IDENTIFICATION OF TUMOUR-ASSOCIATED T-CELL EPITOPES FOR VACCINE DEVELOPMENT

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Ten years ago, the first melanoma patient was successfully treated by vaccination with a short peptide, which was, in fact, the first tumour-specific T-cell epitope ever defined — MAGE. Since then, a number of clinical vaccination studies have underlined the potential of tumour-specific T-cell epitopes. But, how can we identify more epitopes to improve their efficacy as an anticancer treatment?

HLAs
Human leukocyte antigens, which are molecules of the human major histocompatibility complex.

CD8
A surface molecule that is expressed exclusively by cytotoxic T cells.

CD4
A surface molecule that is expressed exclusively by T-helper cells.

MHC CLASS II MOLECULES
Peptide receptors, similar to class I molecules in structure and function, but exclusively expressed by a small set of immune cells. They mainly present peptides from extracellular proteins to T-helper cells.

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The first indication that tumours were immunogenic came from animal models, in which tumours were rejected. There are, however, marked differences in the degree of immunogenicity between different tumours. Some are easily rejected, whereas others escape destruction by the immune system. So, can we use this knowledge to increase tumour immunogenicity as a therapeutic strategy? A large number of pre-clinical and clinical trials in humans have demonstrated the feasibility of antitumour vaccinations. Vaccination is usually well tolerated, which highlights the safety of this approach.

Tumour vaccines make use of tumour antigens — which are, in general, derived from proteins that are produced by the tumour — and aim to trigger a cellular immune response that is executed by T cells. Tumour antigens can be either tumour specific, so are expressed exclusively in tumour tissue, or tumour associated, so are highly overexpressed in tumours but can also be found in normal tissue; differentiation antigens are overexpressed in tumours and are found only in certain types of normal tissue (see below, and TABLE 1). Peptides from tumour antigens, like those from every other cellular protein, are released from their source proteins during antigen processing, mainly by the proteasome, and are presented by human leukocyte antigen (HLA) class I molecules on the cell surface. Such HLA–peptide complexes are under the surveillance of the cellular immune system. The cellular immune response consists of a cytotoxic effector function, which is carried out by cytotoxic T cells that are positive for the CD8 surface marker and recognize peptides that are presented by HLA class I molecules, and a helper function, which is executed by CD4-positive T cells that are restricted by major histocompatibility complex (MHC) CLASS II MOLECULES (see BOX 1). The type of immune reaction that is initiated therefore depends on which MHC molecule presents a peptide from the antigen. During the 1990s, when tumour antigens and their peptide fragments — epitopes, which are presented by MHC molecules — were extensively characterized, much of the work focused on CYTOTOXIC T LYMPHOCYTES (CTLs) and the MHC CLASS I antigen-processing system. In fact, owing to experimental limitations, our knowledge of MHC class-II-restricted T-helper epitopes is still rather limited.

Many epitopes have been reported, some of which are derived from the classical tumour antigens that were identified a long time ago — such as p53, RAS and carcinoembryonic antigen — but others are from recently discovered tumour antigens. More than 70 tumour antigens that are recognized by (mainly CD8^{+}) T cells were listed in a recent review[1]. In principle, a short peptide that is derived from the sequence of a tumour antigen and presented by an HLA class I molecule represents a potential tumour vaccine. We do not know exactly how many times an individual overcomes tumours during their lifetime. The risk of immunosuppressed individuals developing certain virus-associated tumours — such as non-Hodgkin's lymphoma and Kaposi's sarcoma —

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Summary

- T-cell epitopes from tumour antigens have been included in many vaccination studies, and their potential to induce antitumour immune responses has become manifest. Nevertheless, the clinical outcome of such studies has to be improved.
- The number of known epitopes is still limited; therefore, some tumours cannot be treated by immunotherapeutic approaches. For others, the efficacy is not yet optimal.
- T-cell epitopes from tumour antigens can be defined by two principal strategies: one starts from an existing T-cell response and identifies the target of the response, whereas the other uses the sequence of a tumour antigen and employs epitope prediction to identify the relevant epitopes.
- New tumour antigens can be discovered by analysing the specificity of existing T-cell responses, or by screening strategies such as the SEREX programme, comparative proteome analysis or gene-expression profiling.
- To improve the clinical outcome of antitumour vaccinations, tumour-escape mechanisms have to be avoided by the use of efficient, multitarget vaccines.
- With the growing number of T-cell epitopes, it will become feasible to design patient-specific, individual vaccines that address several different antigens from one tumour and several HLA specificities, including class-II-restricted epitopes.

is rather high, but, for most kinds of tumour, impaired functions of the immune system do not seem to be important for suppressing tumour formation. Nevertheless, we have to realize that the immune system has failed when tumours develop. This might be because the tumour orginates from 'self' and is therefore not immunogenic. It could also be that the very low number of antigens that are present in the early stages of tumour development does not induce an immune response, as is the case with low doses of pathogens.

The wide variety of antitumour vaccines that have already been applied show that the perfect vaccine — with respect to the selection and number of antigens, and the application strategy — has still to be designed. One of the most favoured applications uses the most efficient professional antigen-presenting cells, DENDRITIC CELLS (DCs), pulsed with peptides from tumour antigens. The number of cells and the number of different — in part, 'engineered' — peptides administered, and the application of adjuvants or enhancers differs among all studies. Although a complete tumour regression has been reported several times[2,3], reports of a poor clinical outcome unfortunately also exist[4,5], and the first critical opinions have already been voiced; these speak of disappointing trials[6]. There are several possible reasons for the failure of antitumour vaccination, one of which is loss of the antigen from the tumour, which leads to tumour-escape variants[7]. But the loss or downregulation of specific molecules in the antigen-processing pathway has also been reported[8,9]. In addition, immune responses occasionally even fail if antigen, MHC and specific T cells are present, which indicates that tumours can also use other mechanisms to exert a certain form of immunosuppression.

The use of T-cell epitopes in immunotherapy of tumours still presents us with the problem that individual differences between patients — both in the type of HLA molecules and in the epitopes that are presented and recognized — have not yet been finely ascertained. Patient-specific expression of tumour-associated antigens, quantitative differences in HLA-restricted presentation of epitopes and T-cell pools that vary among individuals might all have a significant role. For example, in the SYFPEITHI database[10] (see online links box), which lists cancer-related T-cell epitopes, 11 different HLA-A*0201-restricted CTL epitopes from ERBB2 (HER2/neu) are listed, and we do not know which is most efficient in the struggle against an individual tumour (see BOX 2). A significant limitation of many clinical trials is their use of one or a few epitopes that is presented by only one HLA restriction element, as this facilitates tumour escape because loss of only one antigen suffices. To promote the exciting option of defeating tumours by 'self', the basic strategies must now be corrected. The future of a cancer immunotherapy that exploits T-cell epitopes from tumour antigens lies in

CYTOTOXIC T LYMPHOCYTES
(CTLs: killer cells, cytotoxic T cells). These control all major histocompatibility complex class-I-expressing body cells for the presence of abnormal (viral, tumour-associated) peptides.

MHC CLASS I MOLECULES
Highly polymorphic glycoproteins that are expressed by every nucleated body cell of vertebrates, and that are encoded by the gene cluster 'major histocompatibility complex' (MHC). The human MHC molecules are termed HLA (human leukocyte antigen) molecules. MHC class I molecules mainly present peptides from intracellular proteins to cytotoxic T cells.

DENDRITIC CELLS
(DCs). These present T-cell epitopes very efficiently and are able to activate cytotoxic T lymphocytes.

Table 1 | **A selection of tumour antigens and their T-cell epitopes**

Antigen class	Tumour antigen	CTL epitope (sequence)	HLA	Helper epitope known	References
Cancer-testis antigen	MAGE1	EADPTGHSY	A*0101	+	17
	MAGE3	FLWGPRALV	A*0201	+	51
	NY-ESO-1	SLLMWITQC	A*0201	+	52
Mutated protein	CDK4	ACDPHSGHFV	A*0201	–	53
	β-Catenin	SYLDSGIHF	A*2402	–	54
	Caspase-8	FPSDSWCYF	B*3501	–	55
Tumour virus	HPV16 E7	TLGIVCPI	A*0201	–	56
Differentiation antigen	Tyrosinase	SEIWRDIDF	B*4402	+	57
	GP100	ALLAVGATK	A*0301	+	58
	PSMA	ALFDIESKV	A*0201	–	59
Overexpressed protein	ERBB2	KIFGSLAFL	A*0201	–	60
	MUC1	LLLLTVLTV	A*0201	–	30
	CEA	HLFGYSWYK	A*0301	–	61

Three classes of tumour antigens — cancer-testis antigens, mutated antigens and tumour-virus antigens — are termed tumour specific, as they occur exclusively in tumour tissue. All other antigens are referred to as tumour associated. The largest group so far is represented by overexpressed antigens. T-helper-cell epitopes have been characterized for a small number of tumour antigens only. A comprehensive listing of tumour antigens can be found in REF. 1. CDK4, cyclin-dependent kinase 4; CEA, carcinoembryonic antigen; CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; HPV, human papillomavirus; MAGE, melanoma antigen; MUC1, mucin 1; PSMA, prostate-specific membrane antigen.

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Box 1 | **Tumours and the immune system**

The crucial problem of tumour immunology is that tumours develop from 'self', but the immune system has been trained to exclusively recognize 'non-self' structures. Many of the immune system's components therefore remain idle when faced with tumour cells. For the fight against tumours, the immune system must learn to recognize 'self' structures that are usually invisible, but — following mutation, differentiation or overexpression — can become targets for recognition (TABLE 1). The immune system is armed with:

Cytotoxic T lymphocytes (CTLs)
CTLs record every change within the protein reservoir of body cells, as it is presented to them by means of peptide fragments and the human leukocyte antigen (HLA) class I receptors — a highly polymorphic system of cell-surface proteins. If HLA molecules present an abnormal peptide, CTLs are able to recognize this and destroy the target cell, by driving it into apoptosis. CTLs, like all T cells, tolerate all peptides from proteins that belong to the usually expressed self-protein pool of the organism.

Antibodies
Several tumour-reactive antibodies have been described and some of them have been developed into new cancer therapies — for example, Herceptin. However, it is often difficult for antibodies to reach a tumour in the periphery *in vivo*. The B cells, which produce antibodies, cannot act on their own, but require the help of T-helper cells.

T-helper cells
One subgroup — the T-helper 1 (T_H1) cells — is necessary to induce and propagate an inflammatory immune response in which CTLs are the main players. T-helper 2 (T_H2) cells favour the humoral immune response, which is supported by B cells and antibodies. T-helper cells recognize their antigen only in the form of an HLA class-II-presented peptide.

Natural killer (NK) cells
NK cells recognize and destroy body cells that do not express HLA molecules. Nevertheless, tumour cells often escape NK-cell recognition after HLA loss.

MELANOMA
Skin cancer, originating from transformed melanocytes.

MAGE
Melanoma antigen; a family of proteins that are expressed only in testis or tumour cells.

individual, patient-specific analysis and therapy, and in using several tumour antigens and epitopes that are presented by more than two HLA molecules. However, the number of tumour-specific T-cell epitopes that are known today is still too low, with the possible exception of those for malignant MELANOMA, which remains the model system for tumour vaccines. There is an urgent need for more epitopes so that several tumour antigens can be addressed with one vaccine, and to expand the applicability to a larger number of different tumours. So, which strategies will allow us to define new tumour antigens and T-cell epitopes?

Tumour antigens

Tumour immunologists have defined five different classes of antigens that are associated with tumours (TABLE 1) and that can be targets of antitumour T-cell responses if short peptide fragments that are derived from their sequences are presented by HLA class I molecules. However, we still do not know if most of them are also capable of delivering antigenic peptides to MHC class II molecules. So, what are the characteristics of tumour antigens that make them useful in antitumour vaccines? Most important are tumour-specific antigens: cancer-testis antigens, protein variants that are created by somatic mutations within tumour cells, and proteins from tumour viruses. These are found only in tumour tissue[1], and therefore do not encompass the risk of autoimmune reactions during immunotherapy. Antigens that are highly overexpressed in tumours are referred to as tumour associated and might also serve as targets of a cellular immune response. The occurrence of the antigen within the population is also a measure of how successful a vaccine might be. Ubiquitous or shared tumour antigens are expressed by most tumour-bearing individuals and include telomerase[11] and survivin[12], but antigens that are expressed by only a small percentage of tumours — such as the MAGE gene family[13–15] — might also be used. So, in present clinical studies, tumour specificity and widespread expression have to be considered to further the development of tumour vaccines. An ideal treatment would include only tumour antigens that are expressed by the respective tumour.

Identifying T-cell epitopes from tumour antigens

Two experimental strategies are currently being used. One begins with a documented T-cell reaction against tumour cells, and characterizes T-cell specificity and the nature of the antigen that is recognized. The other uses known tumour antigens as its starting point, and uses the 'reverse immunology' approach to predict *in silico* T-cell epitopes from the sequence of such antigens, according to allele-specific peptide motifs of HLA molecules. T cells are subsequently induced by stimulation with synthetic peptides, and peptide-specific T cells are then tested for the recognition and killing of tumour cells (FIG. 1).

From T-cell recognition to T-cell epitopes. The classical approach is to investigate the specificity of T cells that recognize tumour cells in order to define the epitope (FIG. 1a). The first tumour-specific T-cell epitope that was identified was from MAGE1 (REF. 16), and was presented by HLA-A*0101. It was defined by narrowing down the complexity of antigens that produced the immune response in the target melanoma cell. Thierry Boon and colleagues expressed human cDNA libraries that were generated from melanoma cells, in COS cells that were transfected with the HLA-A*0101 gene, until the T-cell reaction focused on a single gene. Using truncation experiments, a small region of DNA was identified that harboured the relevant T-cell recognition site[17]. Since then, the methods for expression cloning have been substantially improved; a very efficient expression library has recently been described, which enables identification of T-cell specificity after enrichment steps[18].

An alternative approach, which also starts from T-cell function, uses biochemical methods to purify MHC molecules that present the relevant peptide epitope. After acid elution from the MHC molecule, the highly complex mixture of MHC ligands is fractionated by chromatography (capillary high-performance liquid chromatography) and the fraction that is recognized by T cells is then analysed by mass spectrometry[19]. The low number of tumour-specific peptides among thousands of inconspicuous peptides, the often low affinity of T cells for tumour epitopes, and the very low number of peptides, impede this molecular characterization. Less than 10 picograms of peptide have to be analysed, which requires highly sensitive analytical techniques.

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PROTEOMICS
Analysis of the entirety of proteins from a tissue sample, separated by two-dimensional gel electrophoresis. Each individual protein spot can be identified after tryptic digestion and mass spectrometrical analysis of the resulting peptides.

MICROARRAY ANALYSIS
'DNA chips' contain many thousands of oligonucleotides that represent fragments of genes, immobilized on a solid surface. They are probed with cDNA that is prepared from mRNA of tissue samples and so give quantitative information about gene expression.

Box 2 | **Individual differences in humans**

Humans are largely heterogeneous organisms when compared with inbred laboratory mouse strains. Human leukocyte antigen (HLA) polymorphisms contribute to this: one individual HLA pattern — that consists of two alleles each of HLA-A, -B, -C, -DR, -DQ and -DP — occurs only once among tens of thousands of humans. The major histocompatibility complex class I molecule HLA-A*0201 is the most commonly expressed allele; one-third of Caucasians are positive for this allele. Nevertheless, the density of HLA molecules varies greatly on the surface of normal cells, and especially on tumour cells.

But it is not only in the HLA system — and in minor histocompatibility antigens — that tumours vary; tumours also vary greatly with respect to the expression of tumour-associated antigens. Although each tumour results from a monoclonal situation, the thousands of chromosomal abnormalities that are acquired during tumour development guarantee that each tumour is both unique and heterogeneous. Individual mutations provide us with the opportunity of using these as the target of immunotherapies. For optimal treatment, individual differences between patients must therefore be ascertained with extreme precision. This involves consideration of the following features:

- **Expression of two HLA-A and two HLA-B alleles: type and density**
- **Expression of known tumour antigens**
- **Proteins that are specifically overexpressed in the individual tumour**
- **HLA-presented peptides from known tumour antigens or overexpressed proteins.**

This strategy is very tedious and risky, for all these reasons, and such cases remain rare exceptions in tumour-epitope characterization.

From antigens to T-cell epitopes. Immediately after publication of the first HLA-allele-specific peptide motifs[20], scientists used the motifs to predict T-cell epitopes from antigen sequences[21,22]. This approach has been termed 'reverse immunology'[23] and usually starts from a known tumour antigen, but without pre-existing T cells (FIG. 1b). Epitope prediction has now been carried out for more than ten years, and most tumour-specific T-cell epitopes known today have been defined with the help of peptide motifs. Several programmes offer free epitope predictions for a wide range of MHC class-I- and class-II-restricted epitopes from different organisms — the most commonly used are BIMAS[24] and SYFPEITHI. In addition, programmes have evolved during the past two years that offer the prediction of proteasomal cleavages[25,26]: an overview on prediction programmes is given in REF. 27. The experimental verification of proteasomal cleavage sites[28] or the selected potential epitopes starts with peptide synthesis. Synthetic peptides might be used to immunize transgenic mice to produce a T-cell response[29], or to re-stimulate precursor T cells *in vitro*, which are derived from either healthy blood donors or tumour patients[30]. The final step to establishing tumour-specific T cells that have a distinct specificity is accomplished when the raised T cells recognize tumour cells that express the respective antigen. A direct demonstration of natural processing of the predicted epitopes can be achieved by the 'predict, calibrate and detect' method[31], which uses synthetic peptide candidates as calibrants for a capillary-chromatography–mass-spectrometric system, and by comparing the results with those obtained after acid elution of naturally processed peptides from HLA molecules of tumour cells.

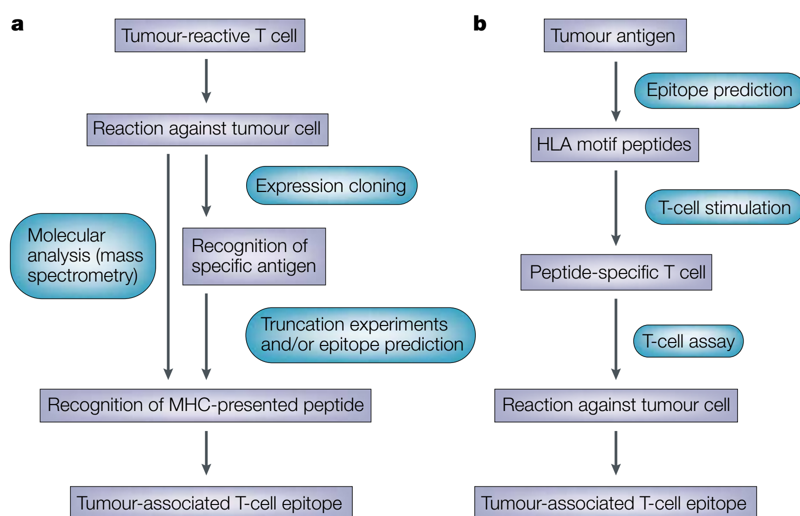

Figure 1 | **Strategies for the characterization of T-cell epitopes.** Depending on the starting point, which is either a T-cell reaction (**a**) or a known tumour antigen (**b**), two different strategies can be followed. **a** | The specificity of a T-cell receptor can be characterized using expression libraries, in order to identify the relevant antigen and its T-cell epitope. **b** | Potential T-cell epitopes can be predicted from the sequence of a tumour antigen. Their ability to induce peptide-specific T cells is assayed, and the tumour specificity of such T cells is verified by assaying tumour-cell recognition. HLA, human leukocyte antigen; MHC, major histocompatibility complex.

New antigens as a source of new epitopes

With the development of screening methods to identify tumour-associated antigens, huge data sets had to be evaluated. The first screening method, SEREX, uses antibodies from sera of tumour patients to determine potential tumour antigens[32]: all proteins recognized by such antibodies (but not by antibodies from healthy persons) have been included in the SEREX database (see online links box), which now contains many hundreds of antigens. Two other screening methods — PROTEOMICS[33–35] and MICROARRAY ANALYSIS[36,37] — investigate the differences between healthy tissue and tumour tissue at the protein or mRNA level, respectively. With this type of differential analysis, several dozen to several hundred tumour-associated antigens can be defined within one assay, depending on the experimental thresholds. After screening a series of samples, scientists need to interpret large amounts of data in the light of published work and database contents. Results from complementary methods have to be

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Table 2 | **Outcome of vaccine trials performed with different modes of application of tumour-associated T-cell epitopes**

Tumour	Source of epitope(s)	Carrier/adjuvant	Cytokine	Helper epitope	Admin.	Patients	PR/CR	References
Melanoma	Tyrosinase	Peptide alone	GM-CSF	–	6 x i.d.	18	0	62
Melanoma	RAS	Peptide alone	GM-CSF	–	6 x i.d.	10	0	63
Melanoma	GP100	Montanide ISA-51/ QS-21	–	TT	2–7 x s.c.	22	n.a.	40
Melanoma	MART1 or GP100 mod.	Autologous DC	–	–	4 x i.v.	10	0	5
Melanoma	Several (pool)	Autologous DC	–	–	4–12 x i.v.	14	1	64
Glioma	Tumour extract	Autologous DC	–	–	3 x s.c.	9	0	65
Pancreatic adenocarcinoma	Mutated RAS	Peptide alone	GM-CSF	–	6 x i.d.	48	5	66
Melanoma	MAGE3 or MART1	Autologous PBMC	IL-12	–	s.c.	8	2	67
Medullary thyroid carcinoma	Calcitonin, CEA	Autologous DC	–	–	7–14 x i.d.	7	1	68
Metastatic- NY-ESO-1- expressing cancers	NY-ESO-1	Peptide alone + DMSO	–	–	4–12 x i.d.	12	0	69
Melanoma	GP100 mod.	Peptide alone	IL-2 or GM-CSF or IL-12	–	s.c.	43	6	70
Prostate cancer	PSMA	Autologous DC	–	–	6 x i.v.	37	11	71
Cervical carcinoma	HPV16 E7	Montanide ISA-51	–	+	s.c.	19	0	39
Gastrointestinal carcinoma	MAGE3	Autologous DC	–	–	4 x i.v.	12	0	72
Breast, ovarian cancer	ERBB2, MUC1	Autologous DC	–	PADRE	3–9 x s.c.	10	1	41

This survey lists a selection of clinical trials that are performed with synthetic peptides. Admin., number and route of applications (i.d., intradermal; i.v., intravenous; s.c., subcutaneous); CEA, carcinoembryonic antigen; DC, dendritic cell; DMSO, dimethyl sulphoxide; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPV, human papillomavirus; IL, interleukin; MAGE, melanoma antigen; MART1, melanoma-associated antigen recognized by T-cells 1; mod., modified; MUC1, mucin 1; n. a., not applicable; PADRE, pan-DR epitope; PBMC, peripheral-blood mononuclear cell; PR/CR, partial response/complete response according to the World Health Organization definitions; PSMA, prostate-specific membrane antigen.

combined to obtain reliable information. For example, the combination of differential display at the protein level, and the recognition of antigens by patients' antibodies might reveal interesting candidate antigens[38], as well as the combination of expression profiling using DNA microarrays with the analysis of peptides presented by HLA molecules of the same tumour sample. After establishing a new tumour-associated antigen, the search for T-cell epitopes from this antigen might start anew by employing reverse immunology[36].

Help is required

Vaccinations that are aimed at recruiting cytotoxic T cells for tumour destruction or prevention have been successful but, unfortunately, the success has been limited to just a few cases. The response rates that have been documented in many clinical studies are encouraging, but must be increased. In addition to tumour-escape mechanisms, inefficient triggering of the T-cell system might be a reason for low response rates. In order to mimic the concerted action of the immune system against pathogens, epitopes that stimulate $CD4^+$ T-helper cells have recently been included in antitumour peptide vaccination studies[39–41]. Ideally, the epitopes that are recognized by CTLs and T-helper cells should be derived from the same tumour antigen. However, although many tumour-specific CTL epitopes are known, only a very small number of HLA class-II-presented, tumour-specific T-helper epitopes have been characterized. Increased efforts are therefore being made to identify tumour-associated epitopes that are presented by class II molecules[42–45], which would activate $CD4^+$ helper cells to induce and maintain $CD8^+$ killer cells[46]. In principle, the strategies that are used for the characterization of CTL epitopes can also be applied to helper epitopes. This holds true for the classical approach in particular, which starts from T-cell recognition, although transfection of MHC class-II-expressing antigen-presenting cells with expression libraries represents a specific problem. An elegant strategy directs the antigen of interest to the lysosomal/endosomal compartment, where antigen processing leads to the generation of MHC class-II-presented epitopes[47–50]. Performing reverse immunology for MHC class-II-restricted antigen presentation is far more difficult, for two reasons. First, the peptide motifs of MHC class II molecules are more degenerate compared with class I motifs and therefore do not allow for similarly precise predictions. Second, it is impossible, at present, to predict the proteolytic events that take place during antigen processing within the MHC class II pathway.

Diverse clinical applications: partial success

The first vaccination that was carried out with a tumour-specific T-cell epitope was in a melanoma patient. Since then, melanoma has represented the model system for cancer immunotherapy. So far, most T-cell epitopes from

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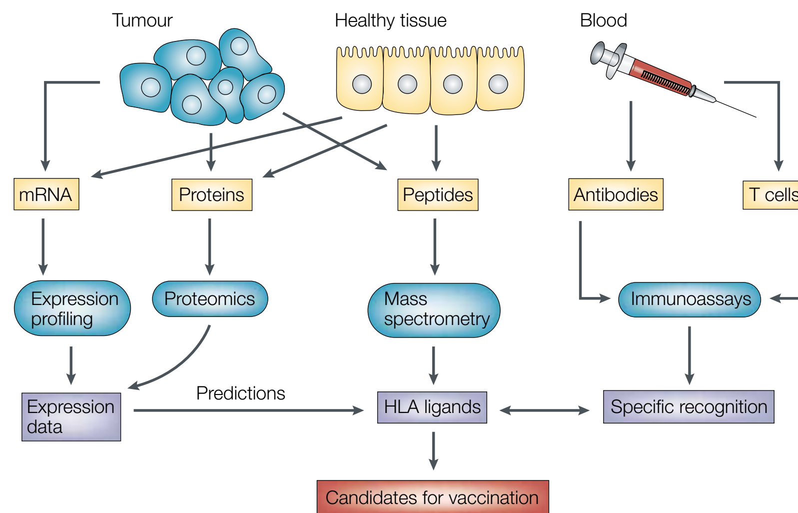

Figure 2 | **Complementary information leads to the identification of useful tumour epitopes.** As much information as possible has to be compiled about the presence of antigens or epitopes in a given tumour in order to identify new tumour antigens, and new tumour-associated T-cell epitopes that can be used in clinical studies. This strategy could lead to the characterization of an individual, patient-specific T-cell epitope pattern, which represents the prescription to treat this tumour. HLA, human leukocyte antigen.

MELANOSOMES
Subcellular organelles in melanocytes, which contain the skin's pigments.

PERIPHERAL-BLOOD MONONUCLEAR CELLS
(PBMCs). Includes B cells, T cells and monocytes. These can be obtained from whole blood after ficoll density-gradient centrifugation.

tumour antigens have been derived from melanomal or MELANOSOMAL proteins. With the growing number of T-cell epitopes identified, the studies have now been expanded to other tumours. Clinical studies have been carried out in more then ten tumour entities, including cancers of the breast, cervix, gastrointestinal duct, lung, ovary, pancreas, prostate, kidney and medullary thyroid.

Although many cancer patients have been treated successfully, these represent just a small percentage of all individuals who underwent vaccine therapy (TABLE 2). Most patients that were included in clinical studies did not benefit from treatment with peptide epitopes that were identified from tumour antigens. We can speculate about the possible reasons that lead to such a disappointing outcome, but it is important to keep in mind that terminal patients — who have already undergone therapies in which their tumours proved to be resistant — are most frequently included in trials. It would be very interesting to compare these results with a first-line immunotherapeutic treatment of low-risk patients.

An additional factor is the individual differences between people (BOX 2), which have never been assessed in their entirety. We are also still unsure of the optimal application of tumour-associated T-cell epitopes: the many possible methods of vaccination have never been compared systematically in humans. Even if we leave out viral or other vectors, proteins and DNA, and only consider the application of synthetic peptides, each protocol that has been applied — use of PERIPHERAL-BLOOD MONONUCLEAR CELLS (PBMCs) or DCs as carriers; peptide only, intradermal or subcutanous administration; or addition of different adjuvants, cytokines or helper epitopes — has a limited, but comparable success (TABLE 2). It is therefore impossible to state that one thing works and another does not. Most application protocols have been shown to induce T-cell responses in patients, but tumour regression has been observed in only a few cases.

New approaches, future directions

There is much scope for the successful application of tumour-associated T-cell epitopes in cancer therapy, as the proof of principle was established long ago: immune responses are induced in most cases, and most tumour types are potential targets of immunotherapy. The problems, however, include the unsolved question of optimal clinical application, unknown escape mechanisms of tumours, and the low number of T-cell epitopes that have been identified for non-melanoma tumours. It has often been argued that HLA polymorphisms are a significant drawback in tumour immunology, but this is not as hopeless as it might seem at first glance: the most abundant HLA molecules have been investigated thoroughly, and their peptide motifs or even crystal structures have been determined. Our knowledge includes peptide motifs of HLA molecules that are expressed in more than 95% of Caucasians.

Activities must now focus on the identification of more tumour-associated antigens, and T-cell epitopes that are derived from their sequences. FIG. 2 shows how many different methods can be used to obtain complementary information about the characteristics of a given tumour. The combination of proteomics and antibody screening has been reported[38], and the combination of expression profiling, HLA-ligand characterization and T-cell assays from blood has been established in our own department (Weinschenk, T. *et al.*, manuscript in preparation). A modern anticancer vaccine should not target one or a few antigens only. It is also important to leave the model system of HLA-A*0201, and to characterize T-cell epitopes that can be presented by many different HLA class I or class II molecules. The most promising strategy seems to be an individual analysis of every tumour that is accessible, and individual treatment of every tumour patient (see also BOX 2 and FIG. 2). The modern peptide vaccine should consist of tumour-associated CTL epitopes and helper epitopes from various tumour antigens that are restricted to more than two HLA class I molecules and one HLA class II molecule. Hopefully, these developments will lead to real benefits for patients with cancer.

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