allow cancer vaccines to become part of the standard treatment regimen for many common tumours within the near future (Svane/Straten, expert opinion).

But doubts remain whether the cancers of a common histogenetic origin are antigenically sufficiently related to allow a common strategy of immunotherapy or vaccination for all or at least a majority of affected patients. This may result in the need for personalised cancer treatment because, in the absence of common tumour antigens, immunotherapy, and especially vaccination, will depend on individual alterations of tumour cells that could be patient-specific.

Thus the target structure for immunotherapy in one patient might not be relevant to most other patients with the same disease. Such therapies would be highly specific and could offer a chance for a cure, but would also be rather cost-intensive.

Moreover, antigen-directed cancer therapy might induce a selection process, promoting the loss of this specific antigen in the tumour cells and resulting in resistant clones, as is observed in recurrence of the disease after initially successful radiotherapy or chemotherapy (Weller, expert opinion).

Also to be considered are the possible adverse side effects connected with new therapies. Compared to the often severe toxic effects of conventional chemotherapy, at least vaccine therapies seem to be in general more compatible (Svane/Straten, expert opinion) – an assessment made with the caveat that experience is still (very) limited. One should keep in mind that the therapeutic potential of activating the immune system could also provoke autoimmune reactions (Weller, expert opinion), especially in the case of tumour antigens which are not completely tumour-specific. And, with regard to the pre-testability and forecast of adverse side effects, it has to be taken into account that animal models are not suitable, as the nature of their antigens is different from that of humans (Svane/Straten, expert opinion).

Another difference in comparison with conventional chemotherapy is that the optimal dose is not necessarily based on the safety profile of the drug, but rather on the ability of the vaccine to induce biological and immunological activity (Svane/Straten, expert opinion). Due to the "biological" nature of immunotherapy, in many cases there will be no linear association between vaccine dose and immune response.

In summary, all three fields of innovative cancer therapy hold significant potentials for the treatment of tumour patients within the next decade, vaccine therapies and immunotherapies being probably the most promising. However, these will be combined with genetic approaches thus fulfilling in part the current definition of gene therapy (Weller, expert opinion). Traditional hormonal and chemotherapy are still important, but will decrease in importance with the use of targeted therapies by which, in the long run, they could possibly be completely substituted. Not only as far as neuro-oncology is concerned, surgery will probably remain important, firstly, because it is necessary to make histological diagnoses and, secondly, because surgery will be an integral part of all those innovative treatments delivered directly to the tumour tissue. This local approach is often pursued in the treatment of brain tumours (Weller, expert opinion). On the other hand, the example of modern breast cancer treatment shows that surgery will become probably less important at least in some cases (Untch, expert opinion).