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(54) **Kriptikus HLA-A24 epitópok azonosítása, és optimalizálása és felhasználása immunterápia céljára**

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IDENTIFIZIERUNG, OPTIMIERUNG UND VERWENDUNG VON KRYPTISCHEN HLA-A24-EPITOPEN FÜR DIE IMMUNTHERAPIE

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Description

[0001] The present invention relates to the field of peptide immunotherapy. In particular, the invention provides novel methods and materials for efficiently treating patients having an HLA- A*2402 phenotype.

[0002] Peptide vaccination or immunotherapy is a therapeutic approach which is currently the subject of a great number of studies in the context of the treatment of cancer. The principle thereof is based on immunization with peptides which reproduce T cell epitopes of tumor antigens recognized by cytotoxic T lymphocytes (CTLs), which play a major role in the elimination of tumor cells.

[0003] It will be recalled that CTLs do not recognize whole protein antigens, but peptide fragments thereof, generally comprising 8 to 10 amino acids, presented by class I major histocompatibility complex (MHC I) molecules expressed on the surface of cells. The presentation of these peptides is the result of the antigen processing which involves three steps:

- cytosolic degradation of the antigen by a multienzyme complex called proteasome,
- translocation of the peptides derived from this degradation in the endoplasmic reticulum (ER) by the TAP transporters,
- association of these peptides with the MHC I molecules and exportation of the peptide/MHC I complexes to the cell surface.

[0004] The peptide/MHC I complexes interact with the specific T cell receptor (TCR) on CTL, inducing the stimulation and amplification of these CTL, which become able to attack target cells expressing the antigen from which the peptide is derived.

[0005] During the antigen processing, a peptide selection takes place, which results in a hierarchy of peptides presentation. Peptides that are preferentially presented by the MHC I molecules are called immunodominant, while peptides which are weakly presented are called cryptic. Immunodominant peptides exhibit a high affinity for the MHC I and are immunogenic while cryptic peptides exhibit a low affinity for MHC I and are non-immunogenic.

[0006] Immunodominant peptides have been widely targeted by tumor vaccines in preclinical and clinical studies with disappointing results (Gross et al., 2004; Rosenberg et al., 2004).

[0007] Tumor antigens are frequently self proteins over-expressed by tumors and expressed at lower levels by normal cells and tissues. The immune system is unable to react against these self antigens because of the self tolerance process. Self-tolerance concerns mainly the immunodominant peptides (Cibotti et al., 1992; Gross et al., 2004), thus explaining the incapacity of these peptides to induce a tumor immunity.

[0008] Cryptic peptides are much less involved in self tolerance process (Cibotti et al., 1992; Gross et al., 2004; Moudgil et al., 1999) and can therefore induce an efficient tumor immunity, provided their immunogenicity is enhanced (Engelhorn et al., 2006; Gross et al., 2004).

[0009] The usual strategy for enhancing the immunogenicity of cryptic peptides, which are non-immunogenic because of their low MHC I affinity, consists in increasing their affinity for the MHC I molecules via amino acids substitutions. Peptide affinity for MHC I molecules mainly depends on the presence at well defined positions (primary anchor positions) of residues called "primary anchor residues". These residues are MHC I allele specific. The presence of primary anchor residues, although often necessary, is not sufficient to ensure a high MHC I affinity. It has been shown that residues located outside the primary anchor positions (secondary anchor residues) may exert a favourable or unfavourable effect on the affinity of the peptide for the MHC I. The presence of these secondary anchor residues makes it possible to explain the existence, within peptides having the primary anchor motifs, of a great variability in the binding affinity (Ruppert et al., 1993).

[0010] Amino acids substitutions aiming at enhancing affinity for MHC I molecule should preserve the antigenicity of such optimized peptides. Indeed, CTL generated by optimized peptides must cross-react with the corresponding native peptides.

[0011] Many teams have succeeded in enhancing immunogenicity of already immunogenic peptides by increasing their affinity for HLA-A*0201 (Bakker et al., 1997; Parkhurst et al., 1996; Valmori et al., 1998). The inventors have previously described a general strategy to enhance affinity and immunogenicity of HLA-A*0201 restricted cryptic peptides (Scardino et al., 2002; Tourdot and Gould, 2002) and HLA-B*0702 (WO 2008/010098).

[0012] HLA-A*2402 is a frequently expressed molecule (27% of the population) and is one of the most common alleles in Japanese and Asian people. Identification and optimization of HLA-A*2402 restricted tumor cryptic peptides is therefore necessary for developing efficient cancer vaccines for HLA-A*2402 expressing patients.

[0013] Several tumor immunogenic peptides presented by HLA-A*2402 have been described to date (table 1).

Table 1: Tumor immunogenic HLA-A24 T cell epitopes

Antigen	Sequence	SEQ ID No:
Beta-catenin	SYLDSGIHF	168

(continued)

Antigen	Sequence	SEQ ID No:
TERT	TYVPLLGSL	169
TERT	CYGDMENKL	170
TERT	AVQVCGPPL	171
KM-HN-1	NYNNFYRFL	172
KM-HN-1	EYSKECLKEF	173
KM-HN-1	EYLSLSDKI	174
MAGE-A2	EYLQLVFGI	175
MAGE-A3	TFPDLESEF	176
MAGE-A3	VAELVHFLL	177
MAGE-A4	NYKRCFPVI	178
SAGE	LYATVIHDI	179
CEA	QYSWFVNGTF	180
CEA	TYACFVSNL	181
gp100 /Pmel17	VYFFLPDHL	182
OA1	LYSACFWWL	183
tyrosinase	AFLPWHRLF	184
Ep-CAM	RYQLDPKFI	185
Her2/neu	TYLPTNASL	186
PRAME	LYVDSLFFL	187
PSMA	NYARTEDFF	188
RNF43	NSQPVWLCL	189
WT1	CMTWNQMNL	190

[0014] As described in the experimental part below, the inventors have now found a strategy to identify, in an antigen, cryptic peptides presented by HLA-A*2402 molecule, and to optimize their immunogenicity, preserving the cross-reactivity with the corresponding native cryptic peptides.

[0015] Hence, the present text first describes a method for identifying an HLA-A*2402-restricted cryptic epitope in an antigen, comprising a step of selecting, in said antigen, a peptide of 8 to 12 amino acids having a tyrosine (Y) in primary anchor position 2, with the proviso that the peptide does not have, simultaneously, a positively charged amino acid (arginine (R) or lysine (K)) in position 1 and a leucine (L), or a phenylalanine (F) or an isoleucine (I) in C-terminal position. Such an epitope hence has the sequence $X_1YX_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID No: 20), wherein X_1 to X_6 are any amino acid, X_7 to X_{10} are any amino acid or none, and $X_{11} \neq$ L or F or I if X_1 = R or K.

[0016] When the above selection step is performed alone, the obtained sequences are those of putative cryptic epitopes. Although epitopes responding to the above criteria have a strong probability to be non immunogenic, functional tests are necessary to identify truly cryptic epitopes with certainty. In particular, the inventors have observed that some peptides having a primary sequence as defined above are in fact immunogenic in individuals expressing HLA-A*2402. Hence, it is herein described a method for identifying a HLA-A*2402-restricted cryptic epitope in an antigen which further comprises step consisting in testing the immunogenicity of each putative cryptic epitope of SEQ ID No: 20, in an appropriate model, and selecting those which are non-immunogenic.

[0017] For performing this aspect of the method, an appropriate model is a model which predicts the immunogenicity of the peptide in an individual who expresses HLA-A*2402. An example of such an appropriate model is described in the experimental part and consists of HLA-A*2402 transgenic mice. In this model, the non-immunogenicity of putative cryptic peptides is checked by vaccinating the mice and testing if specific CTL have been generated, by using human cells expressing HLA-A*2402 and loaded with the peptide as target cells.

[0018] In what follows, the phrases "HLA-A*2402-restricted cryptic epitope" or "native peptide" will be used to designate any peptide of SEQ ID No: 20, whether its non-immunogenicity has been checked or not. When necessary, the phrase "putative HLA-A*2402 -restricted cryptic epitope" will be used to express the fact that the immunogenicity of the peptide has not been tested, and the phrase "confirmed HLA-A*2402-restricted cryptic epitope" will be used for peptides which have been tested and have proved to be non-immunogenic in an appropriate model.

[0019] In the present text, the term "peptide" designates not only molecules in which amino acid residues (in L or D configurations) are joined by peptide (-CO-NH-) linkages, but also synthetic pseudopeptides or peptidomimetics in which the peptide bond is modified, especially to become more resistant to proteolysis, and provided their immunogenicity is

not impaired by this modification.

[0020] According to a preferred embodiment of the above-described method, the selected peptide has 9 to 11 amino acids, more preferably 9 or 10 amino acids and one or more unfavourable amino acids at secondary anchor positions, for example a P (proline) in position 1 and/or a D or E or G or H or P or Q or R or K (glutamic or aspartic acid, glycine, histidine, proline, glutamine, arginine or lysine) at C-terminal position.

[0021] A method for increasing the immunogenicity of a HLA-A*2402-restricted cryptic epitope is also herein described; such a method comprises a step of substituting the N-terminal residue of said epitope with a positively charged amino acid (R or K), and/or substituting the C-terminal residue of said epitope with an L, F or I. Preferentially, the C-terminal modification is the substitution by an L.

[0022] Of course, in this method, the word "substituting" is to be understood as obtaining a peptide the sequence of which is derived from the sequence of said HLA-A*2402-restricted cryptic epitope by the mentioned substitution, whatever the technical method used to obtain said peptide. For example, the peptide can be produced by artificial peptide synthesis or by recombinant expression.

[0023] In particular, the immunogenicity of a HLA-A*2402-restricted cryptic epitope in which the two first residues are RY or KY can be increased by replacing its last amino-acid by an L, F or I, preferentially by an L (or by adding a L, I or F at its C-terminus, provided it is not longer than 11 amino acids). When the sequence of the selected HLA-A*2402-restricted cryptic epitope is $X_1YX_2X_3X_4X_5X_6X_7X_8X_9X_{10}L$ (SEQ ID No: 21), wherein X_1 is any amino acid but R or K, X_2 to X_6 are any amino acid, and X_7 to X_{10} are any amino acid or none, the substitution of X_1 by R or K is sufficient to increase its immunogenicity. More generally, when the sequence of the selected HLA-A*2402-restricted cryptic epitope is $X_1YX_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID No: 22), wherein X_1 is any amino acid but R or K, X_2 to X_6 are any amino acid, and X_7 to X_{10} are any amino acid or none, and X_{11} is not an unfavourable amino acids (D or E or G or H or P or Q or R or K), the substitution of X_1 by R or K can be sufficient to increase its immunogenicity.

[0024] In what follows, the expression "optimized peptide" or "optimized immunogenic A*2402-restricted epitope" will designate an immunogenic peptide derived from a HLA-A*2402-restricted cryptic epitope (called its "cognate native peptide") by the above method.

[0025] In a preferred embodiment of the method, the optimized peptide can trigger an immune response which cross-recognizes its cognate native peptide. The present invention hence pertains to a method for obtaining a HLA-A*2402-restricted epitope able to trigger an immune response against a HLA-A*2402-restricted cryptic epitope of an antigen, comprising the steps of:

(i) identifying, in said antigen, one or several native putative HLA-A*2402-restricted cryptic epitopes, by a method as described above;
(ii) testing the immunogenicity of each native epitope selected in step (i), in HLA-A*2402 transgenic mice, and selecting those which are non-immunogenic;
(iii) for each native epitope selected in step (ii), obtaining an optimized epitope by increasing its immunogenicity, by the method as above-described;
(iv) testing the immunogenicity of each optimized epitope obtained in step (iii), in HLA-A*2402 transgenic mice, and selecting those which are immunogenic;
(v) for each epitope selected in step (iv), testing if the CTLs generated against the optimized epitope also recognize its cognate native epitope, and selecting those for which the test is positive.

[0026] In step (v), the cross-recognition can be performed by any method known by the skilled artisan, for example as described in the experimental part.

[0027] As disclosed in the experimental part below, the inventors have identified in different tumor associated antigens (hTERT, EphA2, MAGE or Her2/neu), a number of putative HLA-A*2402-restricted cryptic epitopes. When testing the immunogenicity of these epitopes, one of them proved to be immunogenic. The inventors have selected the peptides disclosed in Table 2 below, which are confirmed HLA-A*2402-restricted cryptic epitopes. The peptides of SEQ ID Nos: 1, 2, 4, 5 and 6 are part of the present invention.

Table 2: Selected confirmed cryptic HLA-A*2402 restricted peptides

Peptide	Sequence	SEQ ID
TERT 403	PYGVLLKTH	ID N°1
TERT 770	PYMRQFVAH	ID N°2
HER 780	PYVSRLLGI	ID N°3
EphA2 47	PYGKGWDLM	ID N°4
EphA2 502	TYLVQVQAL	ID N°5

(continued)

Peptide	Sequence	SEQ ID
EphA2 817	PYWELSNHE	ID N°6
Her2/neu 922	PYDGIPARE	ID N°7
MAGE 261	RYEFLWGPR	ID N°8
Her2/neu 300	PYNYLSTDV	ID N°9

[0028] The present invention also pertains to optimized peptides derived from the cryptic peptides of SEQ ID Nos: 1 to 6, by a method according to the invention. Preferred examples of optimized peptides are KYGVLLKTL (SEQ ID No: 11), RYMRQFVAL (SEQ ID No: 12), RYVSRLLGI (SEQ ID No: 13), RYGKGWDLL (SEQ ID No: 14), RYLVQVQAL (SEQ ID No: 15), RYWELSNHL (SEQ ID No: 16). Among these peptides, SEQ ID No: 13 and SEQ ID No: 15 have been derived from the cryptic HLA-A*2402-restricted epitopes of SEQ ID NOs: 3 and 5, respectively, by substitution of their N-terminal amino-acid with a R. The peptides of SEQ ID Nos: 11, 12, 14 and 16 have been derived from the peptides of SEQ ID Nos: 1. 2, 4 and 6, respectively, by substituting their N-terminal amino-acid with an R or a K and their C-terminal amino-acid with a L.

[0029] Polyspecific tumor vaccination offers a broader control of tumor cells than monospecific vaccination, thereby reducing the risk of emergence of immune escape variants. In most cases, immunotherapy is then more efficient when targeting several epitopes than when targeting only one epitope, provided the tumour is known to express all targeted antigens. The inventors have previously described a polypeptide composed of HLA-A*0201 restricted optimized cryptic peptides derived from three different universal tumor antigens ($TERT_{988Y}$, $HER\text{-}2/neu_{402Y}$ and $MAGE\text{-}A_{248V9}$), named Vx-006 (WO 2007/073768). Vx-006 is able to induce a polyspecific CD8 cell response both *in vivo* in HLA-A*0201 transgenic HHD mice and *in vitro* in humans, whereas the mixture of $TERT_{988Y}$, $HER\text{-}2/neu_{402Y}$ and $MAGE\text{-}A_{248V9}$ peptides failed to induce a trispecific response. Hence, a chimeric polypeptide comprising several epitopes can be more efficient than a mere mixture of the same epitopes to trigger a response against more than one epitope. Depending on the context, a chimeric polypeptide comprising a repetition of one single epitope can also trigger a stronger response against said epitope than a peptide consisting of said epitope. Indeed, a polypeptide organization (either with several different epitopes or with a repetition of one single epitope) can produce new junctional epitopes, especially CD4 restricted epitopes, able to optimize the targeted peptide(s)-specific immune response. Moreover, when free peptides are subcutaneously injected, peptides bind directly to MHC molecules of every cells present at the site of injection. As polypeptides need to be processed, vaccination with polypeptides is more efficient to target antigenic peptides to professional Antigenic Presenting Cells (APC) as Dendritic Cells.

[0030] A further aspect of the invention is hence a chimeric polypeptide, comprising one, two, three or more different HLA-A*2402-restricted cryptic epitopes selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6). A further aspect of the invention is a chimeric polypeptide comprising two, three or more HLA-A*2402-restricted cryptic epitopes selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYVSRLLGI (SEQ ID NO: 3); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6).

[0031] Another aspect of the present invention is a chimeric polypeptide, comprising one, two, three or more optimized immunogenic HLA-A*2402-restricted epitopes as described above. In a chimeric polypeptide comprising optimized immunogenic HLA-A*2402-restricted epitopes according to the invention, the epitopes can be different from each other, and/or the same epitope can be repeated several times.

[0032] It is to be noted that when several epitopes specific for the same HLA molecule are used together, either in a mix or in a chimeric polypeptide, the epitopes are in competition for the binding to the corresponding HLA molecule. Contrarily, by using a mix of different HLA-restricted epitopes (HLA-A*0201, HLA-A*2402, HLA-B*0702 or others), or a chimeric polypeptide comprising the same different HLA-restricted epitopes, there will be no competition for HLA binding, and a polyspecific response will be obtained with certainty, provided all the HLA molecules are expressed in the vaccinated individual.

[0033] In a chimeric polypeptide according to the invention, HLA-A*2402-restricted cryptic or optimized immunogenic epitopes described above can hence be advantageously associated to previously described HLA-A*0201 (WO 02/02716) and/or HLA-B*0702 peptides (WO 2008/010010 and WO 2008/010098), or to immunogenic epitopes derived from previously described tumor associated antigens, comprising CEA, PRAME, Tyrosinase, TRAG-3, NY-Eso-1, P53, Muc-1, PSA/PSMA, survivin, Melan-A/MART-1, TRP-1, TRP-2, WT1, EphA1, EphA2, EphA3, EphA4, G250/MN/CAIX, STEAP, alphafoetoprotein, RAGE-1, PAGE-1. Of course, a polyallelic peptides mix, comprising at least a peptide according to the present invention and one different HLA-restricted epitope (HLA-A*0201, HLA-A*2402, HLA-B*0702 or others), is also part of the present invention.

[0034] Examples of cryptic epitopes which can advantageously be combined to HLA-A*2402-restricted cryptic epitopes (either in a mix or in a chimeric polypeptide), as well as examples of optimized immunogenic epitopes which can advantageously be combined to optimized immunogenic HLA-A*2402-restricted epitopes, are described in Table 3 below. Of course, these lists are not limitative.

Table 3: epitopes which can be combined to HLA-A*2402-restricted epitopes in chimeric polypeptides according to the invention

HLA-A*0201					
Native peptide			Optimized peptide		
Antigen	Sequence	No	Name	Sequence	No
$Mart\text{-}1_{27}$	AAGIGILTV	23	$Mart\text{-}1_{27Y1}$	YAGIGILTV	24
$Mart\text{-}1_{26}$	EAAGIGILTV	25	$Mart\text{-}1_{26L27}$	ELAGIGILTV	26
$Gp100_{177}$	AMLGTHTMEV	27	$GP100_{177Y1}$	YMLGTHTMEV	28
$Gp100_{178}$	MLGTHTMEV	29	$Gp100_{178Y1}$	YLGTHTMEV	30
$Gp100_{154}$	KTWGQYWQV	31	$Gp100_{154Y1}$	YTWGQYWQV	32
			$Gp100_{154M155}$	KMWGQYWQV	33
$Gp100_{570}$	SLADTNSLAV	34	$Gp100_{570Y1}$	YLADTNSLAV	35
$Gp100_{209}$	TDQVPFSV	36	$Gp100_{209Y1}$	YDQVPFSV	37
			$Gp100_{209M210}$	YMQVPFSV	38
$Gp100_{476}$	VLYRYGSFSV	39	$Gp100_{476Y1}$	YLYRYGSFSV	40
$Gp100_{457}$	LLDGTATLRL	41	$Gp100_{457Y1}$	LLDGTATLRL	42
$HER\text{-}2/neu_{799}$	QLMPYGCLL	43	$HER\text{-}2/neu_{799Y1}$	YLMPYGCLL	44
$HER\text{-}2/neu_{369}$	KIFGSLAFL	45	$HER\text{-}2/neu_{369Y1}$	YIFGSLAFL	46
$HER\text{-}2/neu_{789}$	CLTSTVQLV	47	$HER\text{-}2/neu_{789Y1}$	YLTSTVQLV	48
$HER\text{-}2/neu_{48}$	HLYQGCQW	49	$HER\text{-}2/neu_{48Y1}$	YLYQGCQW	50
$HER\text{-}2/neu_{773}$	VMAGVGSPYV	51	$HER\text{-}2/neu_{773Y1}$	YMAGVGSPYV	52
$HER\text{-}2/neu_{5}$	ALCRWGLL	53	$HER\text{-}2/ne_{5Y1}$	YLCRWGLL	54
$HER\text{-}2/neu_{851}$	VLVKSPNHV	55	$HER\text{-}2/neu_{851Y1}$	YLVKSPNHV	56
$HER\text{-}2/neu_{661}$	ILLVVVLGV	57	$HER\text{-}2/neu_{661Y1}$	YLLVVVLGV	58
$HER\text{-}2/neu_{650}$	PLTSIISAV	59	$HER\text{-}2/neu_{650Y1}$	YLTSIISAV	60
$HER\text{-}2/neu_{466}$	ALIHIINTHL	61	$HER\text{-}2/neu_{466Y1}$	YLIHHNTHL	62
$HER\text{-}2/neu_{402}$	TLEEITGYL	63	$HER\text{-}2/neu_{402Y1}$	YLEEITGYL	64
$HER\text{-}2/neu_{391}$	PLQPEQLQV	65	$HER\text{-}2/neu_{391Y1}$	YLQPEQLQV	66
$HER\text{-}2/neu_{971}$	ELVSEFSRM	67	$HER\text{-}2/neu_{971Y1}$	YLVSEFSRM	68
$EphA_{261}$	DMPIYMYSV	69	$EphA2_{61Y1}$	YMPIYMYSV	70
$HER2_{911}$	TVWELMTFGA	71	$HER_{911Y1V10}$	YVWELMTFGV	74
$HER4_{911}$	TIWELMTFGG	72			
$HER1_{911}$	TVWELMTFGS	73			
$HER2_{722}$	KVKVLGSGA	75	$HER_{722Y1V9}$	YVKVLGSGV	79
$HER3_{722}$	KLKVLGSGV	76			
$HER4_{722}$	RVKVLGSGA	77			
HER 1_{722}	KIKVLGSGA	78			

(continued)

HLA-A*0201					
Native peptide			Optimized peptide		
Antigen	Sequence	No	Name	Sequence	No
$HER2_{845}$	DLAARNVLV	80	HER_{845Y1}	YLAARNVLV	82
$HER3_{845}$	NLAARNVLL	81			
$HER2_{904}$	DVWSYGVTV	83	HER_{904Y1}	YVWSYGVTV	85
$HER4_{904}$	DVWSYGVTI	84			
$HER2_{933}$	DLLEKGERL	86	HER_{933Y1}	YLLEKGERL	88
$HER1_{933}$	SILELKGERL	87			
$HER2_{945}$	PICTIDVYMI	89	HER_{945Y1}	YICTIDVYMV	93
$HER3_{945}$	QICTIDVYMV	90			
$HER4_{945}$	PJCTIDVYMV	91			
$HER1_{945}$	PICTIDVYKI	92			
MAGE-A_{248G9}	YLEYRQVPG	94	MAGE-A_{248G9}	YLEYRQVPV	96
MAGE-A_{248D9}	YLEYRQVPD	95			
$TERT_{988}$	DLQVNSLQTV	97	$TERT_{988Y1}$	YLQVNSLQTV	98
$TERT_{572}$	RLFFYRKSV	99	$TERT_{572Y1}$	YLFFYRKSV	100
HLA-B*0702					
Native peptide			Optimized peptide		
Name	Sequence	No	Name	Sequence	No
$TERT_{444}$	DPRRLVQLL	101	$TERT_{444A1}$	APRRLVQLL	102
$CEA_{188/554}$	SPRLQLSNG	103	$CEA_{188/554L9}$	SPRLQLSNL	104
HER-2/neu_{1069}	APRSPLAPS	105	HER-2/neu_{1069L9}	APRSPLAPL	106
HER-2/neu_{760}	SPKANKEIL	107	HER-2/neu_{760A1}	APKANKEIL	108
HER-2/neu_{246}	GPKHSDCLA	109	HER-2/neu_{246A1}	APKHSDCLA	110

[0035] The skilled artisan can chose any known technique to produce such polypeptides. For example, the polypeptide can be obtained by chemical synthesis, or by using the technology of genetic engineering (Velders et al., 2001).

[0036] Another object of the present invention is an isolated nucleic acid molecule designed to cause the expression of a cryptic HLA-A*2402-restricted epitope, or of an optimized immunogenic HLA-A*2402-restricted epitope, or of a chimeric polypeptide according to the invention. By "designed to cause the expression of" a peptide is herein meant that said peptide is expressed as such, isolated from the whole antigen from which its sequence has been selected (and, in appropriate cases, optimized as above-described), when the nucleic acid is introduced in an appropriate cell. The region encoding the epitope or chimeric polypeptide will typically be situated in the polynucleotide under control of a suitable promoter. Bacterial promoters will be preferred for expression in bacteria, which can produce the polypeptide either *in vitro,* or, in particular circumstances, *in vivo.* An example of bacterium that can be used to produce a peptide or polypeptide according to the invention, directly *in vivo,* is *Listeria monocytogenes,* which is a facultative intracellular bacterium that enters professional antigen-presenting cells by active phagocytosis (Paterson and Maciag, 2005). Alternatively, a nucleic acid according to the invention can be administered directly, using an appropriate vector. In this case, a tissue-specific, a strong constitutive, or an endogenous promoter can be used to control the peptide expression. Suitable vector systems include naked DNA plasmids, liposomal compositions to enhance delivery, and viral vectors that cause transient expression. Examples of viral vectors are adenovirus or vaccinia virus vectors and vectors of the herpes family, especially in a non-replicative form.

[0037] The present invention also pertains to a pharmaceutical composition comprising at least, as an active principle, an HLA-A*2402-restricted cryptic epitope according to the invention, or an optimized immunogenic epitope polypeptide

as mentioned above, or a chimeric polypeptide according to the invention, or a nucleic acid encoding any of these, and/or a vector carrying said nucleic acid. Formulation of pharmaceutical compositions will accord with contemporary standards and techniques. Medicines intended for human administration will be prepared in adequately sterile conditions, in which the active ingredient(s) are combined with an isotonic solution or other pharmaceutical carrier appropriate for the recommended therapeutic use. Suitable formulations and techniques are generally described in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co, Easton PA).

[0038] In particular, a HLA-A*2402-restricted epitope or a chimeric polypeptide or a nucleic acid according to the invention can be used for the preparation of a composition for preventive or curative immunotherapy, especially, for antiviral or anticancer immunotherapy.

[0039] In a particular embodiment, a pharmaceutical composition according to the invention is a vaccine. In this latter case, the components described above can be combined with an adjuvant to potentiate the immune response. Classic adjuvants include oil emulsions, like Incomplete Freund's Adjuvant or Montanide, and adherent surfaces such as alum. Adjuvants that recruit and activate dendritic cells particularly via TLR (such as bacterial DNA or bacterial membrane derived proteins) or help elicit cytotoxic T cells are especially useful. Other factors that otherwise boost the immune response or promote apoptosis or elimination of cancer cells can also be included in the composition, such as IL-2 or IL-12 cytokines or GM-CSF.

[0040] Multiple doses and/or different combinations of the immunogenic compositions of this invention can be packaged for distribution separately or together. Each composition or set of compositions, such as the kits of parts described below, can be accompanied with written instructions regarding the use of the composition or combination for eliciting an immune response and/or for the treatment of cancer.

[0041] In a previous patent application (WO 2006/120038), the Applicant has described a vaccination protocol which enables the initiation and maintenance of a T cell response targeting sub-dominant/cryptic epitopes. The results reported in WO 2006/120038 demonstrate that injection of a native peptide corresponding to a subdominant/cryptic epitope, following vaccination with its cognate optimized peptide, can maintain the immune response initiated by said optimized peptide.

[0042] According to the invention, a HLA-A*2402-restricted cryptic epitope selected amongst SEQ ID Nos: 1 to 6 can hence be used for the preparation of a medicinal composition for maintaining the CTL immune response initiated by its cognate optimized peptide. An immunogenic peptide having an optimized immunogenic HLA-A*2402-restricted epitope sequence derived from a HLA-A*2402-restricted cryptic epitope can also be used, for the preparation of a medicinal composition for initiating a CTL immune response against said HLA-A*2402-restricted cryptic epitope. A method for vaccinating a patient against a tumoral or viral antigen is also described, wherein said method comprises a first step of vaccination with an optimized immunogenic peptide cognate to a native HLA-A*2402-restricted cryptic epitope of said antigen, followed by a second step of vaccination with said native peptide. In such a method, the first step and/or the second step can be performed by using a chimeric polypeptide comprising one, two, three or more optimized or cryptic peptides as above-described, instead of single-epitope peptides.

[0043] The invention also pertains to a kit of parts comprising, in separate formulations or containers (vials, tubes, etc.):

(i) a first peptide comprising a sequence of a HLA-A*2402-restricted cryptic epitope, and
(ii) a second peptide comprising a sequence corresponding to an optimized immunogenic epitope cognate to the cryptic epitope recited in (i).

[0044] Examples of peptides which can be part of a kit according to the invention are the peptides of SEQ ID NOs: 1 to 6, which can constitute the first peptide, the second peptide being then derived from said first peptide by a method for increasing its immunogenicity, as described above. Preferred kits according to the invention can hence comprise peptides of SEQ ID Nos: 1 and 11 (in separate containers), or peptides of SEQ ID Nos: 2 and 12 (in separate containers), or peptides of SEQ ID Nos: 3 and 13 (in separate containers), or peptides of SEQ ID Nos: 4 and 14 (in separate containers), or peptides of SEQ ID Nos: 5 and 15 (in separate containers), or peptides of SEQ ID Nos: 6 and 16 (in separate containers).

[0045] Other kits of parts according to the invention comprise at least one chimeric polypeptide. In this embodiment, the kit also comprises at least a peptide cognate to one of the epitopes comprised in the chimeric polypeptide, wherein said cognate peptide is either isolated or included in another chimeric polypeptide.

[0046] Several preferred variants of such kits are contemplated: in a first embodiment, the kit comprises, in separate formulations, a first chimeric polypeptide comprising one, two, three or more HLA-A*2402-restricted cryptic epitopes, and a second chimeric polypeptide corresponding to its cognate HLA-A*2402-restricted immunogenic chimeric polypeptide (which means that it comprises optimized HLA-A*2402-restricted immunogenic epitopes cognate to the cryptic epitopes comprised in the first chimeric polypeptide). In a second embodiment, the kit comprises one, two, three or more peptides corresponding to distinct HLA-A*2402-restricted cryptic epitopes, wherein said peptides are either mixed in one single formulation, or separated in several formulations and, in a separate formulation, a chimeric polypeptide

comprising the optimized HLA-A*2402-restricted immunogenic epitopes cognate to said cryptic peptides.

[0047] As mentioned above, a polyallelic stimulation (*i.e.*, using epitopes specific for different HLA molecules) can advantageously be performed to obtain a polyspecific response. Accordingly, preferred embodiments of the kits according to the invention comprise, in separate containers:

(i) a polyallelic peptides mix or a polyallelic chimeric polypeptide, comprising at least a HLA-A*2402-restricted cryptic epitope as described above and at least one different HLA-restricted cryptic epitope, and
(ii) a polyallelic peptides mix or a polyallelic chimeric polypeptide, comprising at least a HLA-A*2402-restricted immunogenic epitope cognate to the HLA-A*2402-restricted cryptic epitope recited in (i), and at least another immunogenic epitope cognate to the other cryptic epitope recited in (i).

[0048] Alternatively, the kits according to the invention can comprise, instead of at least part the peptides or chimeric polypeptides, nucleic acid(s) encoding said peptides or chimeric polypeptides. In this case, the nucleic acid(s) is(are) as above-described.

[0049] In the following description of some specific kits according to the invention, mention will be made only of the peptides (native or optimized) included therein; it is understood that chimeric polypeptide(s) (comprising native cryptic epitopes or optimized epitopes) can be enclosed in the kits instead of single-epitope peptides, and that nucleic acid(s) can also be included in addition or instead of at least part of said peptides or chimeric polypeptides.

[0050] In a particular embodiment of the invention, the kit is a vaccination kit, wherein said first (native) and second (cognate optimized) peptides are in separate vaccination doses. In a preferred embodiment, the vaccination kit comprises 2 or 3 doses of optimized peptide, and 3, 4, 5 or 6 doses of native peptide. A particular vaccination kit according to the invention is adapted for the first vaccination sequence of 6 injections, and comprises 2 or 3 doses of optimized peptide, and 4 or 3 doses of native peptide. In case of long-lasting diseases, it is preferable to maintain the level of immunity obtained after this primo-vaccination, by regular recalls. This can be done, for example, by injections performed every 1 to 6 months. Therefore, complementary kits, comprising at least 2 doses, and up to 40 or 50 doses of native peptide, are also part of the present invention.
Alternatively, the vaccination kit can comprise 2 to 3 doses of optimized peptide, and 3 to 40 or up to 50 doses of native peptide. Of course, said native and optimized peptides present in the kit are as described above.

[0051] Each dose comprises between 0.1 and 10 mg of peptide, preferably from 1 to 5 mg, or between 1 and 20 mg of polypeptide. In a preferred embodiment, each dose is formulated for subcutaneous injection. For example, each dose can be formulated in 0.3 to 1.5 ml of an emulsion of aqueous solution emulsified with Montanide ISA51, used as an adjuvant. The skilled artisan can choose any other adjuvant(s) in place of (or in addition to) Montanide ISA51. In a particular embodiment, the doses are in the form of an aqueous solution. Alternatively, the doses can be in the form of a lyophilized peptide, for extemporaneous preparation of the liquid solution to be injected. Other possible components of said kits are one or several adjuvants, to be added to the peptide compositions before administration, and a notice describing how to use said kits.

[0052] The invention is further illustrated by the following figures and examples.

LEGENDS OF FIGURES

[0053]

Figure 1 : Immunogenicity of HLA-A*2402 cryptic peptides. HLA-A*2402 transgenic mice were vaccinated with the cryptic peptides following the described protocol and generated CTL were tested against T2-A24 targets loaded with peptide as indicated (NR non relevant peptide). Percentage of specific lysis was determined as: Lysis = (Experimental Release - Spontaneous Release) / (Maximal Release - Spontaneous Release) x 100. Four CTL dilutions, corresponding to four CTL/target cells ratio were tested.

Figure 2: Immunogenicity of HLA-A*2402 restricted optimized cryptic peptides. HLA-A*2402 transgenic mice were vaccinated with the optimized peptide following the described protocol and generated CTL were tested against T2-A24 targets loaded with the optimized (immunogenicity), the corresponding native (native peptide cross recognition) or an irrelevant (NR) peptide as indicated. Percentage of specific lysis was determined as: Lysis = (Experimental Release - Spontaneous Release) / (Maximal Release - Spontaneous Release) x 100. Four CTL dilutions, corresponding to four CTL/target cells ratio were tested.

EXAMPLES

[0054] The examples have been performed using the following materials and methods:esd

[0055] ***Transgenic Mice.*** The transgenic mice used in the described experiments were obtained by crossing HLA-A24 transgenic mice previously described (Barra et al., 1993) and H2 Kb^- $H2Db^-$ knock out mice, transgenic for both human β2 microglobulin and CD8α chain (Perarnau et al., 1999).

[0056] ***Peptides.*** Peptides were synthesized by Epytop (Nimes, France).

[0057] ***Cells.*** HLA-A*2402 transfected human TAP negative T2-A24 cells were previously described (Miyahara et al., 2005), and were provided by Dr. Lemonnier (Institut Pasteur, Paris, France). All cell lines were grown in FCS 10% supplemented RPMI1640 culture medium.

[0058] ***Measurement of Peptide Relative Affinity to HLA-A*2402.*** The protocol used has been described previously (Rohrlich et al., 2003). Briefly, T2-A24 cells were incubated at 37°C for 16 hours with peptides concentrations ranging from 100 μM to 0.1 μM, and then stained with 0041 HA monoclonal antibody (mAb)(One Lambda, Inc.) to quantify the surface expression of HLA-A*2402. For each peptide concentration, the HLA-A*2402 specific staining was calculated as the percentage of staining obtained with 100 μM of the reference peptide standard A24 (AYIDNYNKF, SEQ ID NO: 111). The relative affinity (RA) was determined as: RA = (Concentration of each peptide that induces 30 % of HLA-A*2402-expression / Concentration of the reference peptide that induces 30 % of HLA-A*2402 expression).

[0059] ***CTL Induction* in vivo *in HLA-A*2402 Transgenic Mice.*** **Mice were** injected subcutaneously with 100 μg of peptide emulsified in Incomplete Freund's Adjuvant (IFA) in the presence of 150 μg of the $I\text{-}A^b$ restricted $HBVcore_{128}$ T helper epitope (TPPAYRPPNAPIL, SEQ ID NO: 112). After 15 days, $5x10^7$ spleen cells were stimulated twice *in vitro* with peptide (10 μM), at 6 days interval. On day 13 of culture, the bulk responder populations were tested for specific cytotoxicity against target cells expressing HLA-A*2402 and loaded with the same peptide.

[0060] ***Cross-recognition assay.*** Mice were injected subcutaneously with 100 μg of optimized peptide emulsified in Incomplete Freund's Adjuvant (IFA) in the presence of 150 μg of the $I\text{-}A^b$ restricted $HBVcore_{128}$ T helper epitope (TPPAYRPPNAPIL, SEQ ID NO: 112). After 15 days, $5x10^7$ spleen cells were stimulated firstly *in vitro* with the optimized peptide (10 μM), and secondly on day 6 of culture with the corresponding native peptide. On day 13, the bulk responder populations were tested for specific cytotoxicity against targets cells expressing HLA-A*2402 and loaded with the optimized, the native or an irrelevant peptide.

[0061] ***Cytotoxic assay***. Targets were labelled with 100 μCi of Cr^{51} for 60 min, plated in 96-well V-bottomed plates ($3x10^3$ cell/well in 100 μL of RPMI 1640 medium) and, when necessary, pulsed with optimized or native peptides (1 μM) at 37°C for 2 hours. Four dilutions of effector cells were then added in the wells and incubated at 37°C for 4 hours. Percentage of specific lysis was determined as: Lysis = (Experimental Release - Spontaneous Release) / (Maximal Release - Spontaneous Release) x 100.

Example 1: Affinity and Immunogenicity of Selected Cryptic Peptides

[0062] The inventors have selected 10 native peptides according to the selection method described above. First, seven peptides were tested for their capacity to bind HLA-A*2402 molecules. All but two peptides were not or weakly able to bind to the HLA-A*2402.

Table 4: HLA-A*2402 affinity of cryptic peptides. RA = Relative Affinity = (Concentration of each peptide that induces 30 % of HLA-A*2402-expression / Concentration of the reference peptide that induces 30 % of HLA-A*2402 expression), (-) means RA>100, (+/-) 10<RA<100, (+) 5<RA<10, (++) RA <5, ND: not determined

Antigen/position	Sequence	RA	SEQ ID No
TERT403	PYGVLLKTH	-	1
TERT 770	PYMRQFVAH	+/-	2
Her2/neu 780	PYVSRLLGI	++	3
EphA2 47	PYGKGWDLM	ND	4
EphA2 502	TYLVQVQAL	ND	5
EphA2 817	PYWELSNHE	ND	6
Her2/neu 922	PYDGIPARE	-	7
MAGE 261	RYEFLWGPR	-	8
Her2/neu 300	PYNYLSTDV	-	9
Her2/neu 802	PYGCLLDHV	+	10

[0063] HLA-A24 transgenic mice were then vaccinated with the selected peptides, and fifteen days later, their spleen cells were *in vitro* stimulated twice at 6 days intervals with the peptide. Peptide-specific CTLs were detected in mice vaccinated with control high affinity peptides selected as having primary Y2 and/or C-terminal anchor motifs (data not

shown). Native peptides, which were not able to bind to the HLA-A*2402 were shown to be also non immunogenic (figure 1) and Her2/neu 802, which binds to the HLA-A*2402, was shown to be immunogenic in transgenic mice. This confirms that there is a correlation between binding affinity and immunogenicity for the HLA-A*2402 restricted peptides.

[0064] Nevertheless, as Her2/neu 780 strongly binds to HLA-A*2402 but is finally non immunogenic, the inventors decided to select native peptides only on their incapacity to induce a specific immune response in HLA-A24 transgenic mice. Finally, only one native peptide selected according to the described selection method was able to generate a specific immune response in HLA-A*2402 transgenic mice, confirming that the described method allows to efficiently select putative cryptic peptides. Immunogenicity of selected native peptides is shown in table 5.

Table 5: HLA-A*2402 immunogenicity of selected cryptic peptides. (-) means that none of the mice vaccinated with the corresponding native peptides develops a specific immune response, (+) that less to 50% of vaccinated mice responded, (++) that more that 50% responded. ND: not determined

Antigen/position	Sequence	Immunogenicity	SEQ ID No
TERT403	PYGVLLKTH	-	1
TEXT770	PYMRQFVAH	-	2
Her2/neu 780	PYVSRLLGI	-	3
EphA2 47	PYGKGWDLM	-	4
EphA2 502	TYLVQVQAL	-	5
EphA2 817	PYWELSNHE	-	6
Her2/neu 922	PYDGIPARE	ND	7
MAGE 261	RYEFLWGPR	-	8
Her2/neu 300	PYNYLSTDV	-	9
Her2/neu 802	PYGCLLDHV	++	10

Example 2: Enhancement of Immunogenicity of the Selected Cryptic Peptides

[0065] To enhance HLA-A*2402 affinity and consequently immunogenicity of low affinity peptides with the HLA specific anchor motifs, it was necessary to identify unfavourable secondary anchor motifs and substitute them with favourable motifs. These substitutions must however preserve the conformation of the peptide segment which interacts with the TCR (position 4 to position 8). The interest was, therefore, focused on secondary anchor position 1. Positively charged amino acids (lysine (K) or arginine (R)) are favourable motifs at position 1 whereas a proline (P) is an unfavourable amino acid.

[0066] Moreover, as shown in table 6 below, more than 50% of HLA-A*2402 CD8 epitope identified both in tumors and HIV cells, have a leucine (L) in C-terminal position. The inventors hence decided to use L as the C terminal modification to enhance immunogenicity of peptides preferentially having an unfavourable amino acids in this position (aspartic or glutamic acid (D,E), glycine (G), histidine (H), glutamine (Q), lysine (K), proline (P) or arginine (R)).

Table 6: Tumor and HIV derived HLA-A*2402 restricted epitopes

Antigen	Sequence	No	reference
Beta-catenin	SYLDSGIHF	113	http://www.cancerimmunity.org/peptidedatabase/mutation.htm http://www.cancerimmunity.org/peptidedatabase/tumorspecific.htm http://www.cancerimmunity,org/peptidedatabase/differentiation.htm http://www.cancerimmunity.org/peptidedatabase/overexpressed.htm
KM-HN-1	NYNNFYRFL	114	
KM-HN-1	EYSKECLKEF	115	
KM-HN-1	EYLSLSDKI	116	
MAGE-A2	EYLQLVFGI	117	
MAGE-A3	TFPDLESEF	118	
MAGE-A3	VAELVHFLL	119	
MAGE-A4	NYKRCFPVI	120	
SAGE	LYATVIHDI	121	

(continued)

Antigen	Sequence	No	reference
CEA	QYSWFVNGTF	122	
CEA	TYACFVSNL	123	
gp100 / Pmel17	VYFFLPDHL	124	
OA1	LYSACFWWL	125	
tyrosinase	AFLPWHRLF	126	
Ep-CAM	RYQLDPKFI	127	
Her2/neu	TYLPTNASL	128	
PRAME	LYVDSLFFL	129	
PSMA	NYARTEDFF	130	
RNF43	NSQPVWLCL	131	
TERT	TYVPLLGSL	132	Ref peptides TERT
TERT	CYGDMENKL	133	
TERT	AVQVCGPPL	134	
WT1	CMTWNQMNL	135	
p17	HYMLKHLVW	136	http://hiv-web.lanl.gov/content/immunology/tables/ctl summary.html
p17	KYKLKHIVW	137	
p17	LYNTVATL	138	
p17	LYGVHQKI	139	
p17-p24	NYPIVQNL	140	
p24	EIYKRWIIL	141	
p24	IYKRWIIL	142	
p24	IYKRWIILGL	143	
p2p7p1p6	LYPLASLRSL	144	
RT	DAYFSVPL	145	
RT	VYYDPSKDL	146	
RT	IYQEPFKNL	147	
Integrase	GYIEAEVI	148	
gp160	LFCASDAKAY	149	
gp160	RYLRDQQL	150	
gp160	RYLKDQQLL	151	
gp160	RYLRDQQLL	152	
gp160	RYLRDQQLLGI	153	
gp160	YLKDQQLL	154	
gp160	YLRDQQLL	155	
gp160	WYIKIFIMI	156	
gp160	SYRRLRDLL	157	
Nef	TYKAAVDL	158	

(continued)

Antigen	Sequence	No	reference
Nef	HSQRRQDIL	159	
Nef	RQDILDLWI	160	
Nef	GYFPDWQNY	161	
Nef	NYTPGPGVRY	162	
Nef	RYPLTFGW	163	
Nef	RYPLTFGWCF	164	
Nef	RYPLTFGWCY	165	
Nef	DSRLAFHHM	166	
Nef	AFHHVAREL	167	

[0067] Optimized peptides were tested for their immunogenicity (table 7, figure 2), showing that the chosen modification enhances the capacity to induce specific immune response in HLA-A24 transgenic mice for six native peptides. HLA-A24 transgenic mice vaccinated with the TERT 403KIL9, TERT 770R1L9, HER 780R1, EphA2 47R1L9, EphA2 502R1 and EphA2 817R1L9 peptides, developed peptide specific CTLs.

[0068] Importantly, CTLs generated in mice vaccinated with optimized peptides recognized target cells loaded with the corresponding native peptide (figure 2).

Antigen/position	Modification	Sequence	Immunogenicity	Native peptide cross recognition	Seq ID N°
TERT 403		PYGVLLKTH	- (0/3)		1
TERT 403	K1L9	KYGVLLKTL	+ (4/15)	+ (3/15)	11
TERT 770		PYMRQFVAH	- (0/3)		2
TERT 770	R1L9	RYMRQFVAL	++ (12/18)	+ (5/18)	12
HER 780		PYVSRLLGI	- (0/8)		3
HER 780	R1	RYVSRLLGI	+ (4/9)	+ (3/9)	13
EphA2 47		PYGKGWDLM	- (0/6)		4
EphA2 47	R1L9	RYGKGWDLL	++ (7/9)	++ (7/9)	14
EphA2 502		TYLVQVQAL	- (0/3)		5
EphA2 502	R1	RYLVQVQAL	++ (3/3)	++ (2/3)	15
EphA2 817		PYWELSNHE	- (0/3)		6
EphA2 817	R1L9	RYWELSNHL	++ (2/3)	++ (2/3)	16
Her2/neu 922		PYDGIPARE	ND		7
Her2/neu 922	R1L9	RYDGIPARL	- (0/9)		17
MAGE 261		RYEFLWGPR	- (0/3)		8
MAGE 261	L9	RYEFLWGPL	- (0/9)		18
Her2/neu 300		PYNYLSTDV	- (0/3)		9
Her2/neu 300	R1L9	RYNYLSTDL	- (0/9)		19

Table 7: Native and modified peptides immunogenicity and native peptide cross recognition. (-) means that none of the mice vaccinated with the corresponding native peptides develops a specific immune response, (+) that less to 50% of vaccinated mice responded, (++) that more that 50% responded. (X/Y) means that X mice developed a specific response for a total of Y mice vaccinated. ND: not determined

[0069] In conclusion, the inventors describe a method to optimize immunogenicity of HLA-A*2402 restricted cryptic peptides. It consists of a) selecting cryptic peptides with Y2 and unfavourable amino acids in secondary anchor position

1 and/or 9; and b) substituting the unfavourable amino acids at the N-terminal position with a positively charged amino acid (R or K) and the C-terminal residue with a L when this later substitution is necessary.

[0070] Using these methods of selection/optimization, the inventors also described 6 optimized cryptic peptides that induce specific CTLs in transgenic mice able to recognize cells presenting the corresponding native peptide.

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Claims

1. A method for obtaining a HLA-A*2402-restricted epitope able to trigger an immune response against a HLA-A*2402-restricted cryptic epitope of an antigen, comprising the steps of

(i) selecting, in said antigen, a peptide of 9 to 11 amino acids having a tyrosine in position 2 and one or more unfavourable amino acid(s) at secondary anchor position(s), selected in the group consisting of a proline in position 1 and a glutamic acid, an aspartic acid, a glycine, a histidine, a proline, a glutamine, an arginine and a lysine at C-terminal position;
(ii) testing, in HLA-A*2402 transgenic mice, the immunogenicity of the peptide selected in step (i);
(iii) selecting said peptide if it failed to induce a specific immune response in HLA- A*2402 transgenic mice

(iv) for each native epitope selected in step (iii), obtaining an optimized epitope by increasing its immunogenicity, by substituting the N-terminal residue of said epitope with an arginine or a lysine, and/or substituting the C-terminal residue of said epitope with a leucine or an isoleucine or a phenylalanine, preferentially with a leucine;
(v) testing the immunogenicity of each optimized epitope obtained in step (iv), in HLA-A*2402 transgenic mice, and selecting those which are immunogenic;
(vi) for each epitope selected in step (v), testing if the CTLs generated against the optimized epitope also recognize its cognate native epitope, and selecting those for which the test is positive.

2. An isolated peptide consisting of a cryptic HLA-A*2402-restricted epitope, wherein said isolated peptide is selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6).

3. An isolated peptide consisting of an immunogenic HLA-A*2402-restricted epitope, wherein said isolated peptide is selected from the group consisting of KYGVLLKTL (SEQ ID No: 11); RYMRQFVAL (SEQ ID No: 12); RYVSRLLGI (SEQ ID No: 13); RYGKGWDLL (SEQ ID No: 14); RYLVQVQAL (SEQ ID No: 15); and RYWELSNHL (SEQ ID No: 16).

4. A chimeric polypeptide comprising several different epitopes or a repetition of one single epitope, **characterized in that** it comprises one, two, three or more HLA-A*2402-restricted cryptic epitopes selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6).

5. A chimeric polypeptide comprising several different epitopes, **characterized in that** it comprises two, three or more HLA-A*2402-restricted cryptic epitopes selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYVSRLLGI (SEQ ID NO: 3); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6).

6. A chimeric polypeptide comprising several different epitopes or a repetition of one single epitope, **characterized in that** it comprises one, two, three or more immunogenic HLA-A*2402-restricted epitopes according to claim 3.

7. An isolated nucleic acid molecule designed to cause the expression of a cryptic HLA-A*2402-restricted epitope according to claim 2, an immunogenic epitope according to claim 3, or a chimeric polypeptide according to any of claims 4 to 6.

8. A pharmaceutical composition comprising at least, as an active principle, an HLA-A*2402-restricted cryptic epitope according to claim 2, or an immunogenic epitope polypeptide according to claim 3, or a chimeric polypeptide according to any of claims 4 to 6, or a nucleic acid according to claim 7.

9. The pharmaceutical composition of claim 8, which is a vaccine.

10. A kit of parts comprising, in separate containers:

(i) a first peptide comprising a sequence of a HLA-A*2402-restricted cryptic epitope, and
(ii) a second peptide comprising a sequence consisting of a HLA-A*2402-restricted immunogenic epitope,

wherein said first and second peptides are selected from the group consisting of the following pairs of peptides:

- PYGVLLKTH (SEQ ID NO: 1) and KYGVLLKTL (SEQ ID No: 11);
- PYMRQFVAH (SEQ ID NO: 2) and RYMRQFVAL (SEQ ID No: 12);
- PYVSRLLGI (SEQ ID NO: 3) and RYVSRLLGI (SEQ ID No: 13);
- PYGKGWDLM (SEQ ID NO: 4) and RYGKGWDLL (SEQ ID No: 14);
- TYLVQVQAL (SEQ ID NO: 5) and RYLVQVQAL (SEQ ID No: 15); and
- PYWELSNHE (SEQ ID NO: 6) and RYWELSNHL (SEQ ID No: 16).

11. A kit of parts comprising, in separate containers:

(i) a first peptide comprising a sequence of a HLA-A*2402-restricted cryptic epitope, and
(ii) a second peptide comprising a sequence consisting of a HLA-A*2402-restricted immunogenic epitope derived from the HLA-A*2402-restricted cryptic epitope recited in (i) by a method according to claim 1,

wherein said first peptide is a chimeric polypeptide according to claim 4 or claim 5, and said second peptide is a chimeric polypeptide according to claim 6, wherein at least one immunogenic epitope comprised in the second peptide is cognate to at least one HLA-A*2402-restricted cryptic epitope comprised in the first peptide.

12. A kit of parts comprising, in separate containers:

(i) a first peptide comprising a sequence of a HLA-A*2402-restricted cryptic epitope selected from the group consisting of PYGVLLKTH (SEQ ID NO: 1); PYMRQFVAH (SEQ ID NO: 2); PYVSRLLGI (SEQ ID NO: 3); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO: 5) and PYWELSNHE (SEQ ID NO: 6), and
(ii) a second peptide comprising a sequence consisting of a HLA-A*2402-restricted immunogenic epitope derived from the HLA-A*2402-restricted cryptic epitope recited in (i) by a method according to claim 1, wherein said second peptide is a chimeric polypeptide according to claim 6.

13. An isolated peptide according to claim 2 or claim 3, or a chimeric polypeptide according to any of claims 4 to 6, or a nucleic acid according to claim 7, for use as a medicament for preventive or curative immunotherapy.

Patentansprüche

1. Verfahren zum Erhalten eines HLA-A*2402-restringierten Epitops, das fähig ist, eine Immunantwort gegen ein HLA-A*2402-restringiertes kryptisches Epitop eines Antigens auszulösen, umfassend die Schritte:

(i) Auswählen, in dem Antigen, ein Peptid mit 9 bis 11 Aminosäuren, das ein Tyrosin in Position 2 hat und eine oder mehrere ungünstige Aminosäure(n) an sekundärer Ankerposition(en), ausgewählt aus der Gruppe, bestehend aus einem Prolin in Position 1 und einer Glutaminsäure, einer Asparaginsäure, einem Glycin, einem Histidin, einem Prolin, einem Glutamin, einem Arginin und einem Lysin an C-terminaler Position, hat;
(ii) Untersuchen der Immunogenität des in Schritt (i) ausgewählten Peptids in HLA-A*2402-transgenen Mäusen;
(iii) Auswählen des Peptids, wenn es keine spezifische Immunantwort in HLA-A*2402-transgenen Mäusen induzieren konnte;
(iv) Erhalten eines optimierten Epitops für jedes native Epitop, das in Schritt (iii) ausgewählt wurde durch Erhöhen seiner Immunogenität, indem der N-terminale Rest des Epitops durch ein Arginin oder ein Lysin ersetzt wird, und/oder der C-terminale Rest des Epitops durch ein Leucin oder ein Isoleucin oder ein Phenylalanin, vorzugsweise ein Leucin, ersetzt wird;
(v) Untersuchen der Immunogenität jedes optimierten Epitops, das in Schritt (iv) erhalten worden war, in HLA-A*2402-transgenen Mäusen und Auswählen derjenigen, die immunogen sind;
(vi) für jedes Epitop, das in Schritt (v) ausgewählt wurde, Untersuchen, ob die CTLs, die gegen das optimierte Epitop erzeugt wurden, auch sein verwandtes natives Epitop erkennen, und Auswählen derjenigen, für die der Test positiv ist.

2. Isoliertes Peptid, bestehend aus einem kryptischen HLA-A*2402-restringierten Epitop, wobei das isolierte Peptid aus der Gruppe, bestehend aus PYGVLLKTH (SEQ ID NO:1); PYMRQFVAH (SEQ ID NO: 2); PYGKGWDLM (SEQ ID NO: 4); TYLVQVQAL (SEQ ID NO:5) und PYWELSNHE (SEQ ID NO:6), ausgewählt ist.

3. Isoliertes Peptid, bestehend aus einem immunogenen HLA-A*2402-restringierten Epitop, wobei das isolierte Peptid aus der Gruppe, bestehend aus KYGVLLKTL (SEQ ID NO:11); RYMRQFVAL (SEQ ID NO:12); RYVSRLLGI (SEQ ID NO:13); RYGKGWDLL (SEQ ID NO:14); RYLVQVQAL (SEQ ID NO:15) und RYWELSNHL (SEQ ID NO:16), ausgewählt ist.

4. Chimäres Polypeptid, das mehrere unterschiedliche Epitope oder eine Wiederholung eines einzelnen Epitops umfasst, **dadurch gekennzeichnet, dass** es ein, zwei, drei oder mehr HLA-A*2402-restringierte kryptische Epitope, ausgewählt aus der Gruppe, bestehend aus PYGVLLKTH (SEQ ID NO:1); PYMRQFVAH (SEQ ID NO:2); PYGKGWDLM (SEQ ID NO:4); TYLVQVQAL (SEQ ID NO:5) und PYWELSNHE (SEQ ID NO:6), umfasst.

5. Chimäres Polypeptid, das mehrere unterschiedliche Epitope umfasst, **dadurch gekennzeichnet, dass** es zwei, drei oder mehr HLA-A*2402-restringierte kryptische Epitope, ausgewählt aus der Gruppe, bestehend aus PYGVLLKTH (SEQ ID NO:1); PYMRQFVAH (SEQ ID NO:2); PYVSRLLGI (SEQ ID NO:3); PYGKGWDLM (SEQ ID NO:4); TYLVQVQAL (SEQ ID NO:5) und PYWELSNHE (SEQ ID NO:6), umfasst.

6. Chimäres Polypeptid, das mehrere unterschiedliche Epitope oder eine Wiederholung eines einzelnen Epitops umfasst, **dadurch gekennzeichnet, dass** es eins, zwei, drei oder mehr immunogene HLA-A*2402-restringierte Epitope gemäß Anspruch 3 umfasst.

7. Isoliertes Nucleinsäuremolekül, das konzipiert ist, um die Expression eines kryptischen HLA-A*2402-restringierten Epitops gemäß Anspruch 2, eines immunogenen Epitops gemäß Anspruch 3 oder eines chimären Polypeptids gemäß einem der Ansprüche 4 bis 6 zu verursachen.

8. Pharmazeutische Zusammensetzung, umfassend wenigstens, als aktiven Wirkstoff, ein HLA-A*2402-restringiertes kryptisches Epitop gemäß Anspruch 2 oder ein immunogenes Epitoppolypeptid gemäß Anspruch 3 oder ein chimäres Polypeptid gemäß einem der Ansprüche 4 bis 6 oder eine Nucleinsäure gemäß Anspruch 7.

9. Pharmazeutische Zusammensetzung gemäß Anspruch 8, die ein Vakzin ist.

10. Kit von Komponenten, umfassend in getrennten Behältern:

(i) ein erstes Peptid, das eine Sequenz eines HLA-A*2402-restringierten kryptischen Epitops umfasst, und
(ii) ein zweites Peptid, das eine Sequenz, die aus einem HLA-A*2402-restringierten immunogenen Epitop besteht, umfasst, wobei das erste und zweite Peptid aus der Gruppe, bestehend aus den folgenden Peptidpaaren:

- PYGVLLKTH (SEQ ID NO:1) und KYGVLLKTL (SEQ ID NO:11);
- PYMRQFVAH (SEQ ID NO:2) und RYMRQFVAL (SEQ ID NO:12);
- PYVSRLLGI (SEQ ID NO:3) und RYVSRLLGI (SEQ ID NO:13);
- PYGKGWDLM (SEQ ID NO:4) und RYGKGWDLL (SEQ ID NO:14);
- TYLVQVQAL (SEQ ID NO:5) und RYLVQVQAL (SEQ ID NO:15) und
- PYWELSNHE (SEQ ID NO:6) und RYWELSNHL (SEQ ID NO:16), ausgewählt sind.

11. Kit von Komponenten, umfassend in getrennten Behältern:

(i) ein erstes Peptid, das eine Sequenz eines HLA-A*2402-restringierten kryptischen Epitops umfasst, und
(ii) ein zweites Peptid, das eine Sequenz, bestehend aus einem HLA-A*2402-restringierten immunogenen Epitop, abgeleitet von dem in (i) genannten HLA-A*2402-restringierten kryptischen Epitop durch ein Verfahren gemäß Anspruch 1, umfasst, wobei das erste Peptid ein chimäres Polypeptid gemäß Anspruch 4 oder Anspruch 5 ist und das zweite Peptid ein chimäres Polypeptid gemäß Anspruch 6 ist, wobei wenigstens ein immunogenes Epitop, das in dem zweiten Peptid enthalten ist, mit wenigstens einem HLA-A*2402-restringierten kryptischen Epitop, das in dem ersten Peptid enthalten ist, verwandt ist.

12. Kit von Komponenten, umfassend in getrennten Behältern:

(i) ein erstes Peptid, umfassend eine Sequenz eines HLA-A*2402-restringierten kryptischen Epitops, ausgewählt aus der Gruppe, bestehend aus PYGVLLKTH (SEQ ID NO:1); PYMRQFVAH (SEQ ID NO:2); PYVSRLLGI (SEQ ID NO:3); PYGKGWDLM (SEQ ID NO:4); TYLVQVQAL (SEQ ID NO:5) und PYWELSNHE (SEQ ID NO:6), und
(ii) ein zweites Peptid, umfassend eine Sequenz, bestehend aus einem HLA-A*2402-restringierten immunogenen Epitop, abgeleitet von dem HLA-A*2402-restringierten kryptischen Epitop, das in (i) genannt ist, durch ein Verfahren gemäß Anspruch 1, wobei das zweite Peptid ein chimäres Polypeptid gemäß Anspruch 6 ist.

13. Isoliertes Peptid gemäß Anspruch 2 oder Anspruch 3 oder chimäres Polypeptid gemäß einem der Ansprüche 4 bis 6 oder Nucleinsäure gemäß Anspruch 7 zur Verwendung als Medikament zur präventiven oder heilenden Immuntherapie.

Revendications

1. Procédé pour l'obtention d'un épitope restreint à HLA-A*2402 apte à déclencher une réponse immunitaire contre un épitope cryptique restreint à HLA-A*2402 d'un antigène, comprenant les étapes de :

(i) sélection, dans ledit antigène, d'un peptide de 9 à 11 amino-acides ayant une tyrosine en position 2 et un

ou plusieurs amino-acides défavorables à une ou plusieurs positions d'ancrage secondaires, choisis dans le groupe consistant en une proline en position 1 et un acide glutamique, un acide aspartique, une glycine, une histidine, une proline, une glutamine, une arginine et une lysine en position C-terminale ;
(ii) test, chez des souris transgéniques HLA-A*2402, de l'immunogénicité du peptide choisi dans l'étape (i) ;
(iii) sélection dudit peptide s'il a été incapable d'induire une réponse immunitaire spécifique chez les souris transgéniques HLA-A*2402 ;
(iv) pour chaque épitope natif sélectionné dans l'étape (iii), obtention d'un épitope optimisé en augmentant son immunogénicité, en substituant le résidu N-terminal dudit épitope par une arginine ou une lysine, et/ou en substituant le résidu C-terminal dudit épitope par une leucine ou une isoleucine ou une phénylalanine, préférentiellement par une leucine ;
(v) test de l'immunogénicité de chaque épitope optimisé obtenu dans l'étape (iv), chez des souris transgéniques HLA-A*2402, et sélection de ceux qui sont immunogènes ;
(vi) pour chaque épitope sélectionné dans l'étape (v), test pour déterminer si les CTL engendrés contre l'épitope optimisé reconnaissent également son épitope natif correspondant, et sélection de ceux pour lesquels le test est positif.

2. Peptide isolé consistant en un épitope cryptique restreint à HLA-A*2402, ledit peptide isolé étant choisi dans le groupe consistant en PYGVLLKTH (SEQ ID N° 1) ; PYMRQFVAH (SEQ ID N° 2) ; PYGKGWDLM (SEQ ID N° 4) ; TYLVQVQAL (SEQ ID N° 5) et PYWELSNHE (SEQ ID N° 6).

3. Peptide isolé consistant en un épitope restreint à HLA-A*2402 immunogène, ledit peptide isolé étant choisi dans le groupe consistant en KYGVLLKTL (SEQ ID N° 11) ; RYMRQFVAL (SEQ ID N° 12) ; RYVSRLLGI (SEQ ID N° 13) ; RYGKGWDLL (SEQ ID N° 14) ; RYLVQVQAL (SEQ ID N° 15) ; et RYWELSNHL (SEQ ID N° 16).

4. Polypeptide chimère comprenant plusieurs épitopes différents ou une répétition d'un épitope unique, **caractérisé en ce qu'**il comprend un, deux, trois ou plus de trois épitopes cryptiques restreint à HLA-A*2402 choisis dans le groupe consistant en PYGVLLKTH (SEQ ID N° 1) ; PYMRQFVAH (SEQ ID N° 2) ; PYGKGWDLM (SEQ ID N° 4) ; TYLVQVQAL (SEQ ID N° 5) et PYWELSNHE (SEQ ID N° 6).

5. Polypeptide chimère comprenant plusieurs épitopes différents, **caractérisé en ce qu'**il comprend deux, trois ou plus de trois épitopes cryptiques restreint à HLA-A*2402 choisis dans le groupe consistant en PYGVLLKTH (SEQ ID N° 1) ; PYMRQFVAH (SEQ ID N° 2) ; PYVSRLLGI (SEQ ID N° 3) ; PYGKGWDLM (SEQ ID N° 4) ; TYLVQVQAL (SEQ ID N° 5) et PYWELSNHE (SEQ ID N° 6).

6. Polypeptide chimère comprenant plusieurs épitopes différents ou une répétition d'un épitope unique, **caractérisé en ce qu'**il comprend un, deux, trois ou plus de trois épitopes immunogènes restreint à HLA-A*2402 suivant la revendication 3.

7. Molécule d'acide nucléique isolée conçue pour provoquer l'expression d'un épitope cryptique restreint à HLA-A*2402 suivant la revendication 2, d'un épitope immunogène suivant la revendication 3 ou d'un polypeptide chimère suivant l'une quelconque des revendications 4 à 6.

8. Composition pharmaceutique comprenant au moins, comme principe actif, un épitope cryptique restreint à HLA-A*2402 suivant la revendication 2, ou un polypeptide épitopique immunogène suivant la revendication 3, ou un polypeptide chimère suivant l'une quelconque des revendications 4 à 6 ou un acide nucléique suivant la revendication 7.

9. Composition pharmaceutique suivant la revendication 8, qui est un vaccin.

10. Kit comprenant, dans des récipients séparés :

(i) un premier peptide comprenant une séquence d'un épitope cryptique restreint à HLA-A*2402, et
(ii) un second peptide comprenant une séquence consistant en un épitope immunogène restreint à HLA-A*2402,

dans lequel lesdits premier et second peptides sont choisis dans le groupe consistant en les paires de peptides suivantes :

- PYGVLLKTH (SEQ ID N° 1) et KYGVLLKTL (SEQ ID N° 11) ;

- PYMRQFVAH (SEQ ID N° 2) et RYMRQFVAL (SEQ ID N° 12) ;
- PYVSRLLGI (SEQ ID N° 3) et RYVSRLLGI (SEQ ID N° 13) ;
- PYGKGWDLM (SEQ ID N° 4) et RYGKGWDLL (SEQ ID N° 14) ;
- TYLVQVQAL (SEQ ID N° 5) et RYLVQVQAL (SEQ ID N° 15) ; et
- PYWELSNHE (SEQ ID N° 6) et RYWELSNHL (SEQ ID N° 16).

11. Kit comprenant, dans des récipients séparés :

(i) un premier peptide comprenant une séquence d'un épitope cryptique restreint à HLA-A*2402, et
(ii) un second peptide comprenant une séquence consistant en un épitope immunogène restreint à HLA-A*2402 dérivé de l'épitope cryptique restreint à HLA-A*2402 indiqué en (i) par un procédé suivant la revendication 1,

dans lequel ledit premier peptide est un polypeptide chimère suivant la revendication 4 ou la revendication 5, et ledit second peptide est un polypeptide chimère suivant la revendication 6, au moins un épitope immunogène présent dans le second peptide étant apparenté à au moins un épitope cryptique restreint à HLA-A*2402 présent dans le premier peptide.

12. Kit comprenant, dans des récipients séparés :

(i) un premier peptide comprenant une séquence d'un épitope cryptique restreint à HLA-A*2402 choisi dans le groupe consistant en PYGVLLKTH (SEQ ID N° 1) ; PYMRQFVAH (SEQ ID N° 2) ; PYVSRLLGI (SEQ ID N° 3) ; PYGKGWDLM (SEQ ID N° 4) ; TYLVQVQAL (SEQ ID N° 5) et PYWELSNHE (SEQ ID N° 6), et
(ii) un second peptide comprenant une séquence consistant en un épitope immunogène restreint à HLA-A*2402 dérivé de l'épitope cryptique restreint à HLA-A*2402 indiqué en (i) par un procédé suivant la revendication 1, ledit second peptide étant un polypeptide chimère suivant la revendication 6.

13. Peptide isolé suivant la revendication 2 ou la revendication 3, ou polypeptide chimère suivant l'une quelconque des revendications 4 à 6, ou acide nucléique suivant la revendication 7, pour une utilisation comme médicament pour une immunothérapie préventive ou curative.

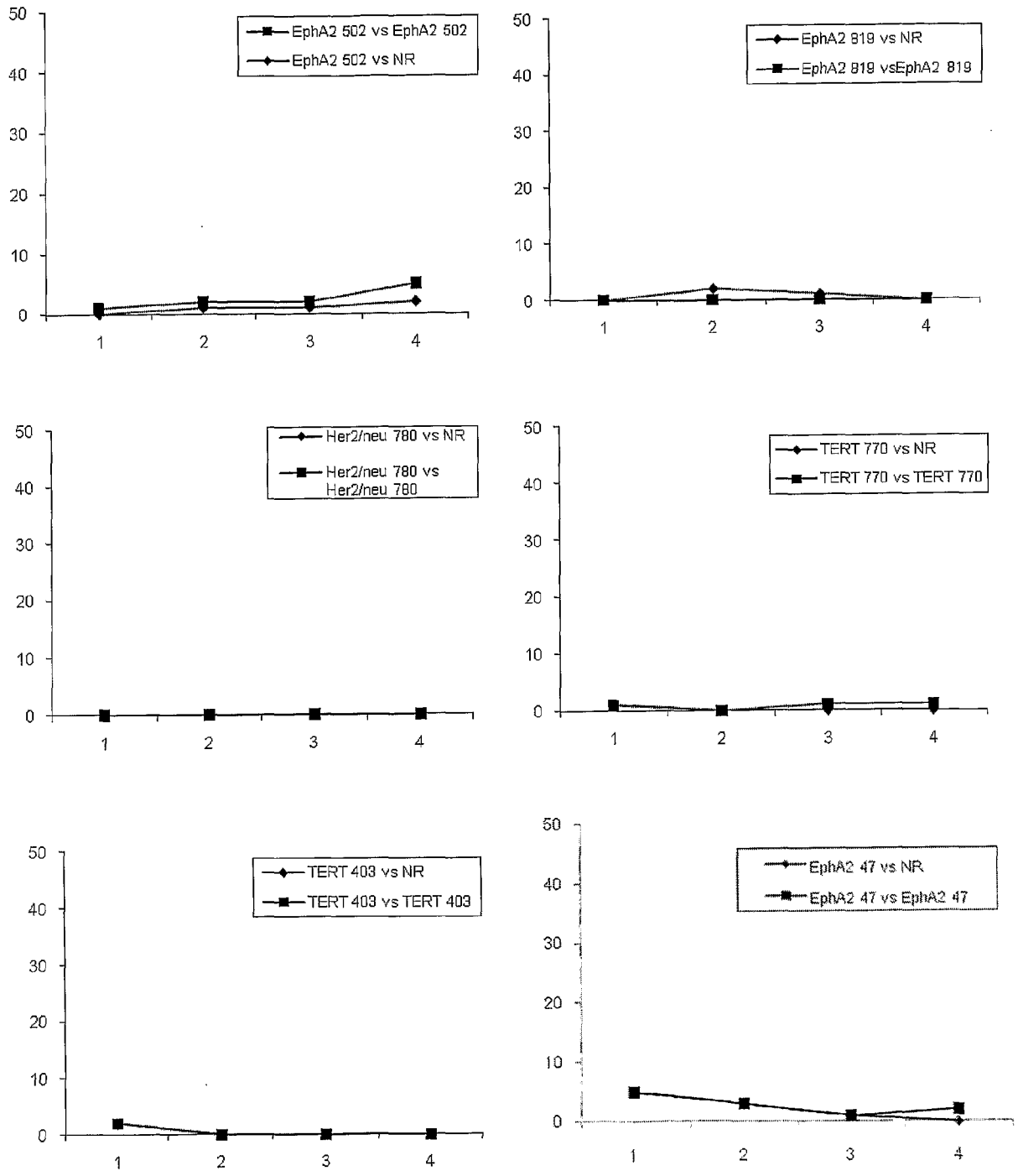
EphA2 502 vs EphA2 502
EphA2 502 vs NR
EphA2 819 vs NR
EphA2 819 vsEphA2 819
Her2/neu 780 vs NR
Her2/neu 780 vs Her2/neu 780
TERT 770 vs NR
TERT 770 vs TERT 770
TERT 403 vs NR
TERT 403 vs TERT 403
EphA2 47 vs NR
EphA2 47 vs EphA2 47

Figure 1

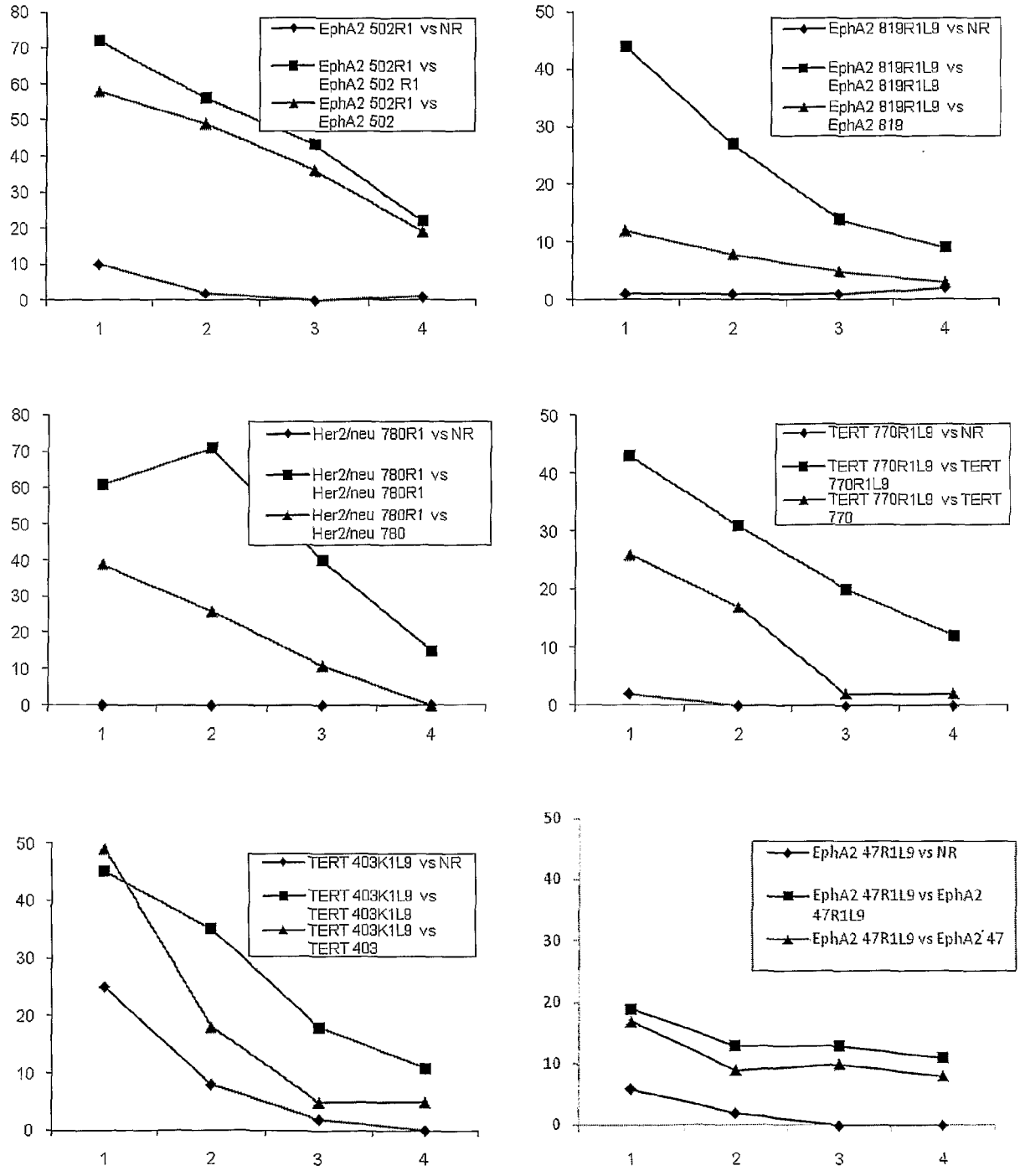
EphA2 502R1 vs NR
EphA2 502R1 vs EphA2 502 R1
EphA2 502R1 vs EphA2 502
EphA2 819R1L9 vs NR
EphA2 819R1L9 vs EphA2 819R1L9
EphA2 819R1L9 vs EphA2 819
Her2/neu 780R1 vs NR
Her2/neu 780R1 vs Her2/neu 780R1
Her2/neu 780R1 vs Her2/neu 780
TERT 770R1L9 vs NR
TERT 770R1L9 vs TERT 770R1L9
TERT 770R1L9 vs TERT 770
TERT 403K1L9 vs NR
TERT 403K1L9 vs TERT 403K1L9
TERT 403K1L9 vs TERT 403
EphA2 47R1L9 vs NR
EphA2 47R1L9 vs EphA2 47R1L9
EphA2 47R1L9 vs EphA2 47

Figure 2

REFERENCES CITED IN THE DESCRIPTION

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KRIPTIKUS HLA-A24 EPITÓPOK AZONOSÍTÁSA, ÉS OPTIMALIZÁLÁSA ÉS FELHASZNÁLÁSA IMMUNTERÁPIA CÉLJÁRA

Igénypontok

1. Egy olyan HLA-A*2402-korlátozott epitóp előállítására szolgáló módszer, amely egy antigén HLA-A*2402-korlátozott kriptikus epitópjával szemben immunválaszt képes kiváltani, és a következő lépéseket foglalja magában:

   (i) az említett antigénben egy olyan, 9-11 aminosavból álló peptid kiválasztása, amely tirozinnal rendelkezik a 2. pozícióban, valamint egy vagy több kedvezőtlen aminosav a másodlagos horgonypozíció(k)ban, a következő csoportból kiválasztva: egy prolin az 1. pozícióban, és egy glutaminsav, egy aszparaginsav, egy glicin, egy hisztidin, egy prolin, egy glutamin, egy arginin és egy lizin a C-terminális pozícióban.
   (ii) az (i) lépésben kiválasztott peptid immunogenitásának tesztelése HLA-A*2402 transzgenikus egerekben;
   (iii) az említett peptid kiválasztása, ha nem indukált specifikus immunválaszt a HLA-A*2402 transzgenikus egerekben
   (iv) a (iii) lépésben kiválasztott minden egyes natív epitóphoz egy optimalizált epitóp kiválasztása az immunogenitásának növelésével, úgy, hogy az említett epitóp N-terminális maradékát egy argininnel vagy egy lizinnel, és/vagy az említett epitóp C-terminális maradékát egy leucinnal vagy egy izoleucinnal vagy egy fenilalaninnal, előnyösen egy leucinnal szubsztituálják;
   (v) a (iv) lépésben kapott mindegyik optimalizált epitóp immungenitásának tesztelése HLA-A*2402 transzgenikus egerekben, és azok kiválasztása, amelyek immunogének;
   (vi) az (v) lépésben kiválasztott minden egyes epitóp esetében annak tesztelése, hogy az optimalizált epitópokkal szemben generált CTL-ek felismerik a rokon natív epitópját is, valamint azok kiválasztása, amelyekre nézve a teszt pozitív.

2. Egy kriptikus HLA-A*2402-korlátozott epitópból álló izolált peptid, ahol az említett izolált peptid a következő csoportból kerül kiválasztásra: PYGVLLKTH (SEQ ID NO (szekvencia-azonosító szám): 1); PYMRQFVAH (SEQ ID NO (szekvencia-azonosító szám): 2); PYGKGWDLM (SEQ ID NO(szekvencia-azonosító szám): 4);

TYLVQVQAL (SEQ ID NO (szekvencia-azonosító szám): 5) és PYWELSNHE (SEQ ID NO (szekvencia-azonosító szám): 6).

3. Egy immunogén HLA-A*2402-korlátozott epitópból álló izolált peptid, ahol az említett izolált peptid a következő csoportból kerül kiválasztásra: KYGVLLKTL (SEQ ID No (szekvencia-azonosító szám): 11); RYMRQFVAL (SEQ ID No (szekvencia-azonosító szám): 12); RYVSRLLGI (SEQ ID No (szekvencia-azonosító szám): 13); RYGKGWDLL (SEQ ID No (szekvencia-azonosító szám): 14); RYLVQVQAL (SEQ ID No (szekvencia-azonosító szám): 15) és RYWELSNHL (SEQ ID No (szekvencia-azonosító szám): 16).

4. Több különböző epitópból vagy egyetlen epitóp ismétlődéséből álló kimerikus polipeptid, **azzal jellemezve, hogy** egy, kettő, három vagy több HLA-A*2402-korlátozott kriptikus epitópból áll, a következő csoportból kiválasztva: PYGVLLKTH (SEQ ID NO (szekvencia-azonosító szám): 1); PYMRQFVAH (SEQ ID NO (szekvencia-azonosító szám): 2); PYGKGWDLM (SEQ ID NO(szekvencia-azonosító szám): 4); TYLVQVQAL (SEQ ID NO (szekvencia-azonosító szám): 5) és PYWELSNHE (SEQ ID NO (szekvencia-azonosító szám): 6).

5. Több különböző epitópból álló kimerikus polipeptid, **azzal jellemezve, hogy** kettő, három vagy több HLA-A*2402-korlátozott kriptikus epitópból áll, a következő csoportból kiválasztva: PYGVLLKTH (SEQ ID NO (szekvencia-azonosító szám): 1); PYMRQFVAH (SEQ ID NO (szekvencia-azonosító szám): 2); PYVSRLLGI (SEQ ID NO (szekvencia-azonosító szám): 3); PYGKGWDLM (SEQ ID NO(szekvencia-azonosító szám): 4); TYLVQVQAL (SEQ ID NO (szekvencia-azonosító szám): 5) és PYWELSNHE (SEQ ID NO (szekvencia-azonosító szám): 6).

6. Több különböző epitópból vagy egyetlen epitóp ismétlődéséből álló kimerikus polipeptid, **azzal jellemezve, hogy** egy, kettő, három vagy több immunogén HLA-A*2402-korlátozott kriptikus epitópból áll a 3. igénypont szerint.

7. Egy izolált nukleinsavmolekula, amelynek rendeltetése, hogy egy kriptikus HLA-A*2402-korlátozott epitóp expresszióját okozza a 2. igénypont, egy immunogén

epitópét a 3. igénypont, vagy egy kimerikus polipeptidét a 4-6. igénypontok bármelyike szerint.

8. Olyan gyógyszerészeti készítmény, amely, aktív hatóanyagként legalább egy HLA-A*2402-korlátozott kriptikus epitópot tartalmaz a 2. igénypont, vagy egy immunogén epitóp polipeptidet a 3. igénypont, vagy egy kimerikus polipeptidet a 4-6. igénypontok bármelyike, vagy egy nukleinsavat a 7. igénypont szerint.

9. Az 8. igénypont szerint gyógyszerészeti készítmény, amely egy vakcina.

10. Egy részegységekből álló készlet, amely a következőket tartalmazza, külön edényzetben:

(i) egy első peptid, amely egy HLA-A*2402-korlátozott kriptikus epitóp szekvenciáját tartalmazza, és
(ii) egy második peptid, amely egy HLA-A*2402-korlátozott immunogén epitóp szekvenciáját tartalmazza,

ahol az említett első és második peptidek a következő peptidpárokat tartalmazó csoportból kerülnek kiválasztásra:

- PYGVLLKTH (SEQ ID NO (szekvencia-azonosító szám): 1) és KYGVLLKTL (SEQ ID No (szekvencia-azonosító szám): 11);
- PYMRQFVAH (SEQ ID NO (szekvencia-azonosító szám): 2) és RYMRQFVAL (SEQ ID No (szekvencia-azonosító szám): 12);
- PYVSRLLGI (SEQ ID NO (szekvencia-azonosító szám): 3) és RYVSRLLGI (SEQ ID No (szekvencia-azonosító szám): 13);
- PYGKGWDLM (SEQ ID NO (szekvencia-azonosító szám): 4) and RYGKGWDLL (SEQ ID No (szekvencia-azonosító szám): 14);
- TYLVQVQAL (SEQ ID NO (szekvencia-azonosító szám): 5) és RYLVQVQAL (SEQ ID No (szekvencia-azonosító szám): 15); és
- PYWELSNHE (SEQ ID NO (szekvencia-azonosító szám): 6) és RYWELSNHL (SEQ ID No (szekvencia-azonosító szám): 16).

11. Egy részegységekből álló készlet, amely a következőket tartalmazza, külön edényzetben:

(i) egy első peptid, amely egy HLA-A*2402-korlátozott kriptikus epitóp szekvenciáját tartalmazza, és
(ii) egy második peptid amely egy HLA-A*2402-korlátozott immunogén epitópból álló szekvenciát tartalmaz, amely utóbbit az (i) bekezdésben említett HLA-A*2402-korlátozott kriptikus epitópból az 1. igénypont szerinti módszerrel származtattak.

ahol az említett első peptid a 4. vagy 5. igénypont szerinti kimerikus polipeptid és az említett második peptid a 6. igénypont szerinti kimerikus polipeptid, ahol a második peptidben lévő legalább egy immunogén epitóp rokon az első peptidben lévő legalább egy HLA-A*2402-korlátozott kriptikus epitóppal.

12. Egy részegységekből álló készlet, amely a következőket tartalmazza, külön edényzetben:

(i) egy első peptid, amely egy HLA-A*2402-korlátozott kriptikus epitóp szekvenciáját tartalmazza, amely epitóp a következő csoportból került kiválasztásra: PYGVLLKTH (SEQ ID NO (szekvencia-azonosító szám): 1); PYMRQFVAH (SEQ ID NO (szekvencia-azonosító szám): 2); PYVSRLLGI (SEQ ID NO (szekvencia-azonosító szám): 3); PYGKGWDLM (SEQ ID NO(szekvencia-azonosító szám): 4); TYLVQVQAL (SEQ ID NO (szekvencia-azonosító szám): 5) és PYWELSNHE (SEQ ID NO (szekvencia-azonosító szám): 6), és
(ii) egy második peptid amely egy HLA-A*2402-korlátozott immunogén epitópból álló szekvenciát tartalmaz, amely utóbbit az (i) bekezdésben említett HLA-A*2402-korlátozott kriptikus epitópból az 1. igénypont szerinti módszerrel származtattak, ahol az említett második peptid a 6. igénypont szerinti kimerikus polipeptid.

13. A 2. vagy 3. igénypont szerinti izolált peptid, vagy a 4-6. igénypontok bármelyike szerinti kimerikus polipeptid, vagy a 7. igénypont szerinti nukleinsav, amely gyógyszerként használható megelőző vagy gyógyító immunterápiában.