

# The immune system and cancer

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**The hypothesis of immunologic surveillance of neoplasia is predicted on the theory that the immune system is capable of discriminating self from foreign antigens, and that tumour-specific antigens are regarded by the immune system as nonself. An alternate view proposed was that the immune system has evolved to detect danger by employing 'professional' antigen-presenting cells as sentinels of tissue distress.**

**The field of tumour immunology has witnessed short bursts of great excitement followed by longer periods of pessimism. Enthusiasm appears to be rising again as many tumour-associated antigens have been identified and their use in vaccines is currently the subject of many clinical trials. The development of genetic engineering has allowed the conversion of anti-tumour mouse monoclonal antibodies into mouse-human chimerized antibodies and humanized reagents, which are undergoing clinical trials. Although 'immunogenotherapy' appears to be a promising approach, much work still needs to be done to understand the mechanisms involved so that efficacious treatment modalities can be designed.**

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CANCER is a major health problem worldwide and one of the most important causes of morbidity and mortality in children and adults. Cancers arise from the uncontrolled proliferation and spread of clones of transformed cells. From an immunologic perspective, cancer cells can be viewed as altered self-cells that have escaped normal growth-regulating mechanisms. The possibility that cancers can be eradicated by specific immune responses has been the impetus for a large body of work in the field of tumour immunology.

In the first half of this century, infectious diseases were the major cause of human suffering and death. Medical research was devoted to the conquest of microbes and the objectives focused on understanding how the immune system reacted to the exogenous stimuli. The possibility that cancers are also viewed by immune system as 'non-self' was postulated by Macfarlane Burnet in 1950. The concept of 'immune surveillance' states that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumours

and to kill tumours after they are formed. This view of cancer as nonself gained wide acceptance. Burnet proposed that the immune system defines the 'self' before birth and predicted that allogenic cells injected into a foetus or neonate would automatically be regarded by the immune system as self and therefore tolerated. A necessary consequence of this model is that antigens making their first appearance after the immune system has reached maturity would automatically be regarded as nonself and attacked<sup>1</sup>.

Capitalizing upon Burnet's self marker concept and the discovery of lymphocytes as the mediators of immune response, Joshua Lederberg, a molecular geneticist, proposed in 1959 the first theory of self/nonself discrimination<sup>2</sup>. The model was quite simple, lymphocytes are born in a state in which antigen recognition leads only to inactivation, but then mature to a state where antigen recognition leads only to activation. Since birth was taken to be the rough divider between immunological immaturity and maturity, the theory meshed nicely with Burnet's model. The hypothesis of immune surveillance of neoplasia, first proposed by Lewis Thomas but later championed by Burnet, was a natural outgrowth of the Lederberg's theory of self/nonself discrimination<sup>3</sup>. Because most tumours arise after the immune system has reached maturity, any unique antigens expressed by the tumours should be regarded as nonself. Therefore, a major function of the immune system is to survey the body for the development of malignancy and to eliminate tumours as they arise. A critical feature of the immune surveillance hypothesis is that the default reaction of the mature immune system to new antigens is activation, and so the major question posed to cancer researchers by this hypothesis was how tumours managed to sneak through this surveillance mechanism.

P. Matzinger proposed the 'Danger Model' as an alternate view to answer this query. She argued that it was time to abandon the self/nonself discrimination paradigm and to adopt a more global perspective, one which the need to defend against lethal pathogens (or tumours) and the need to avoid lethal autoimmunity are equally balanced. The 'Danger Model' proposed that to avoid autoimmunity, the default reaction of T cells to antigens on non-hematopoietic tissues is tolerance, and that is the role of blood-derived 'professional' antigen presenting cells (APC), particularly dendritic cells, to detect and report to T cells situations of dangerous tissue distress<sup>4</sup>. Because tissue

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cells induce tolerance in susceptible T cells, it was predicted that the default immune response to tumour-specific antigens occurring on such tissues is tolerance as well.

The 'danger model' was based on a 'two-signal' model of lymphocyte activation put forth by Bretscher and Cohn<sup>5</sup> and later modified by Lafferty and Cunningham<sup>6</sup> in which antigen receptor ligation (signal 1) is an off signal to the lymphocyte unless accompanied by a second signal (signal 2) delivered by an antigen presenting cell. The 'danger-model' starts with the view that antigen-presenting cells (APC) are not constitutively able to offer co-stimulatory signals and that they must first be activated. The activation of an APC depends on the health of the cells in its neighbourhood. If a cell is injured it sends activating signals to its local APC which then take up local antigens, travel to the draining lymphnodes and upregulate the co-stimulatory molecules needed to activate the T cells. Danger signals may be constitutively present within cells or induced only upon stress such as infection, temperature shift, hypoxia or trauma. Cells of the innate immune system, such as granulocytes and macrophages, possess receptors for conserved microbial molecules by which pathogens can be recognized and engulfed. Janeway argued that the 'pattern recognition receptors'<sup>7</sup> for instance the LPS receptor, have been adopted by cells of the adaptive immune system to serve as inducers of signal 2. Heat shock proteins, synthesized by cells in response to a variety of stress, such as heat, trauma and infections, may also be mediators of a danger signal.

According to a self/nonself discrimination paradigm, tumours avoid immune surveillance by hiding from the immune system. Trauma to the tumour bed or infection at the site of the tumour would expose the immune system to tumour-specific antigens for the first time, leading to 'spontaneous' regression. According to the danger model, trauma or infection would provide the stimulus to activate professional APCs near the tumour, and these APCs would present tumour antigen along with the appropriate signal 2 to naïve, tumour-antigen specific T cells. So how do the two models differ? According to the immune surveillance hypothesis, the default response of mature T cells is activation, whereas in the danger model the default response of T cells is inactivation, and a danger signal is constantly required to sustain the immune response. Therefore, according to the self/nonself discrimination paradigm, once the immune system has been activated by tumour-specific antigens, the T cell response to the tumour should be sustained until the tumour is eliminated. But according to the danger model, the response would proceed only so long as danger signals are present. In the absence of a danger signal, memory T cells encountering the tumour-specific antigen on the tumour cell would receive only the signal 1 in the absence of signal 2 and become tolerized<sup>8</sup>.

## Tumour antigens: Candidates for vaccine development

A variety of tumour antigens that are recognized by T and B lymphocytes have been identified in human and animal cancer. The first experimental demonstration that tumours can induce protective immune responses came from studies of transplanted tumours performed in 1950. In these experiments sarcomas were induced in inbred mouse by painting the skin with the chemical carcinogen methylcholanthrene (MCA). If the MCA-induced tumour is excised and transplanted into other syngenic mice, the tumour grows. In contrast, if the tumour is transplanted back into the original host, the mouse rejects the tumour. The mouse that had become immune to its tumour is incapable of rejecting MCA-induced tumours produced in other mice. Furthermore, T cells from the tumour-bearing mice can transfer protective immunity to another tumour-free mice. Thus, it was demonstrated that immune responses to the tumour exhibit the defining characteristics of adaptive immunity specificity and memory and are mediated by lymphocytes.

The earliest classification of tumour antigens was based on their patterns of expression. Antigens that are expressed on tumour cells but not on normal cells were called tumour-specific antigens (TSAs); some of these antigens were unique to individual tumours; whereas others are shared among tumours of the same type. Tumour antigens that are also expressed on normal cells were called tumour associated antigens (TAA). In most cases these antigens are normal cellular constituents whose expression is aberrant or dysregulated in tumours.

An important breakthrough was the development of techniques for identifying antigens that are recognized by tumour-specific T lymphocytes. Tumour antigens that are recognized by T cells are likely to be the major inducers of tumour immunity and most promising candidates for tumour vaccines. The current approach to immunotherapy mainly relies on the role of CD8<sup>+</sup> cytotoxic T lymphocytes (CTL). Tumour antigens recognized by CD8<sup>+</sup> CTL are capable of lysing tumour cells directly and eradicating tumour masses *in vivo* in animal tumour models. Little attention has been paid to CD4<sup>+</sup> T helper cells and only a few MHC Class II restricted tumour antigens have been identified thus far.

### *Tumour-specific shared antigens*

Tumour antigens may be normal cellular proteins that are over expressed or aberrantly expressed in tumour cells. Many such antigens have been identified in human tumours, such as melanomas, by the molecular cloning of antigens that are recognized by T cells from tumour-bearing patients. Melanoma antigen MAGE-1 was the first tumour antigen identified on human melanoma using

a genetic approach<sup>9</sup>. A genomic library derived from the tumour cell line was transfected into an MHC matched antigen loss variant and were screened with CTLs derived from the melanoma patient who had been repeatedly immunized with mutagenized autologous tumour. MAGE-1 and MAGE-3 were identified based on the specific antigenic peptides recognized by HLA allele restricted CTL. In addition to melanomas, MAGE proteins are expressed on carcinomas of the bladder, breast, skin, lung and prostate. In normal tissues, MAGE expression is restricted to the testis and placenta; it is postulated that these are immunologically privileged sites where T cells do not respond effectively to antigens, so the antigens are ignored. Subsequent to the identification of the MAGE, other additional antigens such as BAGE, GAGE and RAGE were identified which varied in their peptide specificity and HLA restriction<sup>10,11</sup>. Several other melanosomal antigens, including tyrosinase, MART-1/Melan-A, gp100, TRP1 and TRP2, have been identified by screening cDNA libraries with tumour-reactive tumour infiltrating lymphocytes (TIL) which induced tumour regression when administered to autologous patients along with interleukin (IL-2). These antigens were expressed in melanoma, normal melanocytes and retina, but not other normal human tissues.

#### *Tumour-specific unique antigens*

Many mutations have been identified in tumour suppressor genes such as *ras*, *p53* and *p16* in tumour samples<sup>12,13</sup>. Because mutated proteins or peptides have the potential to be more immunogenic and be seen as foreign by the host immune system, it has been long assumed that many tumour antigens would be mutated antigens recognized by T cells. Surprisingly, the majority of tumour antigens identified are also non-mutated self-proteins. To test whether mutated *ras* and *p53* are tumour-specific antigens, several groups have raised CTLs against normal or mutated peptides from the *ras* proto-oncogene and *p53* tumour-suppressor gene. However, in most cases these CTLs failed to recognize tumour cells. Several mutated gene products have been recently identified as tumour-specific antigens recognized by CTL derived from patients by screening cDNA libraries using reactive CTLs.

MUM-1 (melanoma-ubiquitous mutated) antigen was isolated following the transient transfection of COS cells with HLA-B44 and pools of cDNAs derived from the LB33 melanoma cell line. The peptide epitope, EEKIVVLF, was found to be recognized by CTL<sup>14</sup>. DNA sequence analysis revealed that a point mutation in the sequence of the cDNA isolated from the tumour led to a change of one amino acid (Ser to Ile) at position 5 of the peptide. Since both the normal and mutated peptides bound efficiently to the class I HLA-B44 molecule, but only the mutated form

could be recognized by T cells, this indicated that the mutation appeared to have an effect on T-cell recognition. Further, analysis indicated that the antigenic peptide spanned the intron-exon boundary of an incompletely spliced mRNA.

A second product of a mutated gene is cyclin-dependent kinase 4 (CDK4), an enzyme involved in cell cycle control. DNA sequence analysis indicated that a point mutation (a C to T transition) led to a substitution of a cysteine for an arginine residue at codon 24, resulting in a new epitope recognized by CTL<sup>15</sup>. The CDK4 protein usually forms a complex with cyclin D1 and phosphorylates the retinoblastoma RB protein, and therefore promotes the cell cycle progression from G1 to S phase. However, assembly of CDK4 with cyclin D1 as well as its kinase activity was found to be inhibited by p16<sup>INK4a</sup>. Interestingly, p16<sup>INK4a</sup> cannot bind to the mutated CDK4 and failed to inhibit the kinase activity of CDK4/cyclin D, implying that the mutation in the CDK4 gene leads to a loss of cell cycle control.

The mutated  $\beta$ -catenin gene product was also recently identified and shown to be recognized by TIL derived from a melanoma patient<sup>16</sup>. Partial cDNA sequence analysis indicated that a point mutation was found to be responsible for a change of serine to phenylalanine in the coding region and constituted a T-cell epitope (SYLDSGIHF) for T-cell recognition. The  $\beta$ -catenin protein has been shown to be a cytoplasmic protein that interacts with the cellular adhesion molecule e-cadherin. A number of mutations have been found in the  $\beta$ -catenin gene product from different tumours. Loss of cell adhesion molecules may play a role in the metastatic process. Recent reports showed that the upregulation of stabilization of  $\beta$ -catenin may contribute to tumorigenesis and cancer progression due to mutations in the adenomatous polyposis coli tumour-suppressor protein or  $\beta$ -catenin. More importantly, the point mutation initially identified by CTL screening of a melanoma cDNA library is identical to that in  $\beta$ -catenin identified in colon cancer.

The mutated CASP-8 was recently identified with CTL specific for human squamous tumour. The antigen encoded by caspase-8 is required for the induction of apoptosis through Fas-FasL and tumour necrosis factor (TNF) pathway. The T-cell epitope was identified from an extended C-terminus portion of the protein resulting from the nucleotide substitution in the stop codon by the point mutation<sup>17</sup>.

#### *Putative tumour antigens expressed on epithelial tumours*

The majority of human melanoma antigens identified thus far are tissue-specific. HER-2/neu was recently identified as a shared tumour antigen recognized by T cells in breast

and ovarian cancers. The HER-2/neu proto-oncogene encodes a tyrosine kinase protein whose expression was shown to be increased in 30% of breast and ovarian cancers. In breast cancer, HER-2/neu overexpression was reported to be associated with aggressive disease. Cytotoxic T lymphocytes isolated from tumour-associated lymphocytes can specifically recognize a synthetic peptide corresponding to amino acids 971–980 of the HER-2/neu protein. This was the first demonstration that CTLs isolated from human tumours recognize HER-2/neu as an ovarian tumour antigen. Recognition and lysis of ovarian cancer cells by CTLs were also shown to correlate with the expression level of HER-2/neu in the tumour cells. Most importantly, the breast and ovarian cancer-specific CTLs recognized the same epitope peptide (GP2: amino acids 654–662) derived from the HER-2/neu protein in the context of HLA-A2. It appears that the GP2 peptide represents a common epitope shared by different epithelial tumours because it was recognized by CTL lines derived from breast, ovarian, non-small lung and pancreatic cancers<sup>18</sup>.

#### *Tumour-associated mucins*

Altered glycosylation appears to be a constant phenomenon associated with oncogenic transformation in experimental systems as well as in essentially all types of naturally occurring human cancers. Most of the biochemical or, more recently, immunological methods used to identify tumour-associated antigens have resulted in the isolation of glycolipids or glycoproteins (mucins) with altered glycosylation patterns. Mucins are large (> 200 kDa) glycoproteins with a high carbohydrate content. They are expressed by a variety of normal and malignant epithelial cells.

Expression of *MUC-1* gene product has been shown to be associated with breast and pancreatic adenocarcinomas. The *MUC-1* gene is expressed on epithelial cells, fibroblasts and B cells, and can serve as a target for T-cell recognition. However, T-cell recognition of the *MUC-1* gene product appeared to be non-MHC restricted. The epitopes for T-cell recognition were found in the tandem repeat of the MUC-1 protein. MHC-restricted MUC1-specific CTL have been generated and shown to recognize breast tumours. One peptide derived from the tandem repeat was found to be recognized by HLA-A2 restricted CTL and the other recognized by HLA-A11, A1 and A3 restricted CTL<sup>19</sup>.

#### **Mechanisms of immune evasion by tumours**

The long lasting paradox in tumour immunology has been 'why do antigenic tumours escape immune destruction'. The tumour cells have developed a process of 'immune

evasion' often called 'tumour escape' which may be a result of several mechanisms<sup>20</sup>.

#### *Downregulation of MHC class I expression*

Malignant transformation of cells is often associated with a reduction (or even a complete loss) of class I MHC molecules and a number of tumours have been shown to express decreased levels of class I MHC molecules. Since CD8<sup>+</sup> CTL recognize only antigen associated with class I MHC molecules, any alteration in the expression of class I MHC molecules on tumour cells may exert a profound effect on CTL-mediated immune response. Tumour viruses have evolved ways to decrease class I MHC expression and assembly with peptides, thereby blocking presentation of viral antigens to CTLs. These mechanisms may be operative in virally induced tumours. Tumour derived interleukin 10 can lead to reductions in MHC and TAP (transporter associated with antigen processing) expression.

#### *Modulation of tumour antigens*

Certain tumour-specific antigens have been observed to disappear from the surface of tumour cells. Such 'antigen loss variants' are common in rapidly growing tumours and can be readily induced in tumour cell lines by culture with tumour-specific antibodies or CTLs.

#### *Lack of expression of co-stimulatory molecules*

T cell activation requires an activating signal, triggered by recognition of a peptide MHC complex by the T cell receptor, and a co-stimulatory signal, triggered by the interaction of B7 on antigen-presenting cells with CD28 on the T cells. Both signals are needed to induce IL-2 production and proliferation of T cells. Without sufficient numbers of antigen-presenting cells in the immediate vicinity of a tumour, the T cells will receive only a partial activating signal, which may lead to clonal anergy.

#### *Loss of signaling molecules*

In cancer patients and in some tumour-bearing mice, alterations in signal transduction molecules such as T cell receptor Zeta chain, p56lck and NF- $\kappa$ B p65 in T and natural killer (NK) cells are observed. These changes although not antigen-specific, do appear to start at the site of the tumour and eventually become detectable in peripheral blood T cells or splenocytes, suggesting that tumour microenvironment induces alterations in the signal transduction pathways. These changes in signalling molecules

can often be related to 'immune dysfunction' observed in the patients.

### *Products of tumour cells suppress anti-tumour immune responses*

Tumour cells secrete large quantities of immunosuppressive cytokine transforming growth factor- $\beta$  (TGF- $\beta$ ), which inhibits the proliferation and effector functions of lymphocytes and macrophages. An additional escape mechanism adopted by tumour cells is expression of Fas ligand (FasL) by some tumours, which can induce apoptosis of TIL.

### **Immunotherapy of cancer**

Activating the immune response against resident cancer cells has been a 'dream' of immunologists since Ehrlich originally proposed his 'magic bullet' strategy for targeting cytotoxic agents to tumour cells via tumour-specific antibodies. Although the concept of harnessing the immune system against autologous tumour is attractive, there has been over the years both skepticism and enthusiasm for cancer immunotherapy. During the past 10 years, however, tumour immunology has undergone a renaissance and there are now numerous experimental strategies that have demonstrated the efficacy in experimental animal models and are in the process of being tested in clinical settings.

'Tumour immunotherapy' was virtually dominated by what has been termed non-specific approaches to manipulating the immune response to cancer. Long-term regressions of cancers associated with concomitant bacterial infection or injection of mixed bacterial vaccines have been reported. The therapeutic benefit of BCG, levamisole and bacterial products have also been investigated. Unfortunately, the overall results of these non-specific immunotherapies have not been encouraging.

More recently, vaccines composed of killed tumour cells or tumour antigens have been administered to patients and strategies for enhancing immune responses against the tumour are being developed. The major question for cancer immunotherapy is 'how can an effective anti-tumour CTL response be elicited'. The universal answer that has emerged is an effective anti-tumour CTL response requires that T cells be stimulated by specific antigen presenting cells that are called dendritic cells (DC). DCs were first described as morphological distinct langerhans cells in the skin and have since been shown to be the most efficient APC for activation of naïve T cells. The development of simple methods to isolate DC precursors from blood and the expansion of these cells *in vitro* to yield potent APCs have enabled their clinical use in cancer immunotherapy<sup>21</sup>. Several approaches have been used to load DCs *ex vivo* with tumour antigens. Antigen

loaded DCs are then given to patients in the hope that they will elicit a specific anti-tumour response. DCs can be loaded with (i) peptides eluted from MHC class I molecules; (ii) tumour-specific idiotype protein; (iii) RNA derived from neoplastic cells or by fusion of DC with tumour cells. Human clinical trials of tumour antigen loaded DC have been initiated for the treatment of B-cell lymphoma, prostate cancer melanoma and renal cell carcinoma.

Although heat shock proteins (hsps) are the best candidates for the 'danger signal' that might trigger the immune response, the specificity of the anti-tumour response that is induced by tumour-derived hsp vaccines suggests that hsps could have a more important role than as just non-specific danger signal. The tumour-specific immunity observed is mediated by tumour-specific peptides that are chaperoned by hsp and it is against these antigens that the immune response is directed. The cancer vaccine studies have shown that APC of the macrophage DC lineage can take up hsp-tumour peptide complexes and efficiently present these chaperoned peptides to CD8<sup>+</sup> T cells, to yield tumour-specific CTLs. Most importantly these studies have shown that the hsp-chaperoned peptides are independent of the MHC type of the tumours from which they are derived, whereas their presentation to the CTLs is MHC class I restricted and is defined by the MHC phenotype of the APC used.

### *Cytokines in immunotherapy of cancer*

Cytokines may also be administered systemically for the treatment of various human tumours. The largest clinical experience is with IL-2 administered in high doses alone or in conjunction with lymphokine-activated killer (LAK) cells. After the administration of IL-2, numbers of blood T and B lymphocytes, NK cells are increased with increase in serum TNF, IL-1 and IFN- $\gamma$  concentrations. The severe toxicities associated with high dose IL-2 and IL-2 + LAK cells include fever, pulmonary oedema and capillary leak syndrome. IL-2 has been effective in inducing measurable tumour regression in patients with advanced melanoma, and renal cell carcinoma.

Currently the potential of IL-12 to enhance anti-tumour effect via T cells and NK cells has aroused great interest and phase I and II trials are being conducted on patients with advanced cancer. Hematopoietic growth factors, including GM-CSF, G-CSF and IL-11 are used in cancer treatment protocols to shorten periods of neutropenia and thrombocytopenia after chemotherapy or autologous bone marrow transplantation.

### *Immunotherapy with anti-tumour antibodies*

The potential for using antibodies as 'magic bullets' against cancer has been alluring investigators since

Kohler and Milstein described the making of monoclonal antibodies (MAbs)<sup>22</sup>.

The development of genetic engineering has been central to the clinical use of antibodies. This technology has allowed the conversion of existing mouse MAbs into mouse human chimerized antibody and humanized reagents where only the antibody complementarity determining regions (CDR) are of murine origin. More recently, the production of fully human monoclonal antibody has been made using phage display technology or transgenic mice<sup>23</sup>. Table 1 lists the monoclonal antibodies that are currently undergoing clinical trials.

Although it is outside the scope of this review, an expanding field in antibody-based cancer therapy is the use of monoclonal antibodies to direct selective cytotoxic agents, radionuclides, toxins and prodrug converting enzymes that have been conjugated to monoclonal antibodies. These are in various stages of development in clinical trials.

### Gene therapy

Initiation of the first gene therapy clinical trial in 1990 for treatment of a genetic disorder, opened up new vistas for its application in tumour immunotherapy. The current efforts focus on chemogene therapy and immunogene therapy for treatment of cancer. Chemogene therapy involves introduction of genes that confer susceptibility to chemotherapeutics while immunogene therapy involves modulation of the patients immune response capacity<sup>24</sup>.

Chemogene therapy involves introduction into tumour cells of suicide genes that convert nontoxic substances (prodrugs) into toxic metabolites in an attempt to avoid the severe systemic side effects of conventional chemotherapy. The main task is to target the genes specifically to the tumour cells and to reach as many tumour cells as

possible. This is achieved by the 'bystander effects', i.e. cytolysis of nontransduced tumour cells, involving both cell-to-cell transfer of the active metabolites and stimulation of immune-mediated responses against the tumour cells. Suicide genes code for enzymes that render cells sensitive to otherwise nontoxic prodrugs. For example, Herpes simplex virus type 1 thymidine kinase (HSV-tk) converts nucleoside analogs such as ganciclovir (GCV) into monophosphate form. The monophosphate is transformed into a triphosphate metabolite that is a potent inhibitor of DNA elongation, thereby causing cell death. In murine tumour models increased expression of immune stimulatory cytokines tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and granulocyte-macrophage colony stimulating factor (GM-CSF) along with T cell infiltration and hemorrhagic tumour necrosis was observed following HSV-tk gene modified tumour cells and GCV.

The first attempts of immunogene therapy of cancer involved modification of TIL by the insertion of marker genes. In the first phase of these studies a bacterial gene coding for neomycin phosphotransferase, which could induce resistance to the antibiotic neomycin, was inserted into TIL. This procedure enabled differentiation of adoptively transferred TIL from endogenous host lymphocytes. The goal of these studies was to demonstrate the feasibility and safety of using retroviral-mediated gene transfer to introduce genes into human and to study the long-term distribution and survival of autologous TIL. More recent approaches involve genetic modification of tumour cells to increase their immunogenicity. The insertion of cytokine genes can increase the immune recognition of tumour cells and can lead to the production by the host of cytolytic cells that are not produced in response to the parental non-modified tumour. Various phase I and phase II clinical trials have been initiated which include insertion of genes for cytokines IL-2, IL-4, TNF, rIFN $\gamma$ , GM-CSF, and IL-12 into tumour cells. Transfection of MHC class I

**Table 1.** Monoclonal antibodies currently in clinical trials

Cancer type	Target antigen	Antibody	Product type	Sponsors	Trial status phase
Cancer (general)	VEGF	Anti-VEGF	Humanized (IgG1)	Genentech	III
Ovarian	CA 125	OvaRex	Murine	Altarex	II/III
Colorectal	17-1A	Panorex	Murine (IgG2a)	Glaxo Wellcome/ Centocor	Approved (1995) in Germany
Lung	Anti-idiotypic GD3 epitope	BEC2	Murine (IgG) Merck KGaA	ImClone Sys	III
Head and neck	EGFR	IMC-C225	Chimeric (IgG)	Imclone Sys	III
Breast	HER2/neu	Herceptin	Humanized (IgG1)	Genentech	FDA approved (1998)
Sarcoma	$\alpha$ V $\beta$ 3 integrin	Vitaxin	Humanized	Applied Molecular Evolution (formerly Ixsys)/Medimmune	II
CLL	CD52	Campath 1H/ (LDP-03)	Humanized (IgG1)	Leukosite	BLA
AML	CD33	Smart M195	Humanized (IgG)	Protein Design Lab/Kanebo	III
NHL	CD20	Rituxan	Chimeric (IgG1)	IDEC Pharm/Genentech	FDA approved (1997)
	CD22	LymphoCide	Humanized (IgG)	Roche/Zenyaku	I/II
	HLA	Smart 1D10	Humanized	Immunomedics	I
	HLA DR	Oncolym (Lym-1)	Radiolabelled murine	Protein design Lab Techniclone	II/III

and II genes, and genes encoding members of the B7 co-stimulatory molecules (B7-1 or B7-2) into tumour cells have also shown to increase tumour cell recognition by effector cells.

The growing number of reports documenting successful immunotherapy of cancer patients and the increasing knowledge of the mechanisms governing immune reactions against tumour cells warrant further experimental efforts in this area. Vaccination strategies might be improved by increasing the arsenal of tumour-specific peptides and by considering the homing and migration characteristics of lymphocytes and APCs. Antibody therapy might benefit from the use of a combination of antibodies directed against different target antigens and from the development of antibody constructs that can efficiently bind and activate tumour directed immune effector cells. It seems likely that more innovation, based on a deeper understanding of the basic biology of tumour-immune interactions, will be required to develop widely efficacious modalities for tumour immunotherapies.

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