

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
SILVANA MARIA LOPEZ DIAZ

Asunto: Radicación: 13-155530- -8
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 12954
Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/JP2011/006551				
Fecha de Presentación Internacional	25/11/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No. 13-155530-00000-0000	02/Julio/2013
Reivindicaciones:	Radicado No. 13-155530-00000-0000	02/Julio/2013
	Radicado No. 13-155530-00007-0000	08/Septiembre/2014
Dibujos:	Radicado No. 13-155530-00000-0000	02/Julio/2013
Secuencias:	Radicado No. 13-155530-00000-0000	02/Julio/2013
Prioridad:	US 61/419,181	02/Diciembre/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. Para la

presente solicitud se estudió el capítulo reivindicatorio completo (Radicado No. 13-155530-00007-0000).

Título de la solicitud: “Péptidos TOMM34 y vacunas que los incluyen”.

El problema planteado en esta solicitud consiste en la necesidad de proporcionar nuevos antígenos asociados a tumores (TAA) como objetivos terapéuticos anticancerígenos.

Para resolver este problema técnico la solicitud en estudio proporciona un péptido sintetizado de menos de 15 aminoácidos caracterizado porque es seguido por a) o b): a) un péptido sintetizado que comprende una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1, 5, 31 y 32; b) un péptido sintetizado que comprende una secuencia de aminoácidos en la cual uno o dos aminoácidos se sustituyen, eliminan, insertan o agregan en una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1, 5, 31 y 32 que mantiene la capacidad de inducción de los linfocitos T citotóxicos.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C12N 15/00, A61K 38/00, A61K 39/00, A61P 35/00, A61P 35/04, A61P 37/04, C07K 16/32, C07K 7/06, C12N 1/15, C12N 1/19, C12N 1/21, C12N 5/0783, C12N 5/0784, C12N 5/10, G01N 33/574.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (c))

El objeto de las reivindicaciones 10 - 13 y 15 no es patentable de acuerdo con el Art citado, puesto que hacen referencia a células que no se encuentran definidas en términos de ‘una línea celular’.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

1. El objeto de la reivindicación 1 no es claro puesto que:

- Un péptido debe estar caracterizado por su función y estructura completa. Se sugiere que en el preámbulo de la reivindicación se indique que los péptidos son derivados de TOMM34.
- La expresión “de menos de” no es concisa y no permite tener claridad sobre el alcance de la invención.
- Con la expresión “caracterizado porque es seguido por a) o b)” se entiende que el péptido del encabezado está adyacente a otro péptido, el cual puede ser seleccionado de a) o b). Esto es un problema de traducción puesto que en la reivindicación de la solicitud internacional se entiende que el objeto reclamado es un péptido caracterizado de acuerdo con a) o a b). Se sugiere hacer las correcciones necesarias.
- La opción b) carece de concisión puesto que se refiere a una o dos mutaciones en la secuencia pero no se indica el cambio realizado. Se sugiere indicar las posiciones y las sustituciones, deleciones o inserciones hechas. Además, la expresión “...que mantiene la capacidad de inducción...” es un resultado a alcanzar y no definen al péptido, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.

2. La reivindicación 3 es redundante puesto que no presenta alguna característica técnica adicional, simplemente reclama la misma materia ya divulgada en la reivindicación 1.

3. La reivindicación 4 no es clara puesto que se reclaman cambios o mutaciones hechos a las secuencias de los péptidos reivindicados sin referirse específicamente a las posiciones donde se realizan dichos cambios. Se deben indicar las posiciones exactas donde se realizan las mutaciones de acuerdo a la numeración de la secuencia (Ver norma ST.25).

4. El polinucleótido de la reivindicación 5 debe estar caracterizado a partir de su estructura completa, es decir, la secuencia nucleotídica del mismo indicando además el número de identificación SEQ ID N°.
5. Una composición debe estar definida por sus ingredientes (compuesto activo y excipientes) y las proporciones de los mismos (Reivindicaciones 6 y 7).
6. En los métodos de las reivindicaciones 8 y 9 se debe definir de forma inequívoca que se trata de un método in vitro, o de lo contrario se considerará materia exenta de patentabilidad por tratarse de un método de tratamiento. Además, se recuerda a la solicitante que las reivindicaciones de procedimiento deben estar definidas por una serie ordenada de etapas y se deben especificar las condiciones necesarias para que el método se lleve a cabo.
7. Un vector debe reclamarse con una identificación y una estructura definida, indicando al menos el tipo de vector y la secuencia de ácido nucleico que contiene (reivindicación 14).
8. En el caso de la reivindicación 16 se debe caracterizar completamente al exosoma, indicando el tipo de células de las cuales es aislado, el tamaño del mismo, los ligandos específicos y el tipo de antígeno HLA.
9. Un anticuerpo no se debe caracterizar por el antígeno al cual se une. La forma correcta de reclamar un anticuerpo es definirlo a partir de su estructura completa, esto es, de las secuencias de las regiones variables de la **cadena liviana y cadena pesada** ó secuencias **de seis regiones CDR's** (3 de la cadena liviana y 3 de la cadena pesada) (reivindicación 17).
10. La reivindicación 18 aunque encabece como kit, no cumple con los criterios definidos para la caracterización de éste. Se recuerda a la solicitante que un kit-de-partes debe estar caracterizado por una preparación farmacéutica o medicamento A y una preparación farmacéutica o medicamento B indicándose las cantidades o proporciones definidas de ambas preparaciones, donde tales componentes forman una unidad funcional (combinación verdadera) dirigida a un único propósito con efecto sinérgico. Sin embargo, en el kit reclamado en la presente solicitud no se indican las preparaciones farmacéuticas A y B, pues sólo se menciona que comprende ya sea un péptido, un polinucleótido o un anticuerpo. Según lo anterior, el objeto reclamado en la reivindicación 18 no corresponde a un kit pues comprende un solo componente, además no existe en la descripción el sustento para el kit. Se sugiere hacer las aclaraciones necesarias.

6. Unidad de invención (Art 25 D 486)

Al realizar un alineamiento para los 4 péptidos reclamados se encontró que los péptidos no guardan una estructura similar para la actividad común, agrupándose en dos grupos de péptidos:

	1 ·
	31
	5 ·
	32

Por lo tanto, la solicitud se refiere a 2 grupos inventivos:

- Grupo inventivo 1: Péptido derivado de TOMM34 de secuencia SEQ ID NO: 1 o 31. Polinucleótido que lo codifica, composición farmacéutica, anticuerpo, exosoma que lo comprenden.
- Grupo inventivo 2: Péptido derivado de TOMM34 de secuencia SEQ ID NO: 5 o 32. Polinucleótido que lo codifica, composición farmacéutica, anticuerpo, exosoma que lo comprenden.

No hay unidad de invención entre los dos grupos inventivos ya que el concepto común, péptidos derivados de TOMM34, no cumple con el requisito de novedad y nivel inventivo de acuerdo con los documentos D1 y D2 (Análisis de patentabilidad para la reivindicación 1).

Se le sugiere al solicitante restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica. Esto se logra entre otras formas definiendo las características esenciales de la invención y mostrando las otras características particulares como dependientes, pero sin perjuicio de la concisión de las nuevas reivindicaciones tanto independientes como dependientes.

El solicitante también puede realizar una o más de las divisionales de acuerdo a los grupos de conceptos técnicos inventivos presentados en esta solicitud, esto claro, sin ampliar el objeto inicial de la invención.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 02 de Diciembre de 2010, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO 2007013576	"Colon cancer related gene tom34"	01/Febrero/2007
D2	Parker et al. 1994	"HLA Peptide Binding Predictions"	1994

El documento D1¹ divulga la utilidad de TOM34 como un antígeno asociado a tumores (TAA) en el desarrollo de terapias anticancerígenas. Da a conocer péptidos TOM34 que se unen a HLA-A2 (Resumen, Ejemplos, tabla 3).

El documento D2² enseña la herramienta BIMAS, la cual es un sitio web para la predicción de péptidos que se unen a moléculas del complejo mayor de histocompatibilidad (http://www-bimas.cit.nih.gov/cgi-bin/molbio/ken_parker_comboform).

8. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

Grupo inventivo 1:

Se entiende por reivindicación 1: "un péptido sintetizado de menos de 15 aminoácidos caracterizado de acuerdo con a) o b): a) un péptido sintetizado que comprende una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1, y 31; b) un péptido sintetizado que comprende una secuencia de aminoácidos en la cual uno o dos aminoácidos se sustituyen, eliminan, insertan o agregan en una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1 y 31".

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Un péptido sintetizado de menos de 15 aminoácidos caracterizado de acuerdo con a) o b):	Péptido de unión a HLA derivado de TOM34 (Pág. 54)
a) un péptido sintetizado que comprende una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1, y 31;	
b) un péptido sintetizado que comprende una secuencia de aminoácidos en la cual uno o dos aminoácidos se sustituyen, eliminan, insertan o agregan en una secuencia	De secuencia SEQ ID NO: 22: LYGRALRVL (tabla 3)

1 <http://patentscope.wipo.int/search/en/WO2007013576>

2 Parker, K.C., Bednarek, M.A., Coligan, J.E., 1994. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J. Immunol. 152, 163.

HLA Peptide Binding Predictions: Recurso en línea: http://www-bimas.cit.nih.gov/cgi-bin/molbio/ken_parker_comboform

de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1 y 31	
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Las características esenciales del péptido de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual enseña péptidos de unión a HLA derivados de TOM34, los cuales se han predicho con la herramienta bioinformática BIMAS y estimulan las células T citotóxicas (Págs. 54 - 55).

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2 – 18 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel inventivo (Art18 D486)

Grupo inventivo 2:

Se entiende por reivindicación 1: “un péptido sintetizado de menos de 15 aminoácidos caracterizado de acuerdo con a) o b): a) un péptido sintetizado que comprende una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 5, y 32; b) un péptido sintetizado que comprende una secuencia de aminoácidos en la cual uno o dos aminoácidos se sustituyen, eliminan, insertan o agregan en una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 5 y 32”.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Un péptido sintetizado de menos de 15 aminoácidos caracterizado de acuerdo con a) o b):	Péptido de unión a HLA derivado de TOM34 (Pág. 54)	
a) un péptido sintetizado que comprende una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1, y 31;	No menciona	Péptidos predichos con BIMAS a partir de las secuencias de las proteínas anotadas en Genbank para el gen TOMM34 (No. NM_006809)
b) un péptido sintetizado que comprende una secuencia de aminoácidos en la cual uno o dos aminoácidos se sustituyen, eliminan, insertan o agregan en una secuencia de aminoácidos seleccionada del grupo que consiste de SEQ ID NO: 1 y 31	No menciona	

El documento D1 se considera el estado de la técnica cercano a la invención reclamada en la reivindicación 1 porque enseña péptidos de unión a HLA derivados de TOM34 (Págs. 3- 4), los cuales se han predicho con la herramienta bioinformática BIMAS y estimulan las células T citotóxicas (Págs. 54 - 55).

La diferencia entre la invención, definida en la reivindicación 1 y los péptidos divulgados en le documento D1 consiste en la estructura de aminoácidos de los péptidos específicos seleccionados.

No existe un efecto inesperado que dichos péptidos confieran a la invención puesto que indiquen a las células T citotóxicas, igual que los péptidos divulgados en el documento D1 (Pág. 55).

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar los péptidos conocidos en el documento D1 para conseguir péptidos alternativos a los ya conocidos”.

Sin embargo, a partir de lo divulgado en el documento D1, ya era conocido que el gen TOM34

tenía una expresión desregulada en tumores y sus secuencias estaban plenamente identificadas, como se menciona en la memoria descriptiva (folio 5). En ese sentido, para una persona medianamente versada en la materia habría sido previsible llevar a cabo el proceso de identificación de péptidos de antígenos tumorales divulgado en el documento D1, a partir del uso de herramientas bioinformáticas como BIMAS y su subsecuente verificación y validación in vitro (Págs. 54 - 55). De esta manera, por medio de la herramienta BIMAS (D2) se habría introducido la secuencia de aminoácidos de TOM34 (SEQ ID NO: 42) y los péptidos predichos con el programa se habrían llevado a verificación experimental, como lo sugiere el documento D1. Además, es del conocimiento del conjunto que la verificación experimental de los epítopes predichos permite reducir el número de péptidos candidatos potenciales para el desarrollo de composiciones inmunogénicas (Stevanovic 2002)³. Para ejemplificarlo, se muestra a continuación la tabla de resultados arrojados por BIMAS al realizar la búsqueda para la proteína TOM34 (los péptidos correspondientes a las secuencias reclamadas en la presente solicitud están sombreados).

Scoring Results			
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	143	KLPSIPLVPV	559.894
2	97	ALEKYPMAVY	56.309
3	79	ALVPFSIKPL	49.134
4	237	YLVLKQYTEA	34.279
5	135	SLGPEWRLKL	21.362
6	219	SLLCNLESA	20.716
7	238	LVLKQYTEAV	18.757
8	127	RMTRALMDSL	17.388
9	113	QIDDNVTSAV	15.684
10	241	KQYTEAVKDC	12.975
11	30	ALYGRALRVL	12.893
12	220	LLCSNLESAT	12.668
13	195	VLKEEGNELV	8.314

En consecuencia, una persona medianamente versada en la materia llevaría a cabo la identificación de péptidos derivados de TOM34 como lo sugiere el documento D1 a partir de los péptidos predichos con el documento D2, valiéndose de las habilidades rutinarias en el campo técnico. Por lo cual, la solución propuesta por la solicitante en la reivindicación 1 se considera obvia y sin altura inventiva.

Las reivindicaciones dependientes 2 – 18 tampoco cumplen con el requisito de nivel inventivo.

9. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 2007013576	1 – 18 parcialmente	Novedad
D1	WO 2007013576	1 – 18	Nivel inventivo
D2	Parker et al. 1994	1 – 18	Nivel inventivo

³ Stevanovic S. 2002. Identification of tumour-associated T-cell epitopes for vaccine development. Nat Rev Cancer 2(7):514-20.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



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Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-10-14

Elaboró: Edna Carolina Bonilla Leon

Revisó: Consuelo Leguizamon Leguizamon

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

Stefan Stevanovic

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 review¹. 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 —

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 originates 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 trials⁶. 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 variants⁷. 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 database¹⁰ (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	FLWGPRLV	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.

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.

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 tissue¹, 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 site¹⁷. 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 steps¹⁸.

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 spectrometry¹⁹. 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.

MELANOMA
Skin cancer, originating from transformed melanocytes.

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

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 motifs²⁰, 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 BIMAS²⁴ 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 sites²⁸ or the selected potential epitopes starts with peptide synthesis. Synthetic peptides might be used to immunize transgenic mice to produce a T-cell response²⁹, or to restimulate precursor T cells *in vitro*, which are derived from either healthy blood donors or tumour patients³⁰. 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' method³¹, 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.

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 antigens³²: 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

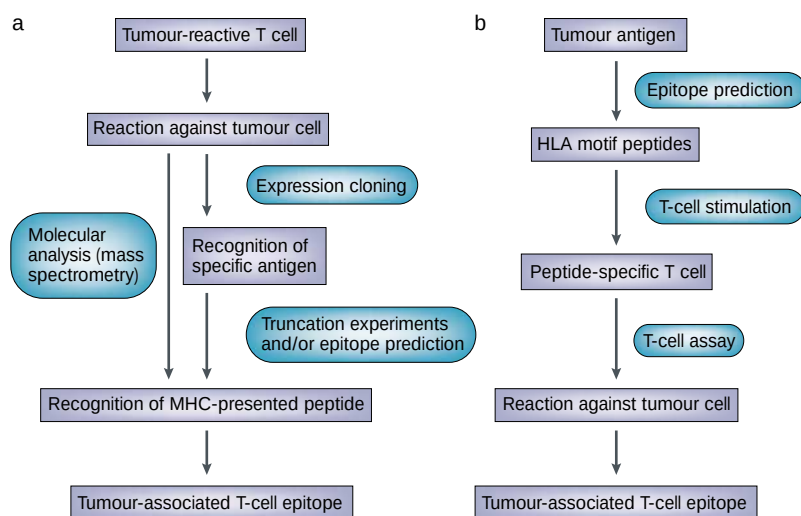


Figure 1 | Strategies for the characterization of T-cell epitopes. Depending on the starting point, which is either a T-cell reaction (a) or a known tumour antigen (b), two different strategies can be followed. **a** | The specificity of a T-cell receptor can be characterized using expression libraries, 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.

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 antigens³⁸, 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 immunology³⁶.

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 cells⁴⁶. 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

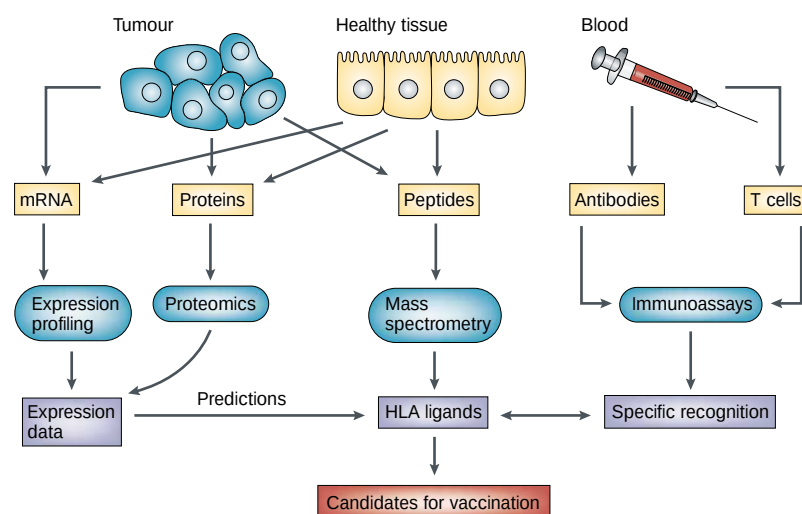


Figure 2 | Complementary information leads to the identification of useful tumour epitopes. As much information as possible has to be compiled about the presence of antigens or epitopes in a given tumour in order to identify new tumour antigens, and new tumour-associated T-cell epitopes that can be used 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 than 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 subcutaneous 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 reported³⁸, 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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