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Biological Therapy—Where do we Stand?

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THE POSSIBILITY to influence malignant processes with biological approaches provides preclinical and clinical scientists with a fascinating new challenge and is expected to revolutionise cancer therapy. Biological therapy of cancer encompasses cytokines and haematopoietic growth factors (HGFs) as well as treatment strategies using (humanised) antibodies, vaccines or the various gene therapeutic approaches. The spectrum of *in vivo* effects of biologicals covers modulation of immune response, stimulation/inhibition of haematopoiesis, direct regulation of cellular growth and differentiation, and modulation of tumour vascularisation. Rapid progress towards the understanding of the mechanisms of action and the evaluation of the clinical relevance of this broad range of agents is requiring new research strategies with close collaboration among preclinical and clinical scientists. As the final therapeutic potential of a biological agent and its network interactions within the integral human body can only be assessed following careful studies in man, the effectiveness of clinical trials must be improved by closely relating them to '*ex vivo*' research programmes utilising material from patients.

FROM BENCH TO CLINICAL APPLICATION

Compared to cytotoxic agents a variety of differences exist not only in the principles of preclinical investigation but also in the *in vivo* application of biologicals [1]. Treatment frequently leads to the modulation of a cascade of events which often cannot be foreseen from experimental animal studies. Modulation of complex networks, we are only beginning to understand, means that unexpected effects including unwelcome side-effects can occur which must be carefully monitored. To further aggravate the dilemma, induction of secondary effects may be variable and related to timing of application and to dosage.

The problem of dosage is a major difficulty and a potential pitfall in the set-up of clinical trials with biologicals. In biological therapy, the aim is usually less the definition of the maximally tolerated dose (MTD) which, from our experience with cytotoxic agents, we are accustomed to using to eradicate a tumour. Much more relevant is the definition of the optimal biologically active dose (OBAD) [2], which is the minimal dose resulting in the significant augmentation of effector cell activity correlated with the therapeutic response. An example of the possible pitfalls of dosage is represented by IFNγ, where a bell-shaped curve of activity has been shown *in vivo* [3], suggesting that too high a dosage may suppress a desired response and too low a dosage may fail to invoke it. The implications for the set-up of early clinical trials are significant: biological endpoints need to be defined allowing the investigators to obtain proof of concept and potentially move to phase III within a short period of time.

The Biological Therapeutics Development Group (BTDG) of the European Organization of Research and Treatment of Cancer (EORTC) was created to meet the challenge of bringing new biological agents and concepts into the EORTC drug development pipeline. To date, most of the clinical trials are being initiated with (humanised) monoclonal antibodies and with anticancer vaccines.

CONCEPTS OF BIOLOGICAL THERAPY

Monoclonal antibodies

Monoclonal antibodies (MAbs) are in the process of becoming an integral component of medical cancer therapy. Because of their specific antigen-binding, MAbs represent a potentially extremely effective form of cancer treatment causing only minor side-effects (fever, flu-like symptoms, etc.). Moreover, genetic engineering makes it possible to produce 'humanised' antibodies which correspond with human antibody molecules and have the advantage of remaining in the body for long periods of time. Therapeutic MAbs act on tumour-associated surface structures in a manner similar to the immune response induced by antitumour vaccines. Binding of the antibody to the cell surface recruits cytotoxic effector cells such as lymphocytes and macrophages while also activating the complement system. In parallel most therapeutically effective antibodies use a transmembrane transfer of signals into the neoplastic cell and induce mechanisms that can cause programmed cell death (apoptosis).

The murine MAb 17-1A recognises a glycoprotein that occurs mainly on the surface of adenocarcinomas. It is produced with the help of a murine cell line derived from the fusion of a myeloma cell line with murine spleen cells previously immunised with human colorectal carcinoma cells.

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The mode of action of the 17-1A antibody is complex and mediated by inducing antibody- as well as the complement-dependent cell-mediated cytotoxicity. As an additional mode of action it is assumed that the antibody leads to the production of anti-idiotypic antibodies which bind to certain structures of the 17-A1 antibody. In the ensuing cascade, immunoglobulins against the anti-idiotypic antibodies are formed, which can possess a cross-reacting antitumour activity and thus further enhance the 17-A1 antibody effect. In principle, the therapeutic use of murine MAbs can lead to the development of human antibodies directed acting against them. This reaction is known as human anti-mouse antibody (HAMA) formation and in principle could negatively influence or even prevent the desired effect of the mouse antibody in humans. Nevertheless the 17-A1 antibody has proven to be effective in a phase III trial in locally advanced colorectal carcinoma (Dukes C) [4].

The first 'humanised' antibody, directed at the CD20 surface structure on B lymphocytes has been approved for administration to patients with CD20 positive non-Hodgkin's lymphoma (NHL). The CD20 antigen is expressed on the surface of mature B lymphocytes and by the majority of malignant lymphomas derived from B cells. In contrast, the CD20 antigen is not expressed by haematopoietic stem cells or very early B cells and T cells. Clinical studies involving the intravenous administration of this CD20 antibody (Rituximab) in patients with low-grade NHL most of whom had previously undergone chemotherapy [5] showed enhanced removal of B cells expressing CD20 with an overall response rate of approximately 40%.

Another antibody proven to be effective in clinical trials is a humanised anti-HER-2 antibody (trastuzumab). The interaction between epidermal growth factor (EGF) and its receptor (EGFR) correlates with proliferation and prognosis in several types of cancer. About 25 to 30% of breast and ovarian carcinomas express a mutated form of EGFR, the so-called HER-2, which is activated without ligand binding and which indicates poor prognosis. Clinical phase II and III trials performed to date show that Herceptin induces durable objective tumour responses as single agent and in addition to chemotherapy is able to significantly increase response rate and progression free survival in HER-2 positive metastatic breast cancer [6].

Further humanised antibodies that are currently being investigated are anti-CD33 in acute myeloid leukaemia and myelodysplastic syndrome and anti-VEGF directed against vascular endothelial growth factor.

Anti-cancer vaccines

The identification of a variety of tumour-associated or tumour-specific antigens presents a unique opportunity to induce antitumour immunological responses *in vivo* [7]. Therefore, numerous early clinical trials have been initiated on potential cancer vaccines. Although the relative antitumour efficacy of antibody versus cell-mediated immune responses is not yet known, the vast majority of cancer vaccines entering into clinical trials have been designed to induce cell-mediated immune responses. This strategy is based on recent developments in cancer immunology which demonstrate the critical role of T cells in anticancer responses and the antitumour activity of cancer vaccines both in animal models and in patients. Antigens recognised by T cells are peptide fragments of intracellular proteins, bound to the MHC molecules and then expressed on the cell surface. Although the antitumour responses observed in clinical trials with vaccines were better than expected, these agents can be potentially active even if they do not show activity in early clinical trials with heavily pretreated patients.

Antitumour vaccines are in principle based on three concepts: peptide vaccination, vaccination with genetically modified organisms (GMO), or by application of autologous tumour cells which may be genetically modified. Peptide vaccination implies the possibility to produce a tumour-associated antigen by means of gene technology and apply it as a drug subcutaneously. Clinical trials are underway mainly in cancers like melanoma where the tumour-associated antigenic structures on the cell surface are well defined. Although the first results seem encouraging, vaccination with peptides has the potential disadvantage to require a more or less intact immune system, a supposition that is often not fulfilled in progressed stages of cancer. Another important problem is the antigen restriction by the major histocompatibility complex (MHC). The MHC comprises a number of genes coding for membrane-bound proteins that play an important role in T cell activation. For specific recognition of a foreign cell by a T lymphocyte not only antigen expression of the cell is important but also antigen presentation by the respective MHC molecule. Therefore, immune responses underlie the control of MHC-coded membrane-bound proteins and a successful peptide vaccination is restricted by the fact that cytotoxic T lymphocytes can only recognise the respective tumour associated antigen (TAA) in patients with the correct HLA phenotype.

Other approaches which have been introduced into early clinical trials using the vaccination strategy focus on the application of genetically modified organisms (GMO). A GMO usually is represented by a virus transfected with the genetic information to code for a tumour-associated antigen. Therapy with GMO must be distinguished from somatic gene therapy as although new genes are transfected in a recombinant virus no somatic cells are altered regarding their genetic information. One advantage in favour of vaccination with GMO is the fact that the mechanism of MHC restriction may be avoided as proteins expressed by the virus in the human cells can be processed by the immune system and expressed on the cell surface without underlying the diversity of the MHC complex.

Another key development that has created substantial new interest in cancer vaccines is the ease with which cytokine genes can be introduced into cancer cells to modulate their immunogenicity. These techniques of somatic gene therapy allow the generation of immunogenic cancer vaccines from the patient's own tumour and seek to locally alter the immunological environment of the tumour. In particular, genes that express cytokines like IL-2 or GM-CSF are already being used in clinical trials. The concept underlying the use of cytokine gene-transduced tumour cells is that the cytokine is produced at very high concentrations local to the tumour. As systemic concentrations of cytokines are usually very low, this paracrine physiology much more closely mimics the natural biology of cytokine action than does the systemic application of cytokines.

Four previous meetings 'Biological Therapy of Cancer—From Basic Research to Clinical Application' have been organised in Innsbruck, Austria in 1991 and Munich, Germany in 1993, 1995 and 1997 by the Biological Therapeutics

Development Group (BTDG) of the EORTC, the National Cancer Institute (NCI), the Cancer Research Campaign (CRC) and the Society for Biological Therapy (SBT). The last two meetings were organised in collaboration also with the German Society for Haematology and Oncology (DGHO).

Since the previous meetings which had focused on concepts based on preclinical research, some fundamental changes have occurred: the first humanised antibodies have demonstrated proof of concept and are FDA-approved. Furthermore our knowledge based on the experiences with early clinical trials applying anticancer vaccines has expanded considerably in terms of defining biological endpoints. The fifth international meeting 'Biological Therapy of Cancer—From Basic Research to Clinical Application' will again focus on stimulation of contacts among basic scientists and clinicians through discussion of clinical trials and the rationales for the development of biological cancer therapy.

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