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# Immunotherapy of malignant diseases: cancer therapy beyond chemotherapy

Following its development in the 1950s, chemotherapy with cytotoxic substances of in most cases relatively simple chemical structure was for many years the only available option for the treatment of advanced malignant disease. The past two decades, however, have seen the development of complementary approaches to cancer therapy which, by attacking more precisely defined targets, act more specifically against tumour cells and therefore come closer to the goal of achieving better disease control at a lower cost in terms of side effects. Immuno-therapeutic agents, signal transmission inhibitors, and gene therapy are considered to be especially promising in this respect.



On the basis of advances in our understanding of the biological basis of immunity and of ways in which this can potentially be influenced, three different new approaches to the immunotherapy of malignant diseases have been adopted over the past two decades. These are

- use of recombinant cytokines,
- use of monoclonal antibodies, and
- T cell-based cancer therapy.

These three approaches (Fig. 1), which are based on three distinct immunological mechanisms, are explained in brief below.

### Cytokines

Cytokines are regulatory molecules that control interactions between cells of different origin in many different ways. Cytokines are especially important in the regulation of blood formation, the immune response, and inflammatory processes. When used in cancer therapy, cytokines can bring about tumour rejection via direct inhibition of tumour growth and via indirect actions such as

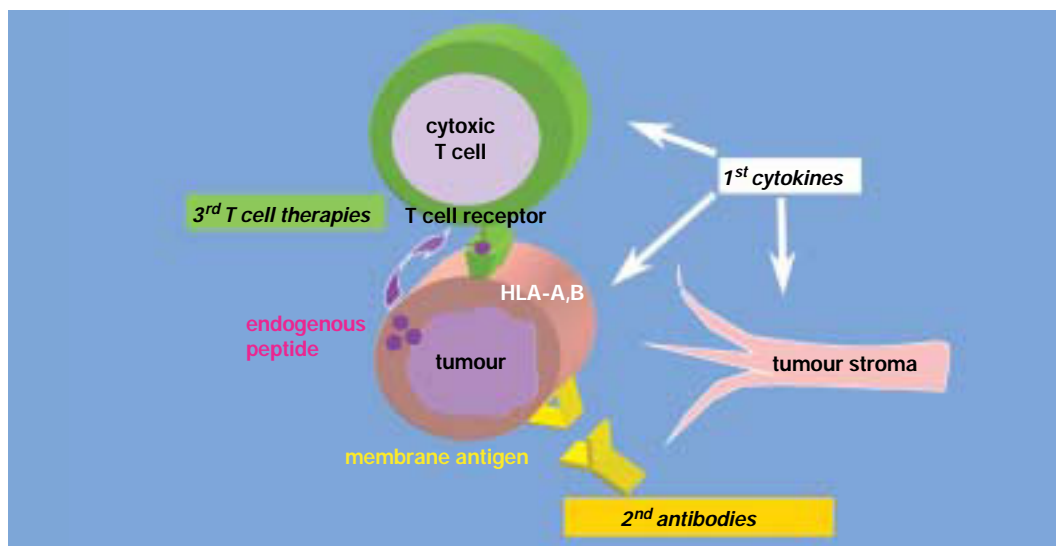


FIGURE 1: Approaches to the immunotherapy of malignant tumours adopted over the past 20 years (HLA = human lymphocyte antigen).

- an antiangiogenic action,
- inhibition of growth factor production in the vascular connective tissue (stroma) of tumours, and
- enhancement of the immune response.

On the basis of the technique for manufacturing recombinant proteins (recombinant DNA technology<sup>1</sup>) developed in the 1970s, more than a dozen recombinant cytokines have been developed to the stage of clinical trials over the past twenty years. Whereas growth-stimulating cytokines such as G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-monocyte colony-stimulating factor) have become widely used for restoration of granulocytopoiesis<sup>2</sup> after chemotherapy and in particular for obtaining stem cells for stem cell transplantation, only two recombinant cytokines have become firmly established in the treatment of malignant disease. These are

- interferon alfa, which is used in chronic myeloid leukemia and hairy cell leukemia and under some circumstances also in malignant lymphomas, Kaposi's sarcoma, essential thrombocythemia, and polycythemia, and
- the T cell growth factor interleukin-2, which is used in the treatment of advanced melanoma and renal cell carcinoma.

It is unsatisfactory that the precise mechanism of action of these two cytokines remains unknown. Despite promising preclinical profiles of action, no other cytokine has proved able to induce remissions<sup>3</sup> in malignant diseases. Only erythropoietin, which selectively stimulates red blood cell formation, has won a place in therapy, namely to treat the anemia that typically accompanies advanced malignant disease.

## Monoclonal antibodies

Over the past twenty years, monoclonal antibodies have become indispensable tools in the immunohistochemical diagnosis of malignant diseases. The principle of their manufacture is shown in Figure 2. In recent years, technical improvements relating to the manufacture of mono-

1 A genetic technique for creating new (recombinant) DNA molecules. Among other things, this permits genetic modification of mammalian cells and microorganisms in such a way that desired proteins can be manufactured on an industrial scale.

2 The formation in the hematopoietic (blood-forming) bone marrow of cells that provide nonspecific cellular immunity.

3 remission = subsidence of disease manifestations without cure

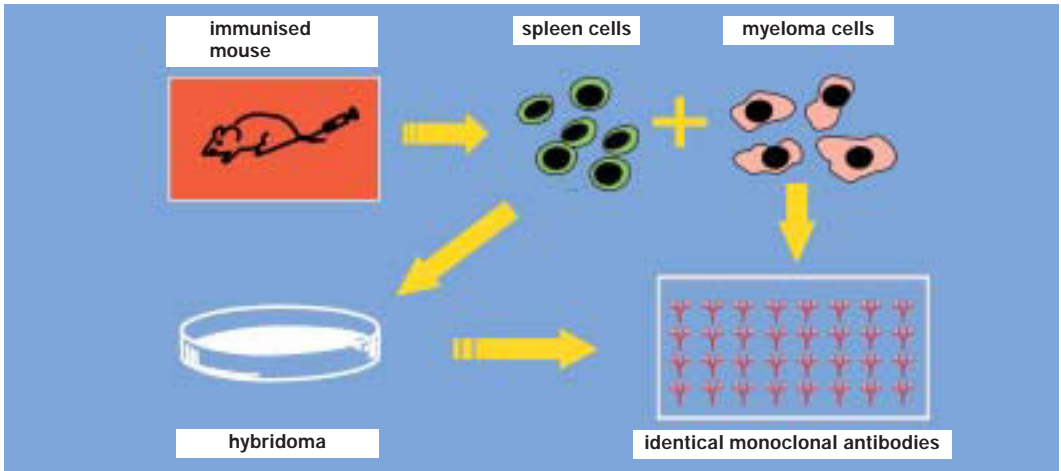


FIGURE 2: In order to produce monoclonal antibodies in large amounts and with a high degree of purity, short-lived antibody-producing cells from the mouse spleen are fused with immortal, continuously dividing tumour cells to form hybridomas. These genetically uniform cell lines can be kept and propagated in cell

cultures, each cell line producing a single type of (monoclonal) antibody [1]. The uniform structure and composition of monoclonal antibodies (MAbs) makes them suitable for research, diagnostic, and therapeutic purposes. Humanised MAbs can be used as highly effective drugs in the treatment of cancer.

clonal antibodies via

- DNA recombination,
- humanisation of murine<sup>4</sup> antibodies, and
- conjugation of antibodies to toxins and radionuclides

have led to their first successful clinical use and thus to their approval for use as medicines. A list of antibodies that have been approved for routine clinical use, together with their target antigens and indications, is given in Table 1. The first two of these antibodies are merely humanised. In the case of Mylotarg<sup>TM</sup>, by contrast, a toxin (calicheamycin) is bound to a CD33-specific chimeric antibody. This drug is thus a synthetic immunotoxin. Common to all three antibodies is the fact that

- they can induce remission even in chemotherapy-refractory<sup>5</sup> diseases,
- they act specifically on their target tissues, and
- their toxicity is low.

<sup>4</sup> murine = derived from mice

<sup>5</sup> refractory = largely or entirely unresponsive to treatment

**TABLE 1: Monoclonal antibodies approved for clinical use.**

antibody	proprietary name	target antigen	indication	literature ref.
Rituximab	Mabthera®	CD20	treatment of relapsed or refractory low-malignancy non-Hodgkin's lymphomas	[2]
Trastuzumab	Herceptin®	HER-2	treatment of chemotherapy-refractory breast cancer in combination with cisplatin	[3]
Gemtuzumab	Mylotarg™	CD33	treatment of relapsed acute myeloid leukemia in elderly patients	[4]

A large number of other antibodies directed against tumour-associated membrane components are currently undergoing clinical trials. Altogether, they promise to significantly broaden, but at the same time make more expensive, the available range of biological anticancer agents.

### T cell therapies

A steadily growing number of cell therapies are based on the use of tumour-reactive T lymphocytes. Figure 3 lists T cell therapies and illustrates the principle on which they are based. All the cells of the body are regulated by T lymphocytes, which function as an external monitoring system to mediate the immunological destruction of 'altered' tissues. The principal evolutionary function of the T cell monitoring system is to destroy virus-infected cells, however, the mechanisms by which this is achieved may also help to control many human cancers and leukemias. Endogenous peptides that result from intracellular breakdown of virus-encoded proteins or tumour proteins are bound in the endoplasmic reticulum to HLA-A, B histocompatibility antigens and anchored to the cell membrane in the form of a peptide/HLA complex. T lymphocytes with a cytotoxic function recognise this complex as foreign and kill the cells that bear it.

### Donor lymphocyte infusions

It has long been known that T lymphocytes mediate a powerful immunological protective mechanism directed against residual leukemia in allogeneic bone marrow transplantation<sup>6</sup>. The central role of T

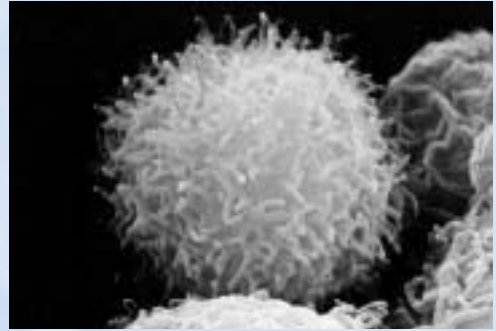
### Versatile T cells

Our blood contains 0.07 % white blood cells (leukocytes). They are divided into granulocytes, monocytes and lymphocytes. All these cells are formed from blood stem cells in the bone marrow. B lymphocytes differentiate into plasma cells and produce antibodies after contact with an antigen (humoral immunity). T lymphocytes mature in the thymus, where, for example, they learn to differentiate between self and nonself. T lymphocytes are responsible for cellular immunity. They are classified into:

- T helper cells
- cytotoxic T cells (T killer cells)
- T suppressor cells
- so-called memory cells.

T helper cells produce growth factors (cytokines and lymphokines) which aid antibody production by B lymphocytes. In cell-mediated immune responses cytotoxic T cells trigger apoptosis (programmed cell death) by releasing lytic enzymes (perforins and granzymes). However, this process begins only after prior binding between T cell surface receptors and a peptide/class 1 major histocompatibility complex (MHC) on the surface of the cells to be destroyed. T suppressor cells are a T lymphocyte subgroup which can suppress the immune response by T helper and cytotoxic

cells. Memory cells are responsible for immunologic memory. They are formed from B and T lymphocytes.



**Electron micrograph of a human T lymphocyte (x 20,000). Picture taken from [6].**

lymphocytes in providing protection against leukemia relapses after allogeneic bone marrow transplantation is clear from the following two observations:

- Removal of T lymphocytes from bone marrow grafts results in an increased incidence of leukemia relapses.
- In certain leukemia relapses, repeated administration of donor lymphocyte infusions leads once again to regression of the disease in a majority of patients.

### The concept of 'mini-transplantation'

The notion that allogeneic bone marrow and stem cell transplantation act as a form of T cell-based immunotherapy has led in the past few years to the concept of 'mini-transplantation'. In mini-transplantation to combat high-risk leukemia, the intensity of preliminary total body irradiation and/or chemotherapy is reduced

6 In allogeneic blood stem cell transplantation (BSCT), the hemopoietic stem cells of the patient are destroyed by chemotherapy and/or irradiation and replaced by

those of a histocompatible donor. In autologous BSCT, the patient's own hemopoietic stem cells are reinfused.

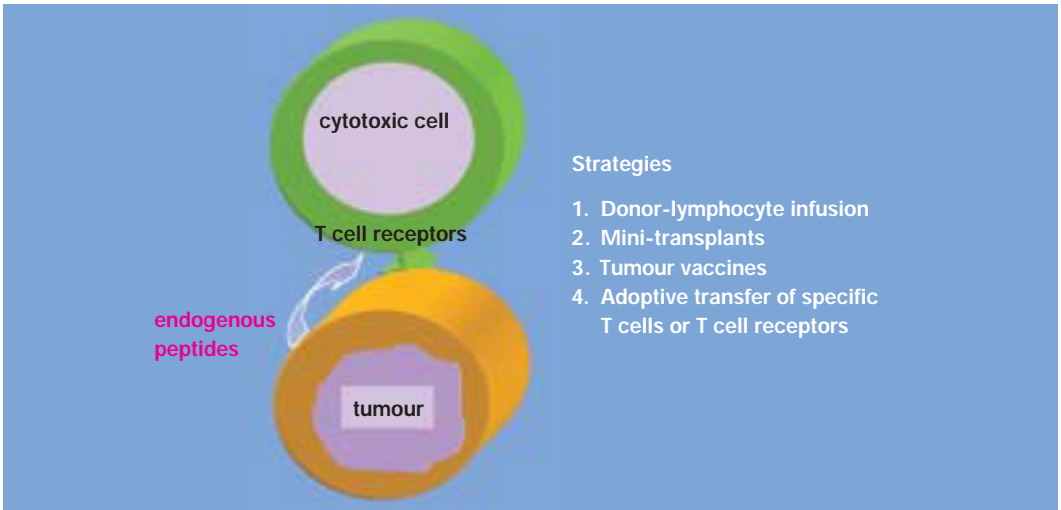


FIGURE 3: Summary of T cell therapies.

to such an extent that eradication of the leukemia is no longer possible. The principal effects of this form of transplantation are mediated by the immunological action of leukemia-reactive lymphocytes. This form of transplantation is now being used increasingly, especially in elderly patients and in patients in whom conventional allogeneic transplantation cannot be performed because of concomitant disease of other organ systems.

### Cancer vaccines

It has long been hoped that vaccines might be developed for use in the treatment of advanced cancer and in particular to prevent relapse of cancer after surgical removal. So far, however, the results achieved with autologous tumour cell vaccines have been rather disappointing. Nevertheless, three advances made within the past ten years now make it far more likely that this approach can be successfully implemented:

- Firstly, expression cloning has in the past few years resulted in the characterisation of an increasing number of molecularly well-defined tumour antigens that are target structures for cytotoxic T lymphocytes. A series of overexpressed tumour proteins such as HER-2, P53, and CEA, differentiation antigens such as tyrosinase, 'cancer-testis' antigens such as MAGE, mutant tumour proteins such as CDK4R24C, and viral onco-

proteins such as HPV-E6 and HPV-E7 are now available for testing in formal cancer vaccination studies.

- Secondly, the technology for producing vaccines has been considerably improved. Defined tumour antigens such as recombinant tumour proteins, tumour peptides, tumour DNA in the form of recombinant vaccine viruses that encode tumour antigens, and also tumour RNA are now being used as immunogens. In the past few years it has also been shown that ex-vivo charging of antigen-presenting dendritic cells is an especially efficient method of triggering potent immune reactions and even inducing tumour regression in isolated cases, at least in melanoma patients.
- Thirdly, innovative techniques such as ELISPOT<sup>7</sup> and tetramer technologies have been developed that permit quantification of tumour antigen-specific T lymphocytes in clinical trials. In a new generation of innovative cancer vaccine studies in which molecularly defined tumour antigens are used for vaccination, the success of vaccination is to be assessed both in terms of clinical tumour regression and in terms of induction of tumour-specific T cells.

It is to be hoped that these innovative approaches will lead to better clinical results in the next few years.

### Adoptive immunotherapy

In the past few years, significant advances have also been made in the so-called adoptive immunotherapy of malignant diseases by administration of tumour-specific lymphocytes or gene transfer of tumour-specific T cell receptors. A number of working groups have succeeded in propagating virus- or tumour antigen-specific T lymphocytes ex vivo. In isolated cases the transfer of such tumour-specific T lymphocytes to patients has led to a reduction in viral load, e.g. in post-transplantation Epstein-Barr virus disease, or in the number of tumour cells, e.g. melanocytes in patients with melanoma. Nevertheless, these favourable results have been achieved only with great difficulty and at enormous expense due to the need to produce such cellular therapeutic agents on an individual basis. As yet, therefore, such techniques

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<sup>7</sup> from **ELISA-SPOT**: a method for the quantitative determination of tumour-specific T cells

do not appear to be suitable for routine use. In the past few years, we and other working groups have therefore concentrated our efforts on the technique of T cell receptor gene transfer [5]. T cell receptor genes from tumour antigen-specific T cell lines of mice have been cloned, recombinant T cell retroviruses constructed, and primary human T lymphocytes infected (transduced) *ex vivo* with these viruses. Using this technique, the ability to destroy a wide variety of tumours has been successfully transferred (transduced) to normal human lymphocytes of healthy donors. It remains to be seen whether the favourable results obtained with these techniques in preclinical studies can also be obtained in the adoptive immunotherapy of malignant diseases.

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