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(54) Title: IDENTIFICATION, OPTIMIZATION AND USE OF CRYPTIC HLA-B7 EPITOPES FOR IMMUNOTHERAPY

(57) Abstract: The present invention relates to the field of peptide immunotherapy. In particular, the invention pertains to a method for identifying a HLA-B*0702-restricted cryptic epitope in an antigen, and to a method for increasing its immunogenicity. The invention also provides novel methods and materials for efficiently treating patients having an HLA- B*0702 phenotype.

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IDENTIFICATION, OPTIMIZATION AND USE OF CRYPTIC HLA-B7 EPITOPES FOR IMMUNOTHERAPY

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- B*0702 phenotype.

Peptide vaccination or immunotherapy is a therapeutic approach which is currently the subject of a great deal of interest 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.

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

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.

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 that are weakly presented are called subdominant/cryptic. Immunodominant peptides exhibit a high affinity for the MHC I and are immunogenic while subdominant/cryptic peptides exhibit a low affinity for MHC I and are non-immunogenic.

Immunodominant peptides have widely been targeted by tumor vaccines in preclinical and clinical studies with disappointing results (Bowne et al., 1999; Colella et al., 2000; Gross et al., 2004; Hawkins et al., 2000; Naftzger et al., 1996; Overwijk et al., 1998; Vierboom et al., 1997; Weber et al., 1998).

Tumor antigens are frequently self proteins over-expressed by tumors and expressed at lower levels by normal cells and tissues. 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;

Hernandez et al., 2000; Theobald et al., 1997) thus explaining the incapacity of these peptides to induce a tumor immunity.

Subdominant/cryptic peptides are much less involved in self tolerance process (Anderton et al., 2002; Boisségault et al., 2000; Cibotti et al., 1992; Friedman et al., 2004; Gross et al., 2004; Moudgil et al., 1999; Overwijk et al., 2003; Sinha et al., 2004) and can therefore induce an efficient tumor immunity providing their immunogenicity is enhanced (Disis et al., 2002; Dyall et al., 1998; Engelhorn et al., 2006; Gross et al., 2004; Grossmann et al., 2001; Lally et al., 2001; Moudgil and Sercarz, 1994a; Moudgil and Sercarz, 1994b; Palomba et al., 2005).

The usual strategy for enhancing the immunogenicity of subdominant/cryptic peptides, that because of their low MHC I affinity are non-immunogenic, 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 the peptides having the primary anchor motifs, of a great variability in the binding affinity.

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

Many teams have succeeded in further enhancing immunogenicity of already immunogenic peptides by increasing their affinity for HLA-A*0201 (Bakker et al., 1997; Parkhurst et al., 1996; Sarobe et al., 1998; 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 et al., 2000).

HLA-B*0702 is a frequently expressed molecule (25% of the population). Identification and optimization of HLA-B*0702 restricted tumor cryptic peptides should therefore be necessary in order to develop efficient cancer vaccines for HLA-B*0702 expressing patients.

As described in the experimental part below, the inventors have now found a general strategy to identify, in an antigen, subdominant/cryptic peptides presented by HLA-B*0702 molecule, and to optimize their immunogenicity.

Hence, a first aspect of the present invention is a method for identifying a HLA-B*0702-restricted cryptic epitope in an antigen, comprising a step of selecting, in said antigen, a peptide of 8 to 12 amino acids having a proline (P) in position 2, and an

arginine (R) in position 3, with the proviso that the peptide does not have, simultaneously, an alanine (A) in position 1 and a leucine (L) in C-terminal position.

In what follows, the phrases "HLA-B*0702-restricted cryptic epitope", or "native peptide" will be used to designate a peptide selected according to the above method, *i.e.*, a peptide having the sequence $X_1PRX_2X_3X_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID No: 119), wherein X_1 to X_5 and X_{10} are any amino acid, X_6 to X_9 are any amino acid or none, and $X_{10} \neq L$ if $X_1 = A$. In the present text, the term "peptide" designates not only molecules in which amino acid residues 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. For example, retro-inverso peptidomimetics, in which the peptide bond is reversed, can be advantageously used. These may be made using methods known in the art, for example such as those described in Meziere et al., 1997.

According to a preferred embodiment of the invention, the selected peptide has 9 to 11 amino acids, more preferably 9 or 10 amino acids.

A second aspect of the present invention is a method for increasing the immunogenicity of a HLA-B*0702-restricted cryptic epitope, comprising a step of substituting the N-terminal residue of said epitope with an alanine, and/or substituting the C-terminal residue of said epitope with a leucine. 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-B*0702-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.

The immunogenicity of a HLA-B*0702-restricted cryptic epitope in which the three first residues are APR can be increased only by replacing its last amino-acid by an L (or by adding a leucine at its C-terminus, provided it is not longer than 11 amino acids). When the sequence of the selected HLA-B*0702-restricted cryptic epitope is $X_1PRX_2X_3X_4X_5X_6X_7X_8X_9L$ (SEQ ID No: 120), wherein X_1 is any amino acid but A, X_2 to X_5 are any amino acid, and X_6 to X_9 are any amino acid or none, the substitution of X_1 by A is sufficient to increase its immunogenicity. In what follows, the expression "optimized peptide" will designate an immunogenic peptide derived from a HLA-B*0702-restricted cryptic epitope by the above methods, and having the general sequence $APRX_1X_2X_3X_4X_5X_6X_7X_8L$ (SEQ ID No: 121), wherein X_1 to X_4 are any amino acid, and X_5 to X_8 are any amino acid or none.

The inventors have identified a number of HLA-B*0702-restricted cryptic epitopes, which are disclosed in Table 1 below. Accordingly, another aspect of the present invention is a cryptic HLA-B*0702-restricted epitope, selected amongst the peptides of SEQ ID Nos: 1 to 68, disclosed in Table 1.

Antigen	Peptide	SEQ ID No:
TRAG-3	FPRQLGREK	1
	RPRASSPLS	2
CEA	SPRLQLSNG	3
	SPRLQLSND	4
Tyrosinase	TPRLPSSAD	5
	FPRACVSSK	6
P53	APRMPEAA	7
	APRMPEAAP	8
NY-ESO-1	APRGPHGGA	9
	GPRGAGAAR	10
MelanA/MART-1	MPREDAHFI	11
PRAME	RPRRWKLQV	12
	LPRELFPPL	13
	SPRRLVELA	14
PSMA	MPRISKLGS	15
	RPRRTILFA	16
	RPRWLCAGA	17
TRP-2	FPRVCMTVD	18
	CPRLGAESA	19
	WPRKFFHRT	20
Gp100	LPRIFCSCP	21
	VPRNQDWLG	22
EphA3	PPRQFAAVS	23
	PPRMYCSTE	24
	SPRNVISNI	25
	LPRQFGLTN	26
	VPRNSAQKI	27
EphA1	DPRVTLRLP	28
	APRNLSFSA	29
	EPRQLELTW	30
	RPRSPGANL	31
	EPRVLLTEL	32
	SPRPSGAPR	33
	APRMHCSPD	34
EphA2	EPRMHCAVDG	35
	DPRVSIRLPS	36
	EPRMHCAVD	37
	DPRVSIRLP	38
G250	SPRAAEPVQ	39
	SPREEDPPG	40
	WPRVSPACA	41
	LPRMQEDSP	42
PAP	CPRFQELES	43
Trp-1	FPRQCATVE	44
HER-2/neu	LPREYVNAR	45
	RPRFRELVSE	46
	APRSPLAPS	47
	SPREGPLPAA	48
	RPRFRELVVS	49
	SPREGPLPA	50
MAGE-A	GPRALAETS	51
	GPRALIETS	52
	GPRALVETS	53

Table 1: Selected cryptic HLA-B*0702 restricted peptides

Antigen	Peptide	SEQ ID No:
TERT	GPRRRLGCE	54
	RPRRGAAPEP	55
	PPRPWDTPCP	56
	TPRRLPRLP	57
	TPRRLPRLPQ	58
	LPRLPQRYW	59
	LPRLPQRYWQ	60
	DPRRLVQLL	61
	APRCRAVRS	62
	GPRRRLGCE	63
	RPRRGAAPE	64
	PPRPWDTPC	65
Hsp70	APRGVPQIEV	66
STEAP	KPRRNLEEDD	67
	KPRRNLEED	68

Table 1 (following): Selected cryptic HLA-B*0702 restricted peptides

The present invention also pertains to optimized peptides derived from the cryptic peptides of SEQ ID Nos: 1 to 68, by a method according to the invention. Preferred examples of optimized peptides are APRMHCAVDL (SEQ ID No: 69), APRVSIRLPL (SEQ ID No: 70), APREYVNAL (SEQ ID No: 71), APRSPLAPL (SEQ ID No: 72), APRALVETL (SEQ ID No: 73), APRRRLGCEL (SEQ ID No: 74), RPRRGAAPEL (SEQ ID No: 75), APRPWDTPCL (SEQ ID No: 76), APRRLPRLPL (SEQ ID No: 77), and APRRLVQLL (SEQ ID No: 78).

Other immunogenic HLA-B*0702-restricted epitopes obtained according to the invention are those derived from a cryptic HLA-B*0702-restricted epitope selected amongst SEQ ID NOS: 8, 9, 29, 34, 62 and 66, by substitution of their C-terminal amino-acid with a leucine, as well as those derived from a cryptic HLA-B*0702-restricted epitope selected amongst SEQ ID NOS: 13, 31 and 32, by substitution of their N-terminal amino-acid with an alanine.

Another immunogenic HLA-B*0702-restricted epitope according to the invention, and derived from the cryptic HLA-B*0702-restricted epitope SEQ ID NO 7, is obtained by adding a leucine at its terminus.

Still other immunogenic HLA-B*0702-restricted epitopes according to the invention are obtained by substituting the N-terminal amino-acid with an alanine and the C-terminal amino-acid with a leucine, starting from a cryptic HLA-B*0702-restricted epitope selected amongst the peptides of SEQ ID NOS: 1 to 6, 10 to 12, 14 to 18, 20 to 28, 30, 33, 37 to 44, 46, 48 to 52, 57, 59, 60, 63 to 65, 67 and 68.

The invention also concerns a chimeric polypeptide, comprising two, three or more HLA-B*0702-restricted cryptic epitopes or two, three or more immunogenic HLA-B*0702-restricted epitopes as described above. In such a chimeric polypeptide, the epitopes can be different from each other, or the same epitope can be repeated several times. 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.

Another object of the present invention is an isolated nucleic acid molecule designed to cause the expression of a cryptic HLA-B*0702-restricted epitope or an immunogenic epitope or a chimeric polypeptide as above-described. 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 encoding region for 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. Exemplary are adenovirus or vaccinia virus vectors and vectors of the herpes family, especially in a non-replicative form.

Another embodiment of the present invention is a pharmaceutical composition comprising at least, as an active principle, a HLA-B*0702-restricted cryptic epitope as above-described, or an immunogenic epitope polypeptide derived therefrom 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).

In particular, a HLA-B*0702-restricted cryptic epitope, or an immunogenic epitope polypeptide derived therefrom, or a chimeric polypeptide carrying several such immunogenic or cryptic epitopes, or a nucleic acid encoding any of these, included or not in a vector, can be used for the preparation of a composition for preventive or curative immunotherapy, especially, for antiviral or anti-cancer immunotherapy.

In a particular embodiment, a pharmaceutical composition according to the invention is a vaccine. In this latter case, the compositions of this invention can be

combined with an adjuvant to potentiate the immune response. Classic adjuvants include oil emulsions, like Incomplete Freund's Adjuvant, 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.

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 (in the form of promotional material or a package insert) regarding the use of the composition or combination in eliciting an immune response and/or the treatment of cancer.

In a previous patent application (PCT/EP2006/005325), 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 PCT/EP2006/005325 demonstrate that injection of a native peptide corresponding to a sub-dominant/cryptic epitope, following vaccination with its cognate optimized peptide, can maintain the immune response initiated by said optimized peptide.

According to the invention, a HLA-B*0702-restricted cryptic epitope 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 HLA-B*0702-restricted epitope sequence derived from a HLA-B*0702-restricted cryptic epitope can also be used, for the preparation of a medicinal composition for initiating a CTL immune response against said HLA-B*0702-restricted cryptic epitope. The present invention also encompasses a method for vaccinating a patient against a tumoral or viral antigen, wherein said method comprises a first step of vaccination with an optimized peptide cognate to a native HLA-B*0702-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 two, three or more optimized or cryptic peptides as above-described, instead of single-epitope peptides.

The invention also pertains to a kit of parts comprising, in separate formulations, a first peptide having the sequence of a HLA-B*0702-restricted cryptic epitope, and a second peptide corresponding to its cognate HLA-B*0702-restricted immunogenic epitope. Examples of peptides which can be part of a kit according to the invention are the peptides of SEQ ID NOs: 1 to 68, 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.

Other kits of parts according to the invention comprise at least a chimeric polypeptide. Several variants of such kits are contemplated: in a first embodiment, the kit comprises, in separate formulations, a first chimeric polypeptide comprising two, three or more HLA-B*0702-restricted cryptic epitopes, and a second
5 chimeric polypeptide corresponding to its cognate HLA-B*0702-restricted immunogenic chimeric polypeptide (which means that it comprises optimized HLA-B*0702-restricted immunogenic epitopes cognate to the cryptic epitopes comprised in the first chimeric polypeptide). In a second embodiment, the kit comprises a first chimeric polypeptide comprising two, three or more HLA-B*0702-restricted cryptic epitopes and, in one or
10 several other separate formulations, peptides corresponding to the optimized HLA-B*0702-restricted immunogenic epitopes cognate to the cryptic epitopes comprised in the first chimeric polypeptide. In a third embodiment, the kit comprises two, three or more peptides corresponding to distinct HLA-B*0702-restricted cryptic epitopes, wherein said peptides are either mixed in one single formulation, or separated in several formulations
15 and, in a separate formulation, a chimeric polypeptide comprising the optimized HLA-B*0702-restricted immunogenic epitopes cognate to said cryptic peptides.

In the following description of the kits according to the invention, mention will be made only of the peptides (native or optimized), it being understood that chimeric polypeptides (comprising native cryptic epitopes or optimized epitopes) can be
20 enclosed in the kits instead of single-epitope peptides.

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
25 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 1.5 to 6 months. Therefore, complementary kits, comprising at least 2
30 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.

Each dose comprises between 0.5 and 10 mg of peptide, preferably from
35 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, used as an adjuvant. The skilled artisan can choose any other adjuvant(s) in place of (or in addition to) Montanide. 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.

5 The invention is further illustrated by the following figures and examples.

LEGENDS OF FIGURES

Figure 1: Immunogenicity of HLA-B*0702 restricted peptides. CTL were tested against RMA-B7 targets loaded with peptide as indicated.

10 **Figure 2: Immunogenicity of optimized HLA-B*0702 cryptic peptides.** CTL were tested against RMA-B7 targets loaded with peptide as indicated.

15 **Figure 3: Recognition of endogenous TERT by TERT₄₄₄ specific murine CTL.** A) CTL were tested against RMA-B7 targets loaded with decreasing doses of TERT₄₄₄ peptide. B) CTL were tested against COS cells transfected with HLA-B*0702 and TERT as indicated.

20 **Figure 4: Recognition of endogenous TERT by TERT₄ specific murine CTL.** A) CTL were tested against RMA-B7 targets loaded with decreasing doses of TERT₄ peptide. B) CTL were tested against COS cells transfected with HLA-B*0702 and TERT as indicated.

25 **Figure 5: Induction of TERT₄ specific human CTL.** A) CTL were tested against T2-B7 targets loaded with TERT₄ (■) or an irrelevant (●) peptide. B) CTL were tested against the HLA-B*0702 positive TERT positive SK-MES-1 (■), HBL-100 (●) and the HLA-B*0702 negative TERT positive SW-480 (□), HSS (○) human tumor cell lines.

EXAMPLES

The examples have been performed using the following materials and methods:

Transgenic Mice. The HLA-B7 H-2 class-I knockout mice were previously described (Rohrlich et al., 2003).

30 **Cells.** HLA-B*0702 transfected murine RMA-B7 and human T2-B7 cells were previously described (Rohrlich et al., 2003). COS-7 and WEHI-164 clone 13 cells were provided by F. Jotereau (INSERM 463, Nantes, France). The HLA-B*0201 positive SK-MES-1 (lung cancer), HBL-100 (breast cancer), and the HLA-B*0702 negative SW-480 (colon cancer) and HSS (myeloma) cell lines were used as targets of
35 human CTL. All cell lines were grown in FCS 10% supplemented RPMI1640 culture medium.

Peptides and Plasmids. Peptides were synthesized by Epytop (Nîmes, France). HLA-B*0702 plasmid was provided by Dr. Lemonnier (Institut Pasteur, Paris,

France) (Rohrlich et al., 2003) and TERT plasmid was provided by Dr Weinberg (MIT, Boston, MA) (Meyerson et al, 1997).

Measurement of Peptide Relative Affinity to HLA-B*0702. The protocol used has been described previously (Rohrlich et al., 2003). Briefly, T2-B7 cells were incubated at 37°C for 16 hours with peptides concentrations ranging from 100 µM to 0.1 µM, and then stained with ME-1 monoclonal antibody (mAb) to quantify the surface expression of HLA-B*0702. For each peptide concentration, the HLA-B*0702 specific staining was calculated as the percentage of staining obtained with 100 µM of the reference peptide CMV₂₆₅₋₂₇₄ (R10V; RIPHERNGFTV, SEQ ID NO: 117). The relative affinity (RA) was determined as: $RA = (\text{Concentration of each peptide that induces 20 \% of HLA-B*0702-expression} / \text{Concentration of the reference peptide that induces 20 \% of HLA-B*0702 expression})$.

CTL Induction in vivo in HLA-B*0702 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-A^b restricted HBVcore₁₂₈ T helper epitope (TPPAYRPPNAPIL, SEQ ID NO: 118). After 11 days, 5×10^7 spleen cells were stimulated *in vitro* with peptide (10 µM). On day 6 of culture, the bulk responder populations were tested for specific cytotoxicity.

Peptide Processing Assay on COS-7 Transfected Cells. 2.2×10^4 simian COS-7 cells were plated in flat-bottomed 96 well plates in DMEM+10% FCS, in triplicate for each condition. Eighteen hours later, cells were transfected with 100 ng of each DNA plasmid with DEAE Dextran. After 4 hours, PBS+10% DMSO was added for 2 minutes. Transfected COS cells were incubated in DMEM+10% FCS during 40 hours and then used to stimulate murine CTL in a TNFα secretion assay.

TNFα Secretion Assay. Transfected COS-7 cells at day 4 were suspended in 50 µl of RPMI+10% FCS and used as stimulating cells. 5×10^4 murine T cells were then added in 50 µl RPMI 10% FCS and incubated for 6 hours. Each condition was tested in triplicate. 50 µl of the supernatant was collected to measure TNF-α. Standard dilutions were prepared in 50 µl with final doses of TNF-α ranging from 104 to 0 pg/ml. On both the supernatants and the standard dilutions, 3×10^4 TNF-α sensitive WEHI-164c13 cells in 50 µl were added. They were incubated for 16h at 37°C. Inhibition of cell proliferation was evaluated by the MTT colorimetric method (Espevik and Nissen-Meyer, 1986).

Generation of CTL from human PBMC. PBMC were collected by leukapheresis from healthy HLA-A*0201 volunteers. Dendritic cells (DC) were produced from adherent cells cultured for seven days (2×10^6 cells/ml) in the presence of 500 IU/ml GM-CSF (Leucomax®, Schering-Plough, Kenilworth, NJ) and 500 IU/ml IL-4 (R&D Systems, Minneapolis, MN) in complete medium (RPMI-1640 supplemented with 10% heat inactivated human AB serum, 2 µM L-Glutamine and antibiotics). On day seven, DC

were pulsed with 10 μ M peptides for 2 hrs; maturation agents Poly I:C (Sigma, Oakville, Canada) at 100 ng/ml and anti-CD40 mAb (clone G28-5, ATCC, Manassas, VA) at 2 μ g/ml were added in the culture and DCs were incubated at 37°C overnight or up to 48 hours. Mature DC were then irradiated (3500 rads). CD8⁺ cells were purified by positive selection with CD8 MicroBeads (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. 2×10^5 CD8⁺ cells + 6×10^4 CD8⁻ cells were stimulated with 2×10^4 peptide pulsed DC in complete culture medium supplemented with 1000 IU/ml IL-6 and 5 IU/ml IL-12 (R&D Systems, Minneapolis, MN) in round-bottomed 96 well plates. From day seven, cultures were weekly restimulated with peptide-loaded DC in the presence of 20 IU/ml IL-2 (Proleukin, Chiron Corp., Emeryville, CA) and 10 ng/ml IL-7 (R&D Systems, Minneapolis, MN). After the third *in vitro* restimulation, bulk cell cultures were tested for cytotoxicity.

Cytotoxic assay. Targets were labeled with 100 μ Ci of Cr⁵¹ for 60 min, plated in 96-well V-bottomed plates (3×10^3 cell/well in 100 μ L of RPMI 1640 medium) and, when necessary, pulsed with peptides (1 μ M) at 37°C for 2 hours. Effectors 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 of Peptides

Eight peptides with the HLA-B*0702 specific anchor motifs (P2 and preferentially L/V at C-terminal position), (Sidney et al., 1996), derived from the Hsp70 (Hsp70₁₁₅, Hsp70₁₃₇, Hsp70₃₉₇), TERT (TERT₄ and TERT₄₄₄) and MAGE-A (MAGE-A_{121.1}, MAGE-A_{121.2} and MAGE-A_{121.4}) antigens were tested for binding to the HLA-B*0702 molecule. Only TERT₄ bound to HLA-B*0702 with a high affinity, the remaining seven peptides were very weak or non binders (Table 2). This demonstrates that the presence of anchor motifs is not sufficient to ensure a high binding affinity to HLA-B*0702. Given their low affinity, peptides Hsp70₁₁₅, Hsp70₁₃₇, Hsp70₃₉₇, TERT₄₄₄, MAGE-A_{121.1}, MAGE-A_{121.2} and MAGE-A_{121.4} are considered cryptic peptides.

	peptide	séquence	RA	SEQ ID No
1	Hsp70 115	YP EEISSMVL	>10	79
	115A1	AP EEISSMVL	>10	80
	115A1R3	AP REISSMVL	1.6	81
2	Hsp70 137 (10)	YP VTNAVITV	>10	82
3	Hsp70 397	AP LSLGLET	>10	83
	397R3L9	AP RSLGLEL	1	84
4	TERT4	AP RCRAVRSL	0.74	85
5	TERT444	DP RRLVQLL	>10	61
	444A1	AP RRLVQLL	1.4	78
6	MAGE-A121.1	EP VTKA EML	>10	86
	121.1 A1	AP VTKA EML	>10	87
	121.1 A1R3	AP RTKA EML	2.36	88
7	MAGE-A121.2	EP FTKA EML	>10	89
8	MAGE-A121.4	EP ITKA EIL	>10	90

Table 2: HLA-B*0702 affinity of peptides

Example 2: *In vivo* Immunogenicity of Peptides

HLA-B7 transgenic mice were vaccinated with the selected peptides, and eleven days later, their spleen cells were *in vitro* stimulated with the peptide. Peptide specific CTL were detected in mice vaccinated with the high affinity TERT₄ peptide but not in mice vaccinated with the low affinity Hsp70₁₃₇, Hsp70₁₁₅, Hsp70₃₉₇, and TERT₄₄₄ peptides (Figure 1). This confirms that there is a correlation of binding affinity and immunogenicity for the HLA-B*0702 restricted peptides.

Example 3: Enhancement of Immunogenicity of the Nonimmunogenic Low Affinity Peptides

To enhance HLA-B*0702 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 should however preserve the conformation of the peptide segment that interacts with the TCR (position 4 to position 8). The interest was, therefore, focused on secondary anchor positions 1 and 3. Aliphatic aminoacids are favourable motifs at position 1 (Sidney, Southwood et al., 1996). However, peptides Hsp70₁₁₅ and Hsp70₁₃₇ that have a Y at position 1 are non binders. Moreover, the substitution of the aminoacid at position 1 by an A (present in the strong binder TERT₄) enhances the affinity of the TERT₄₄₄ but not of the Hsp70₁₁₅ and the MAGE-A_{121.1} peptides. This indicates that the presence of favourable aa at position 1 and anchor positions 2 and 9/10 cannot ensure by itself a high binding affinity of all peptides. The R has been described to be favourable at position 3 (Sidney et al., 1996) and eight out of 26 identified tumor and HIV derived immunogenic peptides have an R at position 3 (Table 3). This is also the case of the high affinity TERT₄ and TERT_{444A1} peptides.

Antigen	sequence	No	reference
NY-ESO-1	APRGVRMAN	91	http://www.cancerimmunity.org/peptidedatabase/mutation.htm
ICE	SPRWWPPTCL	92	http://www.cancerimmunity.org/peptidedatabase/tumorspecific.htm
RAGE-1	SPSSNRIRNT	93	http://www.cancerimmunity.org/peptidedatabase/differentiation.htm
RU2AS	LPRWPPPQL	94	http://www.cancerimmunity.org/peptidedatabase/overexpressed.htm
RBAF500	RPHVPESAF	95	
SSX2 fusion protein	QPRYGYDQIM	96	
HIVp17	RPGGKKRYKL	97	http://hiv-web.lanl.gov/content/immunology/tables/ctl_summary.html
HIVp24	SPRTLNAWV	98	
HIVp24	HPVHAGPIA	99	
HIVp24	PPIPVGEIY	100	
HIVp24	GPGHKARVL	101	
HIV-RT	SPIETVPVKL	102	
HIV-RT	GPKVKQWPLT	103	
HIV-RT	SPAIFQSSM	104	
HIV-RT	IPLTEEAEL	105	
HIV-RT	QPDKSESELV	106	
HIV-Vif	HPRISSEVHI	107	
HIV-Vif	KPPLPSVKKL	108	
HIV-Vif	FPRTWLHGL	109	
HIVgp160	KPCVKLTPLC	110	
HIVgp160	KVVSTQLLL	111	
HIVgp160	RPWNNTRKSI	112	
HIVgp160	IPRRIRQGL	113	
HIVnef	FPVTPQVPL	114	
HIVnef	TPQVPLRPM	115	
HIVnef	RPMTYKAAL	116	

Table 3: Tumor and HIV derived HLA-B*0702 restricted epitopes

Hsp70₁₁₅, Hsp70₃₉₇ and MAGE-A_{121.1A1R3}, were therefore modified at position 1 (X→A) and/or position 3 (X→R). For peptide Hsp70₃₉₇ an additional modification at C-terminal position (T→L) has been introduced. All modified peptides

5 (Hsp70_{115A1R3}, Hsp70_{397R3L9}, MAGE-A_{121.1A1R3}) exhibited a strong affinity for HLA-B*0702 (Table 2). High affinity Hsp70_{115A1R3}, Hsp70_{397R3L9} and TERT_{444A1} peptide variants have been tested for their *in vivo* immunogenicity in HLA-B7 mice. All peptides induced an immune response in the majority of vaccinated mice. However, for all peptides but TERT_{444A1}, generated CTL recognized the optimized peptide but not the

10 corresponding native peptide (Figure 2). This strongly suggests that substitution of the aa at position 3 by an R may change the conformation of the peptide segment that interacts with the TCR and guarantees TCR cross-recognition. Since a) all tested peptides with A1P2R3L9/10 have a high affinity and are immunogenic and b) substitution of the aa at position 3 by an R may break the cross-recognition of the native peptide, the inventors

15 selected peptides having P2R3 and they substituted aa at position 1 by an A and at C-terminal position with an L when necessary. Table 1 above shows the list of selected peptides derived from different tumor antigens. Eleven of these peptides have been modified by replacing the first aa with an A and the C-terminal aa with a L when necessary (Table 4). All peptides in their native form had a low affinity for HLA-B*0702

20 (cryptic peptides) while all modified peptides were strong binders.

Antigen	Position	Modifications	Sequence	Affinity	SEQ ID No :
EphA2	242		EPRMHCAVDG	-	35
EphA2	242	A1L9	APRMHCAVDL	++	69
EphA2	888		DPRVSIRLPS	-	36
EphA2	888	A1L9	APRVSIRLPL	++	70
Her2/neu	550		LPREYVNAR	-	45
Her2/neu	550	A1L9	APREYVNAL	+	71
Her2/neu	1070		APRSPLAPS	-	47
Her2/neu	1070	L9	APRSPLAPL	++	72
MAGE A-A	+/-273		GPRALAETS	-	51
MAGE A-I	+/-273		GPRALIETS	-	52
MAGE A-V	+/-273		GPRALVETS	-	53
MAGE A	+/-273	A1L9	APRALVETL	+	73
TERT	192		GPRRRRLGCER	-	54
TERT	192	A1L9	APRRRLGCEL	+	74
TERT	237		RPRRGAAPEP	-	55
TERT	237	A1L9	APRRGAAPEL	+	75
TERT	313		PPRPWDTPCP	-	56
TERT	313	A1L9	APRPWDTPCL	+	76
TERT	375		TPRRLPRLPQ	-	58
TERT	375	A1L9	APRRLPRLPL	+	77
TERT	444		DPRRLVQLL	-	61
TERT	444	A1	APRRLVQLL	+	78

Table 4: Affinity of optimized HLA-B*0702 restricted peptides. - = RA>10, + = 1<RA<10, ++ = RA<1

In conclusion, the inventors describe a method to optimize affinity and immunogenicity of HLA-B*0702 restricted cryptic peptides. It consists of a) selecting peptides with P2R3 and b) substitute the first residue with an A and the C-terminal residue with a L when this later substitution is necessary.

Example 4: CTL Induced by TERT_{444A1} Peptide Recognize Endogenous TERT

HLA-B7 transgenic mice were immunized with the TERT_{444A1} and eleven days later their spleen cells were *in vitro* stimulated with the native TERT₄₄₄ peptide. Generated CTL killed RMA-B7 targets loaded with decreasing concentrations of TERT_{444A1} and TERT₄₄₄ peptides. The half maximal lysis of TERT₄₄₄ loaded and TERT_{444A1} loaded targets was obtained with 5.5nM and 1nM respectively (figure 3A). CTL were then tested for their capacity to recognize COS-7 cells expressing HLA-B*0702 and endogenous TERT. Results presented in figure 3B show that CTL recognized COS-7 cells transfected with both HLA-B*0702 and TERT but not COS-7 cells transfected with either HLA-B*0702 or TERT demonstrating that TERT₄₄₄ is an HLA-B*0702 restricted cryptic epitope naturally processed from endogenous TERT.

Example 5: CTL Induced by the TERT₄ Peptide Recognize Endogenous TERT

HLA-B7 transgenic mice were immunized with the TERT₄ and eleven days later their spleen cells were *in vitro* stimulated with the peptide. Generated CTL killed RMA-B7 targets loaded with decreasing concentrations of TERT₄ peptide. The half maximal lysis of TERT₄ loaded targets was obtained with 1.5nM (Figure 4A). CTL were then tested for their capacity to recognize COS-7 cells expressing HLA-B*0702 and endogenous TERT. Results presented in figure 4B show that CTL recognized COS-7 cells transfected with both HLA-B*0702 and TERT but not COS-7 cells transfected with either HLA-B*0702 or TERT, demonstrating that TERT₄ is an HLA-B*0702 restricted epitope naturally processed from endogenous TERT.

Example 6: TERT₄ Stimulates CTL from Healthy Donors

CD8 cells from healthy donors were *in vitro* stimulated with autologous dendritic cells loaded with TERT₄ peptide. After four stimulations, CTL were tested for cytotoxicity against TERT₄ loaded T2-B7 targets. Three donors were tested and CTL were induced in two of them. Results from one responding donor are presented in Figure 5A. CTL killed T2-B7 targets presenting TERT₄ but not T2-B7 cells presenting the irrelevant Nef peptide. Interestingly, CTL killed the HLA-B*0201+TERT+ SK-MES-1 and HBL-100 but not the HLA-B*0702-TERT+ SW-480 and HSS human tumor cell lines confirming the HLA-B*0702 restricted presentation and the endogenous processing of the TERT₄ epitope (Figure 5B).

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CLAIMS

1. A method for identifying a HLA-B*0702-restricted cryptic epitope in an antigen, comprising a step of selecting, in said antigen, a peptide of 8 to 12 amino acids having a proline in position 2, and an arginine in position 3, with the proviso that the peptide does not have, simultaneously, an alanine in position 1 and a leucine in C-terminal position.
2. The method of claim 1, wherein the selected peptide has 9, 10 or 11 amino acids.
3. A method for increasing the immunogenicity of a HLA-B*0702-restricted cryptic epitope, comprising a step of substituting the N-terminal residue of said epitope with an alanine.
4. The method of claim 3, further comprising a step of substituting the C-terminal residue of said epitope with a leucine.
5. A method for increasing the immunogenicity of a HLA-B*0702-restricted cryptic epitope, wherein the three first residues of said cryptic epitope are APR, comprising a step of substituting the C-terminal residue of said epitope with a leucine.
6. A cryptic HLA-B*0702-restricted epitope, selected in the group consisting of FPRQLGREK (SEQ ID NO: 1); RPRASSPLS (SEQ ID NO: 2); SPRLQLSNG (SEQ ID NO: 3); SPRLQLSND (SEQ ID NO: 4); TPRLPSSAD (SEQ ID NO: 5); FPRACVSSK (SEQ ID NO: 6); APRMPEAA (SEQ ID NO: 7); APRMPEAAP (SEQ ID NO: 8); APRGPHGGA (SEQ ID NO: 9); GPRGAGAAR (SEQ ID NO: 10); MPREDAHFI (SEQ ID NO: 11); RPRRWKLQV (SEQ ID NO: 12); LPRELFPPPL (SEQ ID NO: 13); SPRRLVELA (SEQ ID NO: 14); MPRISKLGs (SEQ ID NO: 15); RPRRTILFA (SEQ ID NO: 16); RPRWLCAGA (SEQ ID NO: 17); RPRRWKLQV (SEQ ID NO: 18); LPRELFPPPL (SEQ ID NO: 19); SPRRLVELA (SEQ ID NO: 20); LPRIFCSCP (SEQ ID NO: 21); VPRNQDWLG (SEQ ID NO: 22); PPRQFAAVS (SEQ ID NO: 23); PPRMYCSTE (SEQ ID NO: 24); SPRNVISNI (SEQ ID NO: 25); LPRQFGLTN (SEQ ID NO: 26); VPRNSAQKI (SEQ ID NO: 27); DPRVTLRLP (SEQ ID NO: 28); APRNLSFSA (SEQ ID NO: 29); EPRQLELTW (SEQ ID NO: 30); RPRSPGANL (SEQ ID NO: 31); EPRVLLTEL (SEQ ID NO: 32); SPRPSGAPR (SEQ ID NO: 33); APRMH CSPD (SEQ ID NO: 34); EPRMHCAVDG (SEQ ID NO: 35); DPRVSIRLPS (SEQ ID NO: 36); EPRMHCAVD (SEQ ID NO: 37); DPRVSIRLP (SEQ ID NO: 38); SPRAAEPVQ (SEQ ID NO: 39); SPREEDPPG (SEQ ID NO: 40); WPRVSPACA (SEQ ID NO: 41); LPRMQEDSP (SEQ ID NO: 42); CPRFQELES (SEQ ID NO: 43); FPRQCATVE (SEQ ID NO: 44); LPREYVNAR (SEQ ID NO: 45); RPRFRELVSE (SEQ ID NO: 46); APRSPLAPS (SEQ ID NO: 47); SPREGPLPAA (SEQ ID NO: 48); RPRFRELVs (SEQ ID NO: 49); SPREGPLPA (SEQ ID NO: 50); GPRALAETS (SEQ ID NO: 51); GPRALIETS (SEQ ID NO: 52); GPRALVETS (SEQ ID NO: 53); GPRRRRLGCER (SEQ ID NO: 54); RPRRGAAPEP (SEQ ID NO: 55);

PPRPWDTPCP (SEQ ID NO: 56); TPRRLPRLP (SEQ ID NO: 57); TPRRLPRLPQ (SEQ ID NO: 58); LPRLPQRYW (SEQ ID NO: 59); LPRLPQRYWQ (SEQ ID NO: 60); DPRRLVQLL (SEQ ID NO: 61); APRCRAVRS (SEQ ID NO: 62); GPRRRLGCE (SEQ ID NO: 63); RPRRGAAPE (SEQ ID NO: 64); PPRPWDTPC (SEQ ID NO: 65);
 5 APRGVPQIEV (SEQ ID NO: 66); KPRRNLEEDD (SEQ ID NO: 67); and KPRRNLEED (SEQ ID NO: 68).

7. An immunogenic HLA-B*0702-restricted epitope, which is derived from a cryptic HLA-B*0702-restricted epitope selected in the group consisting of APRMPEAAP (SEQ ID NO: 8); APRGPHGGA (SEQ ID NO: 9); APRNLSFSA (SEQ ID NO: 29); APRMHCSPP (SEQ ID NO: 34); APRSPLAPS (SEQ ID NO: 47);
 10 APRCRAVRS (SEQ ID NO: 62); and APRGVPQIEV (SEQ ID NO: 66), by substituting its C-terminal amino-acid with a leucine.

8. An immunogenic HLA-B*0702-restricted epitope, which is derived from a cryptic HLA-B*0702-restricted epitope APRMPEAA (SEQ ID NO: 7), by adding a
 15 leucine at its C-terminus

9. An immunogenic HLA-B*0702-restricted epitope, which is derived from a cryptic HLA-B*0702-restricted epitope selected in the group consisting of LPRELFPP (SEQ ID NO: 13); RPRSPGANL (SEQ ID NO: 31); EPRVLLTEL (SEQ ID NO: 32); AND DPRRLVQLL (SEQ ID NO: 61), by substituting its N-terminal amino-
 20 acid with an alanine.

10. An immunogenic HLA-B*0702-restricted epitope, which is derived from a cryptic HLA-B*0702-restricted epitope selected in the group consisting of FPRQLGREK (SEQ ID NO: 1); RPRASSPLS (SEQ ID NO: 2); SPRLQLSNG (SEQ ID NO: 3); SPRLQLSND (SEQ ID NO: 4); TPRLPSSAD (SEQ ID NO: 5); FPRACVSSK
 25 (SEQ ID NO: 6); GPRGAGAAR (SEQ ID NO: 10); MPREDAHFI (SEQ ID NO: 11); RPRRWKLQV (SEQ ID NO: 12); SPRLVELA (SEQ ID NO: 14); MPRISKLG (SEQ ID NO: 15); RPRRTILFA (SEQ ID NO: 16); RPRWLCAGA (SEQ ID NO: 17); RPRRWKLQV (SEQ ID NO: 18); CPRLGAESA (SEQ ID NO: 19); SPRLVELA (SEQ ID NO: 20); LPRIFCSCP (SEQ ID NO: 21); VPRNQDWLG (SEQ ID NO: 22);
 30 PPRQFAAVS (SEQ ID NO: 23); PPRMYCSTE (SEQ ID NO: 24); SPRNVISNI (SEQ ID NO: 25); LPRQFGLTN (SEQ ID NO: 26); VPRNSAQKI (SEQ ID NO: 27); DPRVTLRPL (SEQ ID NO: 28); EPRQLELTW (SEQ ID NO: 30); SPRPSGAPR (SEQ ID NO: 33); EPRMHCAVDG (SEQ ID NO: 35); DPRVSIRLPS (SEQ ID NO: 36); EPRMHCAVD (SEQ ID NO: 37); DPRVSIRLP (SEQ ID NO: 38); SPRAAEPVQ (SEQ
 35 ID NO: 39); SPREDPPG (SEQ ID NO: 40); WPRVSPACA (SEQ ID NO: 41); LPRMQEDSP (SEQ ID NO: 42); CPRFQELES (SEQ ID NO: 43); FPRQCATVE (SEQ ID NO: 44); LPREYVNAR (SEQ ID NO: 45); RPRFRELVS (SEQ ID NO: 46); SPREGPLPAA (SEQ ID NO: 48); RPRFRELVS (SEQ ID NO: 49); SPREGPLPA (SEQ ID NO: 50); GPRALAETS (SEQ ID NO: 51); GPRALIETS (SEQ ID NO: 52);

GPRALVETS (SEQ ID NO: 53); GPRRRLGCER (SEQ ID NO: 54); RPRRGAAPEP (SEQ ID NO: 55); PPRPWDTPCP (SEQ ID NO: 56); TPRRLPRLP (SEQ ID NO: 57); TPRRLPRLPQ (SEQ ID NO: 58); LPRLPQRYW (SEQ ID NO: 59); LPRLPQRYWQ (SEQ ID NO: 60); GPRRRLGCE (SEQ ID NO: 63); RPRRGAAPE (SEQ ID NO: 64);
 5 PPRPWDTPC (SEQ ID NO: 65); KPRRNLEEDD (SEQ ID NO: 67); and KPRRNLEED (SEQ ID NO: 68), by substituting its N-terminal amino-acid with an alanine and its C-terminal amino-acid with a leucine.

11. The immunogenic HLA-B*0702-restricted epitope of any of claims 7 to 10, which is selected amongst APRMHCAVDL (SEQ ID NO: 69); APRVSIRLPL (SEQ ID NO: 70); APREYVNAL (SEQ ID NO: 71); APRSPLAPL (SEQ ID NO: 72);
 10 APRALVETL (SEQ ID NO: 73); APRRRLGCEL (SEQ ID NO: 74); APRRGAAPEL (SEQ ID NO: 75); APRPWDTPC (SEQ ID NO: 76); APRRLPRLPL (SEQ ID NO: 77); and APRRLVQLL (SEQ ID NO: 78).

12. A chimeric polypeptide, comprising two, three or more HLA-B*0702-restricted cryptic epitopes according to claim 6.

13. A chimeric polypeptide, comprising two, three or more immunogenic HLA-B*0702-restricted epitopes according to any of claims 7 to 11.

14. An isolated nucleic acid molecule designed to cause the expression of a cryptic HLA-B*0702-restricted epitope according to claim 6, an immunogenic epitope according to any of claims 7 to 11, or a chimeric polypeptide according to claim 12 or claim 13.

15. A pharmaceutical composition comprising at least, as an active principle, a HLA-B*0702-restricted cryptic epitope according to claim 6, or an immunogenic epitope polypeptide according to any of claims 7 to 11, or a chimeric polypeptide according to claim 12 or claim 13, or a nucleic acid according to claim 14.

16. The pharmaceutical composition of claim 15, which is a vaccine.

17. A kit of parts comprising, in separate formulations, a first peptide having the sequence of a HLA-B*0702-restricted cryptic epitope, and a second peptide corresponding to its cognate HLA-B*0702-restricted immunogenic epitope.

18. The kit according to claim 17, wherein said first peptide is a cryptic epitope according to claim 6, and said second peptide is derived from said first peptide by a method according to any of claims 3 to 5.

19. A kit of parts comprising, in separate formulations, a first chimeric polypeptide comprising two, three or more HLA-B*0702-restricted cryptic epitopes, and a second chimeric polypeptide comprising HLA-B*0702-restricted immunogenic epitope cognate to the HLA-B*0702-restricted cryptic epitopes comprised in the first chimeric polypeptide.

20. The kit according to claim 19, wherein said first chimeric polypeptide is a chimeric polypeptide according to claim 12, and said second peptide is a chimeric polypeptide according claim 13.

5 21. The kit according to any of claims 17 to 20, which is a vaccination kit, wherein said first and second peptides or chimeric polypeptides are in separate vaccination doses.

22. The vaccination kit according to claim 21, which comprises 2 or 3 doses of second peptide or chimeric polypeptide, and 3, 4, 5, 6 or up to 50 doses of first peptide or chimeric polypeptide.

10 23. The vaccine according to claim 16, or the vaccination kit according to claim 21 or claim 22, wherein each dose comprises 1 to 5 mg of peptide or 1 to 20 mg of chimeric polypeptide.

24. The vaccine according to claim 16 or claim 23, or the vaccination kit of any of claims 21 to 23 wherein the vaccination doses are formulated for subcutaneous injection.

25. Use of a HLA-B*0702-restricted cryptic epitope according to claim 6, or an immunogenic epitope polypeptide according to any of claims 7 to 11, or a chimeric polypeptide according to claim 12 or claim 13, or a nucleic acid according to claim 14, for the preparation of a composition for preventive or curative immunotherapy.

20 26. The use of claim 25, for antiviral or anti-cancer immunotherapy.

27. The use of claim 25 or claim 26, for the preparation of a vaccine.

28. Use of a peptide having a sequence of a HLA-B*0702-restricted cryptic epitope, for preparing a medicinal composition for maintaining the CTL immune response initiated by its cognate optimized peptide.

25 29. Use of a chimeric polypeptide comprising two, three or more HLA-B*0702-restricted cryptic epitopes, for preparing a medicinal composition for maintaining the CTL immune response initiated by either its cognate optimized chimeric polypeptide, or by optimized peptides cognate to said cryptic epitopes.

30 30. Use of an immunogenic peptide having an optimized HLA-B*0702-restricted epitope sequence derived from a HLA-B*0702-restricted cryptic epitope, for preparing a medicinal composition for initiating a CTL immune response against said HLA-B*0702-restricted cryptic epitope.

35 31. Use of a chimeric polypeptide comprising two, three or more optimized HLA-B*0702-restricted epitopes derived from a HLA-B*0702-restricted cryptic epitopes, for preparing a medicinal composition for initiating a CTL immune response against said HLA-B*0702-restricted cryptic epitope.

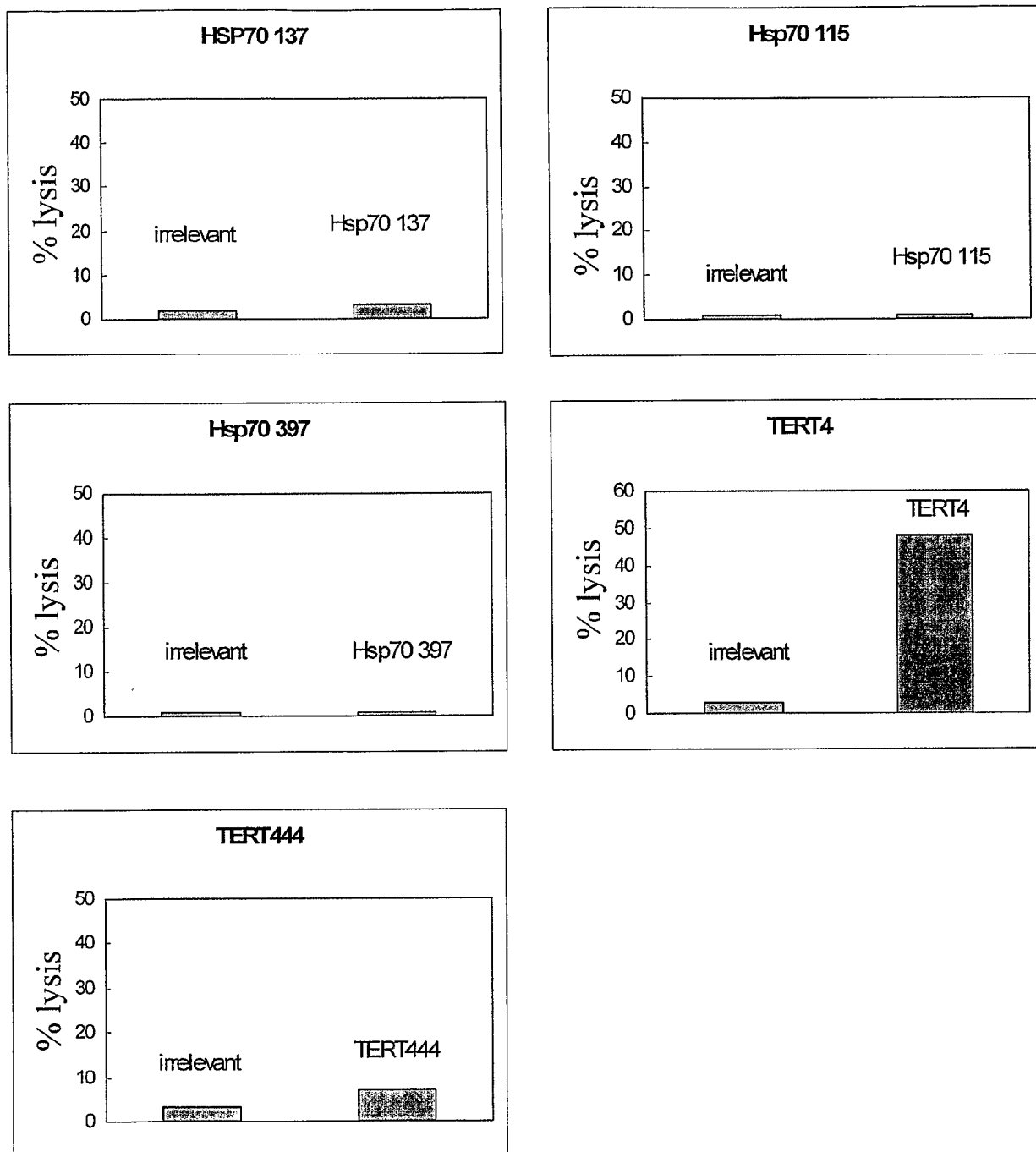


Figure 1

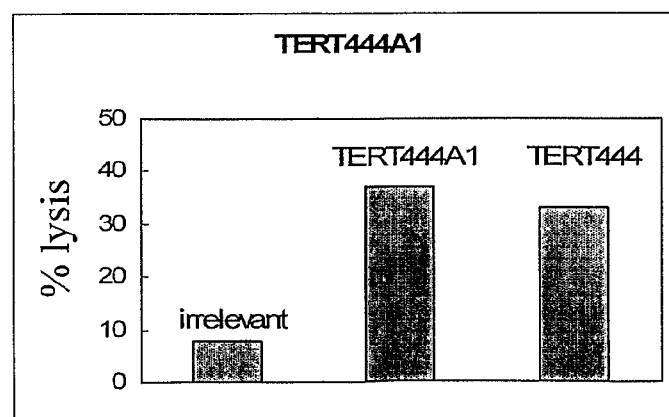
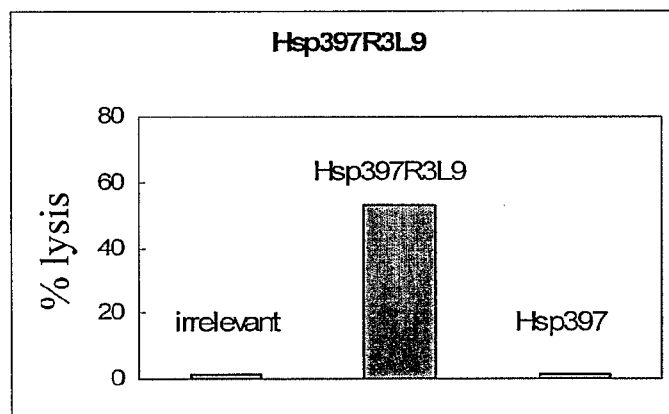
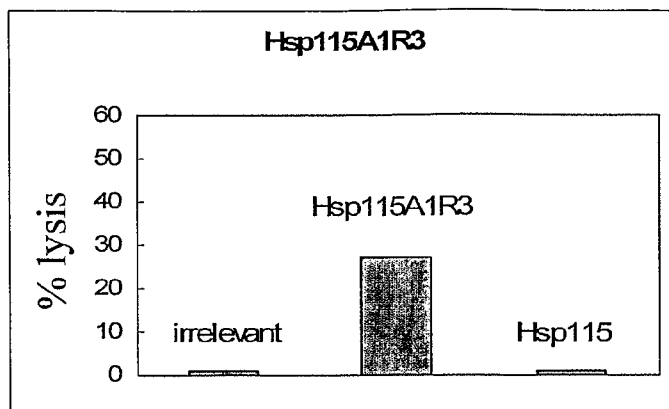
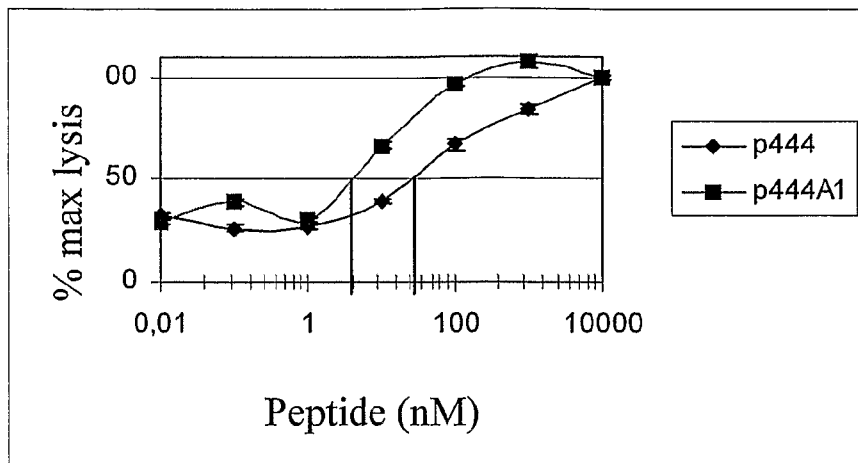


Figure 2

A



B

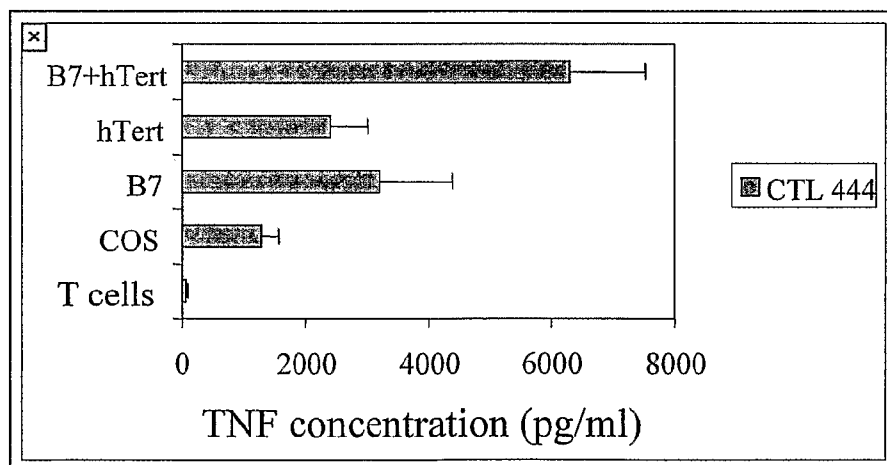
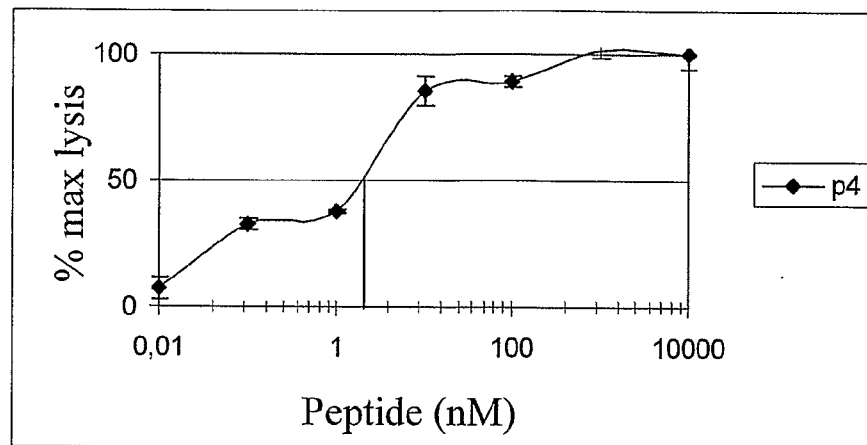


Figure 3

A



B

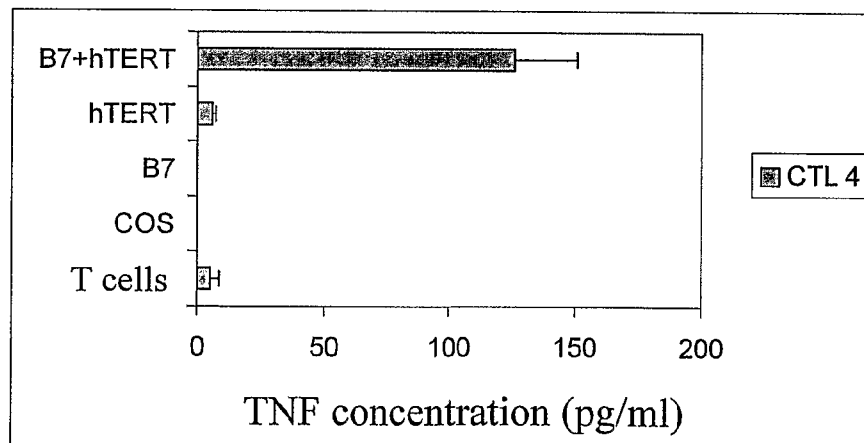
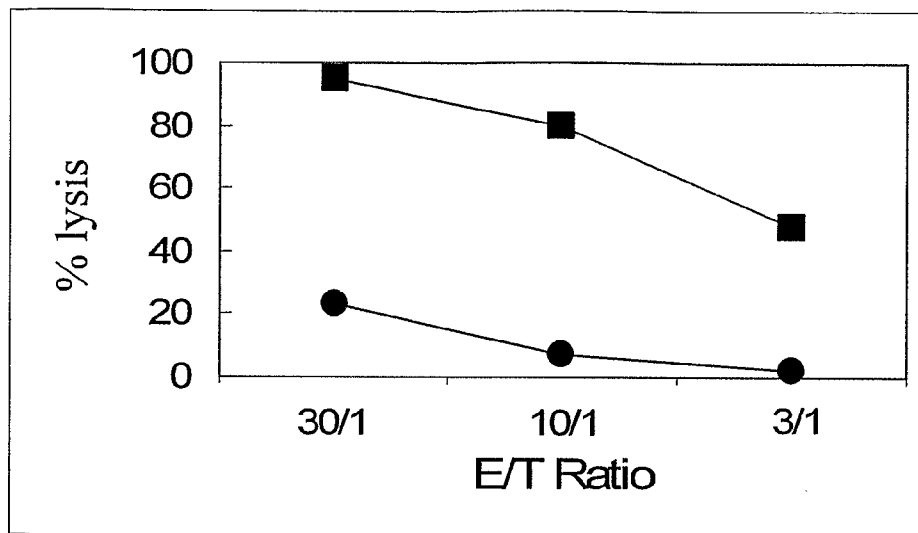


Figure 4

A



B

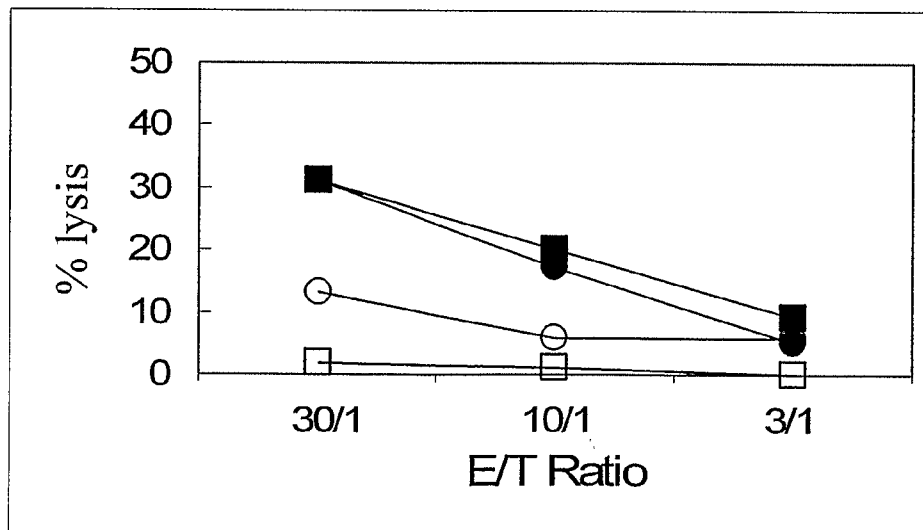


Figure 5

1

SEQUENCE LISTING

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KOSMATOPOULOS, Kostantinos (Kostas)
GRAFF-DUBOIS, Stéphanie
MENEZ-JAMET, Jeanne

<120> IDENTIFICATION, OPTIMIZATION AND USE OF CRYPTIC HLA-B7 EPITOPES
FOR IMMUNOTHERAPY

<130> VMA/bv-F1788/3PCT

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<210> 71
<211> 9
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<213> Artificial

<220>
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<400> 71

Ala Pro Arg Glu Tyr Val Asn Ala Leu
1 5

<210> 72
<211> 9
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<220>
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16

<400> 72

Ala Pro Arg Ser Pro Leu Ala Pro Leu
1 5

<210> 73

<211> 9

<212> PRT

<213> Artificial

<220>

<223> MAGE-A-derived immunogenic epitope

<400> 73

Ala Pro Arg Ala Leu Val Glu Thr Leu
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<210> 74

<211> 10

<212> PRT

<213> Artificial

<220>

<223> TERT-derived immunogenic epitope

<400> 74

Ala Pro Arg Arg Arg Leu Gly Cys Glu Leu
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<210> 75

<211> 10

<212> PRT

<213> Artificial

<220>

<223> TERT-derived immunogenic epitope

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Ala Pro Arg Arg Gly Ala Ala Pro Glu Leu
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<211> 10

<212> PRT

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<223> TERT-derived immunogenic epitope

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Ala Pro Arg Pro Trp Asp Thr Pro Cys Leu
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<210> 77

<211> 10

<212> PRT

<213> Artificial

17

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<223> TERT-derived immunogenic epitope

<400> 77

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<210> 78

<211> 9

<212> PRT

<213> Artificial

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<223> TERT-derived immunogenic epitope

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Ala Pro Arg Arg Leu Val Gln Leu Leu
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<212> PRT

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<220>

<223> Peptide from Hsp70_115

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<210> 80

<211> 10

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<223> Peptide from 115A1

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<210> 81

<211> 10

<212> PRT

<213> Artificial

<220>

<223> Peptide from 115A1R3

<400> 81

Ala Pro Arg Glu Ile Ser Ser Met Val Leu
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<210> 82

<211> 10

<212> PRT

18

<213> Artificial

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<223> Peptide from Hsp70 137 (10)

<400> 82

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<210> 83

<211> 9

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<223> Peptide from Hsp70 397

<400> 83

Ala Pro Leu Ser Leu Gly Leu Glu Thr
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<210> 84

<211> 9

<212> PRT

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Ala Pro Arg Ser Leu Gly Leu Glu Leu
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<210> 85

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<220>

<223> Peptide from TERT4

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Ala Pro Arg Cys Arg Ala Val Arg Ser Leu
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<210> 86

<211> 9

<212> PRT

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<223> Peptide from MAGE-A121.1

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Glu Pro Val Thr Lys Ala Glu Met Leu
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<210> 87
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<220>
<223> Peptide from 121.1 A1

<400> 87

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1 5

<210> 88
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<212> PRT
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<220>
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Ala Pro Arg Thr Lys Ala Glu Met Leu
1 5

<210> 89
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<400> 89

Glu Pro Phe Thr Lys Ala Glu Met Leu
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<210> 90
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Glu Pro Ile Thr Lys Ala Glu Ile Leu
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20

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<223> ICE-derived HLA-B7 restricted epitope

<400> 92

Ser Pro Arg Trp Trp Pro Thr Cys Leu
1 5

<210> 93
<211> 10
<212> PRT
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<400> 93

Ser Pro Ser Ser Asn Arg Ile Arg Asn Thr
1 5 10

<210> 94
<211> 9
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<400> 94

Leu Pro Arg Trp Pro Pro Pro Gln Leu
1 5

<210> 95
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<210> 96
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1 5 10

<210> 97

<211> 10

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<223> HIVp17-derived HLA-B7 restricted epitope

<400> 97

Arg Pro Gly Gly Lys Lys Arg Tyr Lys Leu
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<210> 98

<211> 9

<212> PRT

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<220>

<223> HIVp24-derived HLA-B7 restricted epitope

<400> 98

Ser Pro Arg Thr Leu Asn Ala Trp Val
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<211> 9

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<213> Artificial

<220>

<223> HIVp24-derived HLA-B7 restricted epitope

<400> 99

His Pro Val His Ala Gly Pro Ile Ala
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<211> 9

<212> PRT

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<220>

<223> HIVp24-derived HLA-B7 restricted epitope

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Pro Pro Ile Pro Val Gly Glu Ile Tyr
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<220>
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Gly Pro Lys Val Lys Gln Trp Pro Leu Thr
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<210> 104
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Ser Pro Ala Ile Phe Gln Ser Ser Met
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1 5

<210> 106
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<400> 107

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Phe Pro Arg Thr Trp Leu His Gly Leu
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Lys Pro Cys Val Lys Leu Thr Pro Leu Cys
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<210> 111
<211> 9
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<220>
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1 5

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<211> 10
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<400> 112

Arg Pro Trp Asn Asn Thr Arg Lys Ser Ile
1 5 10

<210> 113
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<223> HIVgp160-derived HLA-B7 restricted epitope

<400> 113

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<210> 114
<211> 9
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<400> 114

Phe Pro Val Thr Pro Gln Val Pro Leu
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<210> 115
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<220>
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<400> 115

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<210> 116
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<400> 117

Arg Pro His Glu Arg Asn Gly Phe Thr Val
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<400> 118

Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu
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<220>
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<220>
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<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> MISC_FEATURE
<222> (4)..(7)
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<220>
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<222> (8)..(11)

26

<223> Xaa can be any naturally occurring amino acid or none

<220>

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<222> (12)..(12)

<223> Xaa can be any naturally occurring amino acid, provided Xaa is not L if Xaa(1)..(1) is A

<400> 119

Xaa	Pro	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
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<210> 120

<211> 12

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<223> HLA-B*0702-restricted cryptic epitope

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> Xaa can be any naturally occurring amino acid but A

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<222> (4)..(7)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> MISC_FEATURE

<222> (8)..(11)

<223> Xaa can be any naturally occurring amino acid or none

<400> 120

Xaa	Pro	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu
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<210> 121

<211> 12

<212> PRT

<213> Artificial

<220>

<223> Optimized peptide

<220>

<221> MISC_FEATURE

<222> (4)..(7)

<223> Xaa can be any naturally occurring amino acid

<220>

<221> MISC_FEATURE

<222> (8)..(11)

<223> Xaa can be any naturally occurring amino acid or none

<400> 121

Ala	Pro	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu
1			5					10			

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2006/002937

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K7/04 A61K38/04 A61P31/12 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the International search

21 February 2007

Date of mailing of the International search report

12/06/2007

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Montrone, Marco

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2006/002937

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TURCANOVA V ET AL: "Sustained CD8+ T-cell immune response to a novel immunodominant HLA-B*0702-associated epitope derived from an Epstein-Barr virus helicase-primase-associated protein" JOURNAL OF MEDICAL VIROLOGY, ALAN R. LISS, NEW YORK, NY, US, vol. 72, no. 4, April 2004 (2004-04), pages 635-645, XP002408387 ISSN: 0146-6615 abstract page 636, column 2, paragraph 3 page 638, column 2, paragraph 3 - page 639, column 1, paragraph 1; table 1 page 639, column 2, paragraph 2 - page 640, column 2, paragraph 2 page 641, column 1, paragraph 2 - page 642, column 2, paragraph 2 page 643, column 2, paragraph 2 - page 644, column 1, paragraph 1; figure 5	1,2
X	WO 2005/049072 A2 (KRAEFTENS BEKAEMPELSE [DK]; ANDERSEN MADH HALD [DK]; STRATEN PER THOR) 2 June 2005 (2005-06-02) abstract page 2, lines 13-35 page 4, lines 8-26 page 9, lines 4-8 page 10; sequence 91	6,14-16, 25-27
Y	RAMMENSEE HANS-GEORG ET AL: "SYFPEITHI: Database for MHC ligands and peptide motifs" IMMUNOGENETICS, SPRINGER VERLAG, BERLIN, DE, vol. 50, no. 3-4, November 1999 (1999-11), pages 213-219, XP002183663 ISSN: 0093-7711 abstract	1,2,6, 14-16, 23-28
Y	HEBART H ET AL: "Sensitive detection of CMV peptide-specific CTL-responses by INFg-ELISPOT and flow cytometry in healthy individuals and patients following allogeneic stem cell transplantation" BLOOD, vol. 96, no. 11 Part 1, 16 November 2000 (2000-11-16), page 771a, XP009078709 & 42ND ANNUAL MEETING OF THE AMERICAN SOCIETY OF HEMATOLOGY; SAN FRANCISCO, CALIFORNIA, USA; DECEMBER 01-05, 2000 ISSN: 0006-4971 abstract	1,2,6, 14-16, 23-28

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2006/002937

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PASQUETTO VALERIE ET AL: "HLA-A*0201, HLA-A*1101, and HLA-B*0702 transgenic mice recognize numerous poxvirus determinants from a wide variety of viral gene products" JOURNAL OF IMMUNOLOGY, vol. 175, no. 8, October 2005 (2005-10), pages 5504-5515, XP002419446 ISSN: 0022-1767 abstract page 5505, column 1, paragraph 6 - column 2, paragraph 2 page 5506, column 1, paragraph 3 - column 2, paragraph 2 page 5508; figure 2 page 5511, column 1, paragraph 1; table 3 page 5513, column 2, paragraph 2 - page 5514, column 1, paragraph 3 -----	1,2,6, 14-16, 23-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2006/002937

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2 (complete), 6, 14-16, 23-28 (all partial)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1,2(complete), 6, 14-16,23-28(all partial)

Method for identifying cryptic HLA-B*0702 restricted epitopes by selecting in an antigen, a peptide of 8 to 12 amino acids having a proline in position 2 and an arginine in position 3 with the proviso that the peptide does not have, simultaneously, an alanine in position 1 and a leucine in C-terminal position.
The cryptic HLA-B*0702 restricted epitope of SEQ.ID.NO.:1 and medical uses thereof.

Invention 2: claims 3-5(complete)

A method for increasing the immunogenicity of said epitope by substituting the N-terminal position with an alanine.

Invention 3 to 79: claims 6 to 31(all partially)

Referring to the different cryptic HLA-B*0702 restricted epitopes as referred to in the different SEQ.ID.NO.: 2 to 78 of claims 6 to 11 and chimeric variants thereof, Kits and medical uses thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2006/002937

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005049072	A2	02-06-2005	NONE