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(54) Titre : IDENTIFICATION, OPTIMISATION ET UTILISATION D'EPITOPES HLA-B7 CRYPTIQUES POUR UNE IMMUNOTHERAPIE
(54) Title: IDENTIFICATION, OPTIMIZATION AND USE OF CRYPTIC HLA-B7 EPITOPES FOR IMMUNOTHERAPY

(57) Abrégé/Abstract:
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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.

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.

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 11 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 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.

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.

Cryptic peptides are much less involved in self tolerance process (Anderton et al., 2002; Boisgérault 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 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 (Parker et al., 1994; Rammensee H et al., 1999). 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.

Few tumor peptides presented by HLA-B*0702 have been described to date. Two peptides derived from the CEA (CEA_{632}) (Lu et al. 2000) and TERT ($TERT_{1123}$) (Cortez-Gonzales et al. 2006) antigens have been identified; these peptides exhibited a strong binding affinity for HLA-B*0702 and were immunogenic both in HLA-B*0702 transgenic mice and in vitro tests with human cells. These experimental results show that these peptides are immunodominant peptides.

Two additional peptides derived from MAGE-A1 ($MAGE\text{-}A1_{289}$) (Luiten et al., 2000) and RU2 (a new antigen expressed by renal cell carcinoma) (Van den Eynde et al. 1999) have been identified to be targets of HLA-B*0702 CTL that had been isolated from cancer patients. Although there is no information about the HLA-B*0702 affinity of these two peptides, we can consider them immunodominant because CTL developed in cancer patients are always directed against immunodominant peptides.

As described in the experimental part below, the inventors have now found a general strategy to enhance affinity and immunogenicity of a HLA-B*0702 restricted cryptic peptide.

In a first aspect, the present invention provides 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 (A), or substituting the C-terminal residue of said epitope with a leucine (L).

In what follows, the phrases "HLA-B*0702-restricted cryptic epitope" is used to designate a peptide, having 8 to 11 amino acids, more preferably 9 or 10 amino acids, which exhibits a low affinity for HLA-B*0702, is non immunogenic and has the sequence $X_1PX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 58), wherein P is for proline, X_3 is R (arginine) or K (lysine) or H (histidine) or M (methionine), X_1 and X_4 to X_7 are independently any amino acid, X_8 to X_{10} are independently any amino acid or none, and the C-terminal amino acid X_{11} is any amino acid with the proviso that if the N-terminal amino acid X_1 is A (alanine) then X_{11} is neither L (leucine) nor A, nor I (isoleucine), nor V (valine), and nor M, and if X1 is an amino acid other than A then X_{11} is L or A or I or V or M.

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.

In the present text, the amino acid residues are designated by their one letter codes.

As used herein, 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, when the amino acid sequence of said cryptic epitope does not contain the appropriate amino acid, 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 affinity of a peptide for HLA-B*0702 can be determined by methods known in the art, for instance by the assay described by Rohrlich *et al.*, 2003. Results are expressed as relative affinity (RA) when compared to a reference peptide. Following this method a peptide is said to have a low affinity for HLA-B*0702 when RA is greater than 10. Peptides with RA greater than 10 are therefore considered to be cryptic peptides (or epitopes).

As used herein, the term "non immunogenic" refers to a peptide unable to initiate a HLA-B*0702-restricted CTL immune response when administered to a subject expressing HLA-B*0702 (including a HLA-B*0702 transgenic animal).

In another embodiment, the immunogenicity of a HLA-B*0702-restricted cryptic epitope in which the second and third amino acid residues are PR or PK or PH or PM and the last residue is L or A or I or V or M, can be increased by substituting its first amino-acid by an A (alanine). Indeed, when the sequence of the selected HLA-B*0702-restricted cryptic epitope is $X_1PX_3X_4X_5X_6X_7X_8X_9$ $X_{10}X_{11}$ (SEQ ID NO: 59), wherein the N-terminal amino acid X_1 is any amino acid but A, X_3 is R or K or H or M, the C-terminal amino acid X_{11} is L or A or I or V or M, X_4 to X_7 are independently any amino acid, and X_8 to X_{10} are independently any amino acid or none, the substitution of X_1 by A is sufficient to increase its immunogenicity.

In yet another embodiment, the immunogenicity of HLA-B*0702-restricted cryptic epitopes in which the three first amino acid residues are APX_3 (wherein X_3 is R or K or H or M) can be increased by substituting its last amino-acid by a L (or by adding a leucine at its C-terminus, provided the amino acid sequence of said epitope after having added the leucine is not longer than 11 amino acids). Indeed, when the sequence of the selected HLA-B*0702-restricted cryptic epitope is $APX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 60), wherein X_3 is R or K or H or M, X_4 to X_7 are independently any amino acid, X_8 to X_{10} are independently any amino acid or none, and the C-terminal amino acid X_{11} is an amino acid other than L or A or I or V or M, the substitution of X_{11} by L is sufficient to increase its immunogenicity.

In what follows, the expression "optimized peptide" designates an immunogenic peptide derived from a HLA-B*0702-restricted cryptic epitope by the above methods, and having the general sequence $APX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID

No: 61), wherein X_3 is R or K or H or M, X_4 to X_7 are independently any amino acid, X_8 to X_{10} are independently any amino acid or none, and the C-terminal amino acid X_{11} is L or A or I or V or M.

The inventors have identified a number of HLA-B*0702-restricted cryptic epitopes, some of them are disclosed in Table I 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 4, disclosed in Table I.

Antigen	Peptide	SEQ ID NO:
$TERT_{444}$	DPRRLVQLL	1
HER-2/neu	APRSPLAPS	2
	SPKANKEIL	3
	GPKHSDCLA	4

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

Examples of immunogenic HLA-B*0702-restricted epitopes obtained according to the present invention are those derived from a cryptic HLA-B*0702-restricted epitope SEQ ID NOs 1, 3, 4, by substitution of their N-terminal amino-acid with an A (alanine) and those derived from the cryptic HLA-B*0702-restricted epitope SEQ ID NOs: 2 by substitution of their C-terminal amino-acid with a L (leucine).

Therefore, the present invention also pertains to optimized peptides derived from the cryptic peptides of SEQ ID NOs: 1 to 4, by a method according to the invention. Preferred examples of optimized peptides are APRSPLAPL (SEQ ID NO: 6), APKANKEIL (SEQ ID NO: 7), APKHSDCLA (SEQ ID NO: 8) and APRRLVQLL (SEQ ID NO: 5).

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 a chimeric polypeptide according to the invention, the epitopes can be different from each other, or the same epitope can be repeated several times (two, three or more 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 to 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 cryptic epitopes. The results reported in PCT/EP2006/005325 demonstrate that injection of a native peptide corresponding to a 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 4, 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 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 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 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 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 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 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 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.

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

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

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

Figure 3: *In vivo* immunogenicity of optimized HLA-B*0702 Her2/neu$_{1069L9}$ (A) and Her2/neu$_{1069}$ (B) peptides in HLA-B*0702 transgenic mice. CTL were tested against RMA-B7 targets loaded with peptide as indicated. CTL population induced was diluted 3 (1), 10 (2), 30 (3) and 100 (4) fold.

Figure 4: TERT$_4$ induces TERT specific CTL in HLA-B7 mice and in healthy donors. (A) TERT$_4$ immunogenicity in HLA-B*0702 transgenic mice. CTL were tested against RMA-B7 targets loaded with decreasing doses of TERT$_4$ peptide. **(B) Recognition of endogenous TERT by TERT$_4$ specific murine CTL.** CTL were tested against COS cells transfected with HLA-B*0702 and TERT as indicated. **(C) Induction of TERT$_4$ specific human CTL.** CTL were tested against T2-B7 targets loaded with TERT$_4$ (■) or an irrelevant (●) peptide using the Effector/ Target ratio as indicated (left graph), and 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 (right graph).

Figure 5: Recognition of endogenous TERT by TERT$_{444}$ specific murine CTL. (A) CTL were tested against RMA-B7 targets loaded with decreasing doses of TERT$_{444}$ or TERT$_{444A1}$ peptides as indicated. **(B)** CTL were tested against COS cells transfected with HLA-B*0702 and/or TERT as indicated.

Figure 6: Induction of TERT$_{444A1}$ specific human CTL. CTL were tested against T2-B7 targets loaded with peptides as indicated. CTL maximal activation is obtained by PMA/ionomycin treatment.

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).

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*0702 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 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 $CMV_{265-274}$ (R10V; RPHERNGFTV, SEQ ID NO: 9). The relative affinity (RA) was determined as: RA = (Concentration of each peptide that induces 20 % of HLA-B*0702-expression / 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 $I\text{-}A^b$ restricted $HBVcore_{128}$ T helper epitope (TPPAYRPPNAPIL, SEQ ID NO: 10). After 11 days, $5x10^7$ 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. $2.2x10^4$ 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 50μl of RPMI+10% FCS and used as stimulating cells. $5x10^4$ 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 TNFα. Standard dilutions were prepared in 50μl with final doses of TNFα ranging from 104 to 0 pg/ml. On both the supernatants and the standard dilutions, $3x10^4$ TNFα sensitive WEHI-164c13 cells in 50μl 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-B*0702 volunteers. Dendritic cells (DC) were produced from adherent cells cultured for seven days ($2x10^6$ cells/ml) in the presence of 500 IU/ml GM-CSF and 500IU/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 μM 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 μg/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. $2x10^5$ $CD8^+$ cells + $6x10^4$ $CD8^-$ cells were stimulated with $2x10^4$ 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 ($TERT_4$) or for IFNγ intracellular staining ($TERT_{444A1}$).

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 peptides (1 μM) 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.

IFNγ intracellular staining. T cells (10^5) were incubated with 2×10^5 T2 cells loaded with stimulating peptide in the presence of 20 μg/ml Brefeldin-A (Sigma, Oakville, Canada). Six hours later they were washed, stained with r-phycoerythrin-conjugated anti-CD8 antibody (Caltag Laboratories, Burlingame, CA, USA) in PBS for 25 min at 4 °C, washed again, and fixed with 4% PFA. The cells were then permeabilized with PBS, 0.5% BSA, 0.2% saponin (Sigma, Oakville, Canada), and labeled with an allophycocyanin-conjugated anti-IFNγ mAb (PharMingen, Mississauga, Canada) for 25 min at 4 °C before analysis with a FACSCalibur® flow cytometer.

<u>Example 1: Affinity of peptides</u>

Eight peptides with the HLA-B*0702 specific anchor motifs, i.e. P2 and preferentially L/V at C-terminal position (Sidney et al., 1996) belonging to Hsp70 (Hsp70$_{115}$, Hsp70$_{137}$, Hsp70$_{397}$), TERT (TERT$_4$ and TERT$_{444}$), 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 TERT$_4$ bound to HLA-B*0702 with a high affinity, the remaining seven peptides were very weak or non binders (Table II). 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 Hsp70$_{115}$, Hsp70$_{137}$, Hsp70$_{397}$, TERT$_{444}$, MAGE-A$_{121.1}$, MAGE-A$_{121.2}$, MAGE-A$_{121.4}$, **are considered cryptic peptides.**

	peptide	séquence	RA	SEQ ID No
1	Hsp70 115	YPEEISSMVL	>10	11
	Hsp70 115A1	APEEISSMVL	>10	12
2	Hsp70 137 (10)	YPVTNAVITV	>10	13
3	Hsp70 397	APLSLGLET	>10	14
4	TERT4	APRCRAVRSL	0.74	15
5	TERT444	DPRRLVQLL	>10	1
	TERT 444A1	APRRLVQLL	1.4	5
6	MAGE-A121.1	EPVTKAEML	>10	16
	MAGE-121.1 A1	APVTKAEML	>10	17
7	MAGE-A121.2	EPFTKAEML	>10	18
8	MAGE-A121.4	EPITKAEIL	>10	19

<u>Table II</u>: HLA-B*0702 affinity of peptides

<u>Example 2: Immunogenicity of selected peptides</u>

The low affinity Hsp$_{137}$, Hsp$_{115}$, Hsp$_{397}$, TERT$_{444}$ and the high affinity TERT$_4$ peptides have been tested for their capacity to induce a specific CTL immune response in HLA-B*0702 transgenic mice. Only the high affinity $TERT_4$ was immunogenic confirming that immunogenicity of peptides is strongly related to their affinity for HLA (Figure 1).

Example 3: Enhancement of affinity of Low Affinity Peptides

Since all these cryptic peptides had favourable primary anchor motifs, enhancement of their affinity is a prerequisite for them to be immunogenic. It required the identification of unfavourable secondary anchor motifs and their substitution 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 amino acids are favourable motifs at position 1 (Sidney, Southwood et al., 1996). However, peptides $Hsp70_{115}$ and $Hsp70_{137}$ that have a Y (tyrosine) at position 1 are non binders. Moreover, the substitution of the amino acid at position 1 by an A (alanine) that is also favourable at this position. (Parker et al, 1994) enhances the affinity of the $TERT_{444}$ but not of the $Hsp70_{115}$ and the MAGE-$A_{121.1}$ peptides (Table II). This indicates that the presence of favourable amino acids at position 1 and anchor positions 2 and 9/10 **cannot ensure by itself a high binding affinity of all peptides**. In the other hand, positively charged peptides (R/H/K) have been described to be favourable at position 3 (Sidney et al., 1996) and ten out of 26 identified tumor and HIV derived immunogenic peptides have an R/K/H at position 3 (Table III).

Antigen	sequence	SEQ ID NO :	reference
NY-ESO-1	APRGVRMAV	20	Slager et al, 2004
ICE	SPRWWPTCL	21	Ronsin et al., 1999
RAGE-1	SPSSNRIRNT	22	Gaugler et al., 1996
RU2AS	LPRWPPPQL	23	Van Den Eynde et al., 1999
RBAF500	RPHVPESAF	24	Lennerz et al., 2005
SSX2 fusion protein	QPRYGYDQIM	25	Worley et al, 2001
HIVp17	RPGGKKRYKL	26	HIV Molecular Immunology Database (Operated by Los Alamos National Security, LLC, for the U.S. Department of Energy's National Nuclear Security Administration) http://hivweb.lanl.gov/content/immunology/tables/ctl_summary.html
HIVp24	SPRTLNAWV	27	
HIVp24	HPVHAGPIA	28	
HIVp24	PPIPVGEIY	29	
HIVp24	GPGHKARVL	30	
HIV-RT	SPIETVPVKL	31	
HIV-RT	GPKVKQWPLT	32	
HIV-RT	SPAIFQSSM	33	
HIV-RT	IPLTEEAEL	34	
HIV-RT	QPDKSESELV	35	
HIV-Vif	HPRISSEVHI	36	
HIV-Vif	KPPLPSVKKL	37	
HIV-Vif	FPRTWLHGL	38	
HIVgp160	KPCVKLTPLC	39	
HIVgp160	KVVSTQLLL	40	
HIVgp160	RPWNNTRKSI	41	
HIVgp160	IPRRIRQGL	42	
HIVnef	FPVTPQVPL	43	
HIVnef	TPQVPLRPM	44	

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

According to all these observations, peptides with the sequence $APX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ (SEQ ID N° 61) should have a high affinity for HLA-B*0702. This is confirmed by results shown in Table IV. All eighteen peptides with the above cited sequence had a high affinity and/or were immunogenic in HLA-B*0702 transgenic mice.

Sequence	SEQ ID NOs	RA	Immunogenicity
APRRLVQLL	5	+	+
APRSPLAPL	6	++	+
APKANKEIL	7	ND	+
APKHSDCLA	8	ND	+
APRCRAVRSL	15	+	+
APRMHCAVDL	45	++	+
APRVSIRLPL	46	++	ND
APREYVNAL	47	+	+
APRGVPQIEL	48	ND	+
APRALVETL	49	+	+
APRMPEAAL	50	ND	+
APRRRLGCEL	51	+	+
APRPWTPCL	52	+	+
APRASSPLL	53	ND	+
APRQLGREL	54	ND	+
APREISSMVL	55	+	+
APRSLGLEL	56	++	+
APRTKAEML	57	+	+

Table IV: Affinity and immunogenicity of $APX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ HLA-B*0702 restricted peptides. - = RA>10, + = 1<RA<10, ++ = RA<1, Immunogenicity was tested as described in Exemple 2. + : means that a specific immunoresponse was generated in at least one HLA-B*0702 transgenic mice, ND : Not Determined

Example 4: *In vivo* Immunogenicity of peptides with enhanced affinity and recognition of the native counterpart

HLA-B7 transgenic mice were vaccinated with the selected peptides, and eleven days later, their spleen cells were *in vitro* stimulated with the peptide.

In this context, $Hsp70_{115}$, $Hsp70_{397}$ and $TERT_{444}$, were therefore modified at position 1 (substitution of the amino acid by an A) and/or position 3 (substitution of the amino acid by an R). For peptide $Hsp70_{397}$ an additional modification at C-terminal position (substitution of the T by an L) has been introduced. Modified peptides i.e. $Hsp70_{115A1R3}$ (SEQ ID NO: 55), $Hsp70_{397R3L9}$ (SEQ ID NO: 56), $TERT_{444A1}$ (SEQ ID NO: 5) exhibited a strong affinity for HLA-B*0702 (Table IV) and induced an immune response in the majority of vaccinated mice (Figure 2). However, for all peptides but $TERT_{444A1}$, generated CTL **recognized the optimized peptide but not the corresponding native peptide** (Figure 2). This strongly suggests that substitution of the amino acid 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 $APX_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$ have a high affinity and are immunogenic (Table IV and Figure 1, 2) and b) substitution of the amino acid at position 3 by an R may break the cross-recognition of the native peptide, the inventors selected native peptides having a P and R at positions 2 and 3 respectively, and they substituted the amino acid at position 1 by an A if the last amino acid was favourable (L, A, I, V or M). Given the high importance of position 3 in both affinity and CTL recognition of HLA-B*0702 restricted peptides inventors selected peptides with the sequence X_1PX_3 (wherein X_1 is any amino acid and X_3 is K, R, H or M; these amino acids have been described as being favourable residues at position 3) and a favourable amino acid (A/I/L/V) at C-terminal position. Peptides with this sequence and low affinity for HLA-B*0702 have been modified by substitution of the first residue by an A. This is the case of $TERT_{444}$, Her-2/neu_{760} and Her-2/neu_{246}. Inventors also selected peptides with the sequence APX_3 (wherein X_3 is K, R, H or M) and a non favourable residue at C-terminal position (*i.e.*, an amino acid other than L, A, I, V or M). Peptides with this sequence and low affinity for HLA-B*0702 have been modified by substituting residue at C-terminal position with a L. This is the case of Her-2/neu_{1069}. All these modified peptides had a strong affinity for HLA-B*0702.

Example 5: Immunogenicity of optimized peptides and cross-recognition of the native counterpart

Native Her2/neu_{246}, Her2/neu_{760}, Her2/neu_{1069} and $TERT_{444}$ peptides were not immunogenic, whereas the optimized peptides were immunogenic in HLA-B*0702 transgenic mice. Moreover, CTL induced by all these optimized peptides were able to cross-react with the corresponding native peptide (Figure 3 and Table V).

Peptide	Sequence	SEQ ID NOs :	Immunogenicity	Corresponding native peptide cross-reconnaissance
$TERT_{444}$	DPRRLVQLL	1	-	
$TERT_{444A1}$	APRRLVQLL	5	+	+
Her2/neu_{760}	SPKANKEIL	3	-	
Her2/neu_{760A1}	APKANKEIL	7	+	+
Her2/neu_{246}	GPKHSDCLA	4	-	
Her2/neu_{246A1}	APKHSDCLA	8	+	+
Her2/neu_{1069}	APRSPLAPS	2	-	
Her2/neu_{1069L9}	APRSPLAPL	6	+	+

Table V: Immunogenicity of native and optimized HLA-B*0702 restricted peptides. + for immunogenicity or native peptide cross recognition means that the peptide induced a specific response in at least one HLA-B*0702 transgenic mouse, able to recognized the corresponding native peptide.

In conclusion, the inventors have described a method to optimize immunogenicity (and also affinity) of HLA-B*0702 restricted cryptic peptides. It consists in a) substituting the residue at position 1 with an A in all peptides comprising the sequence X_1PX_3 (wherein X_1 is any amino acid except A and X_3 is R or K or H or M), a favourable amino acid at C-terminal position (*i.e.*, L or A or I or V or M), and a low affinity for HLA-B*0702, or b) substituting the residue at C-terminal position with a L in peptides comprising the sequence APX_3 (X_3 being defined as above), a non favourable residue at C-terminal position (*i.e.*, an amino acid other that L or A or I or V or M), and a low affinity for HLA-B*0702.

Example 6: $TERT_4$ immunodominant peptide induces TERT specific CTL

HLA-B7 transgenic mice were then immunized with the $TERT_4$ (SEQ ID NO: 15) 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 $TERT_4$ peptide (Figure 4A). The half maximal lysis of $TERT_4$ 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 $TERT_4$ dominant peptide is an HLA-B*0702 restricted epitope naturally processed from endogenous TERT.

Moreover, CD8 cells from healthy donors were *in vitro* stimulated with autologous dendritic cells loaded with $TERT_4$ peptide. After four stimulations, CTL were tested for cytotoxicity against $TERT_4$ 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 4C. CTL killed T2-B7 targets presenting $TERT_4$ but not T2-B7 cells presenting the irrelevant Nef peptide (left graph). Interestingly, CTL killed the HLA-B*0702 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 $TERT_4$ epitope (right graph).

Example 7: CTL Induced by $TERT_{444A1}$ Peptide Recognize Endogenous TERT

$TERT_{444A1}$ (SEQ ID NO: 5) was tested for its ability to induce CTL able to recognize endogenous TERT and to induce CTL in healthy donors (Example 6). HLA-B*0702 transgenic mice were then immunized with the $TERT_{444A1}$ and eleven days later their spleen cells were *in vitro* stimulated with the native $TERT_{444}$ peptide (SEQ ID NO: 1). Generated CTL killed RMA-B7 targets loaded with decreasing concentrations of $TERT_{444A1}$ and $TERT_{444}$ peptides. The half maximal lysis of $TERT_{444}$ loaded and $TERT_{444A1}$ loaded targets was obtained with 5.5nM and 1nM respectively (Figure 5A). CTL were then tested for their capacity to recognize COS-7 cells expressing HLA-B*0702 and endogenous TERT. Results presented in Figure 5B 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 $TERT_{444}$ is an HLA-B*0702 restricted cryptic epitope naturally processed from endogenous TERT.

Example 8: $TERT_{444A1}$ Stimulates CTL from Healthy Donors

CD8 cells from healthy donors were *in vitro* stimulated with autologous dendritic cells loaded with $TERT_{444A1}$ peptide. After four stimulations, proliferating cells were divided into 4 pools. Each pool was then tested for intracellular IFNg production upon stimulation with T2-B7 cells loaded with optimized $TERT_{444A1}$ or native $TERT_{444}$. Results from D5609 responding donor are presented in Figure 6. IFNg producing CTL were detected in pools 2 and 4 after stimulation with either $TERT_{444}$ or $TERT_{444A1}$ loaded T2B7 cells (Figure 6).

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CLAIMS

1. 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, or substituting the C-terminal residue of said epitope with a leucine.

2. 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 or APK or APH or APM, comprising a step of substituting the C-terminal residue of said epitope with a leucine.

3. A method for increasing the immunogenicity of a HLA-B*0702-restricted cryptic epitope, wherein the second and the third residues of said cryptic epitope are PR or PK or PH or PM and the amino acid at C-terminal position is L or A or I or V or M, comprising a step of substituting the N-terminal residue of said epitope with an alanine.

4. A cryptic HLA-B*0702-restricted epitope, selected in the group consisting of APRSPLAPS (SEQ ID NO: 2), SPKANKEIL (SEQ ID NO: 3), GPKHSDCLA (SEQ ID NO: 4), DPRRLVQLL (SEQ ID NO: 1).

5. An immunogenic HLA-B*0702-restricted epitope, which is derived from the cryptic HLA-B*0702-restricted epitope APRSPLAPS (SEQ ID NO: 2), by substituting its C-terminal amino-acid with a leucine.

6. An immunogenic HLA-B*0702-restricted epitope, which is derived from a cryptic HLA-B*0702-restricted epitope selected in the group consisting of SPKANKEIL (SEQ ID NO: 3), GPKHSDCLA (SEQ ID NO: 4), DPRRLVQLL (SEQ ID NO: 1), by substituting its N-terminal amino-acid with an alanine.

7. An immunogenic HLA-B*0702-restricted epitope according to claims 5 or 6, which is selected amongst APRSPLAPL (SED ID NO:6), APKANKEIL (SED ID NO:7), APKHSDCLA (SED ID NO:8), APRRLVQLL (SED ID NO:5).

8. A chimeric polypeptide, comprising two, three or more HLA-B*0702-restricted cryptic epitopes according to claim 4.

9. A chimeric polypeptide, comprising two, three or more immunogenic HLA-B*0702-restricted epitopes according to any of claims 5 to 7.

10. An isolated nucleic acid molecule designed to cause the expression of a cryptic HLA-B*0702-restricted epitope according to claim 4, an immunogenic epitope according to any of claims 5 to 7, or a chimeric polypeptide according to claim 8 or claim 9.

11. A pharmaceutical composition comprising at least, as an active principle, a HLA-B*0702-restricted cryptic epitope according to claim 4, or an

immunogenic epitope polypeptide according to any of claims 5 to 7, or a chimeric polypeptide according to claim 8 or claim 9, or a nucleic acid according to claim 10.

12. The pharmaceutical composition of claim 11, which is a vaccine.

13. 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.

14. The kit according to claim 13, wherein said first peptide is a cryptic epitope according to claim 4, and said second peptide is an immunogenic peptide according to claims 5 to 7.

15. 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.

16. The kit according to claim 15, wherein said first chimeric polypeptide is a chimeric polypeptide according to claim 8, and said second peptide is a chimeric polypeptide according claim 9.

17. The kit according to any of claims 13 to 16, which is a vaccination kit, wherein said first and second peptides or chimeric polypeptides are in separate vaccination doses.

18. The vaccination kit according to claim 17, 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.

19. The vaccine according to claim 12, or the vaccination kit according to claim 17 or claim 18, wherein each dose comprises 1 to 5 mg of peptide or 1 to 20 mg of chimeric polypeptide.

20. The vaccine according to claim 12 or claim 19, or the vaccination kit of any of claims 16 to 18 wherein the vaccination doses are formulated for subcutaneous injection.

21. Use of a HLA-B*0702-restricted cryptic epitope according to claim 4, or an immunogenic epitope polypeptide according to any of claims 5 to 7, or a chimeric polypeptide according to claim 8 or claim 9, or a nucleic acid according to claim 11, for the preparation of a composition for preventive or curative immunotherapy.

22. The use of claim 21, for antiviral or anti-cancer immunotherapy.

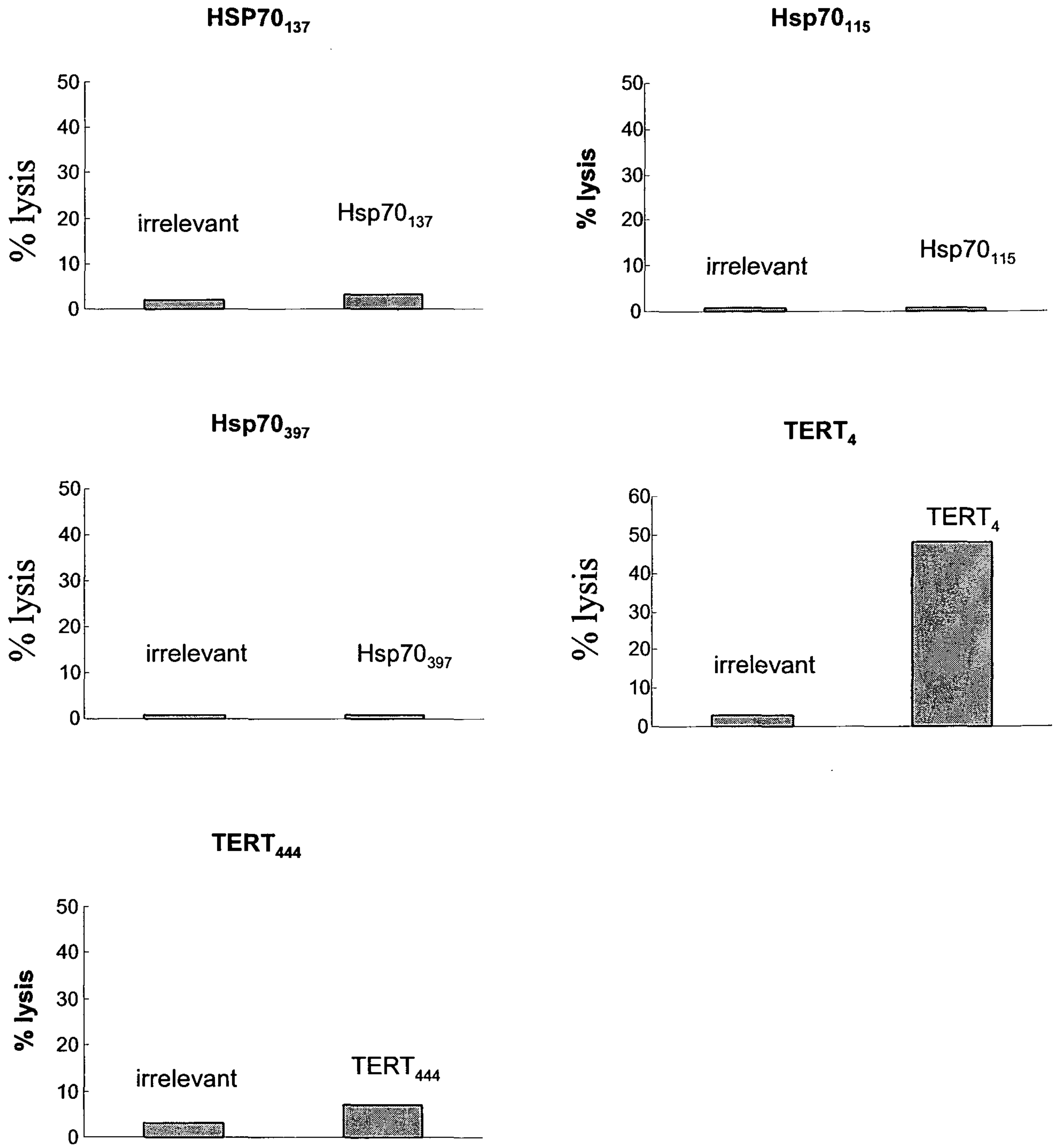
23. The use of claim 21 or claim 22, for the preparation of a vaccine.

24. 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. 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.

26. 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.

27. 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.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10
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irrelevant
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% lysis
60
50
40
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TERT4
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40
30
20
10
0
irrelevant
TERT444

Figure 1

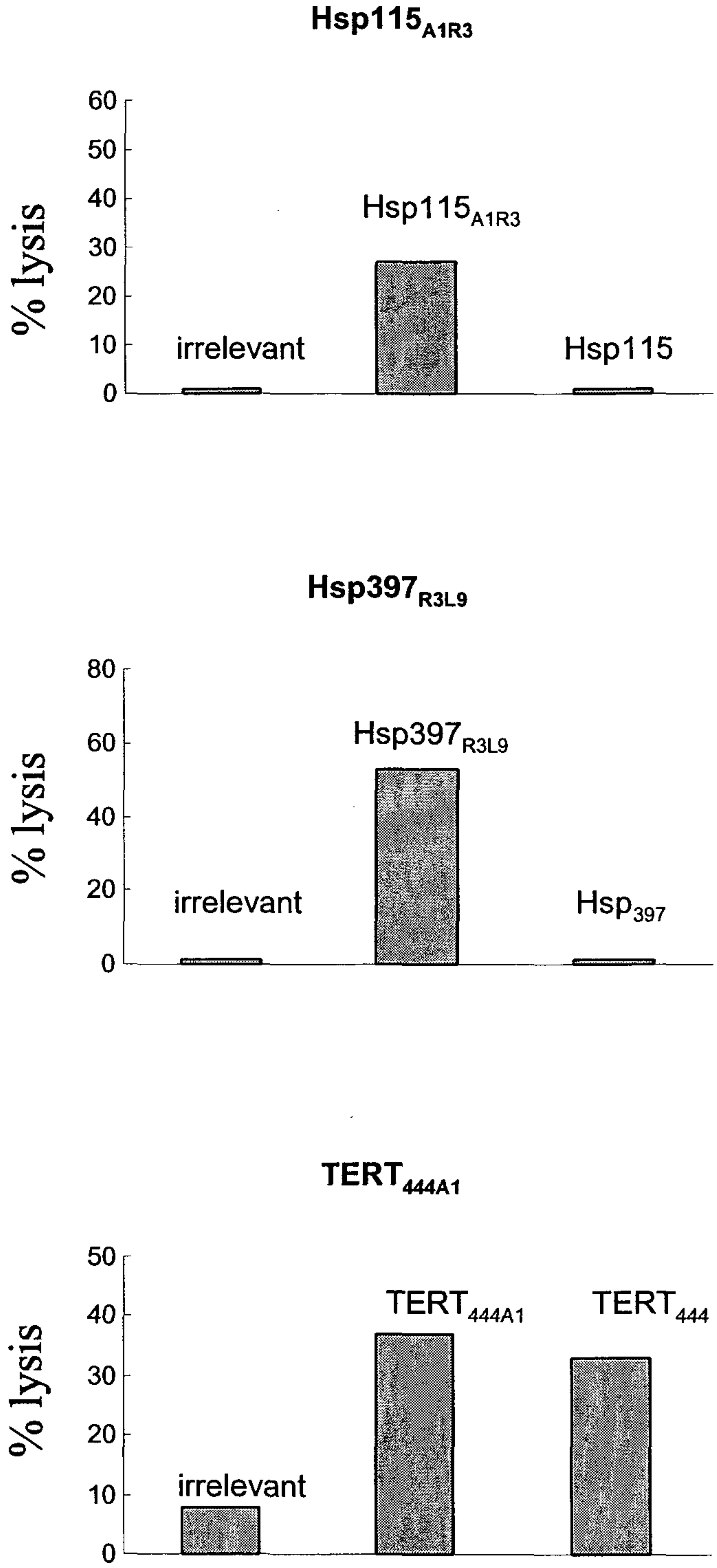
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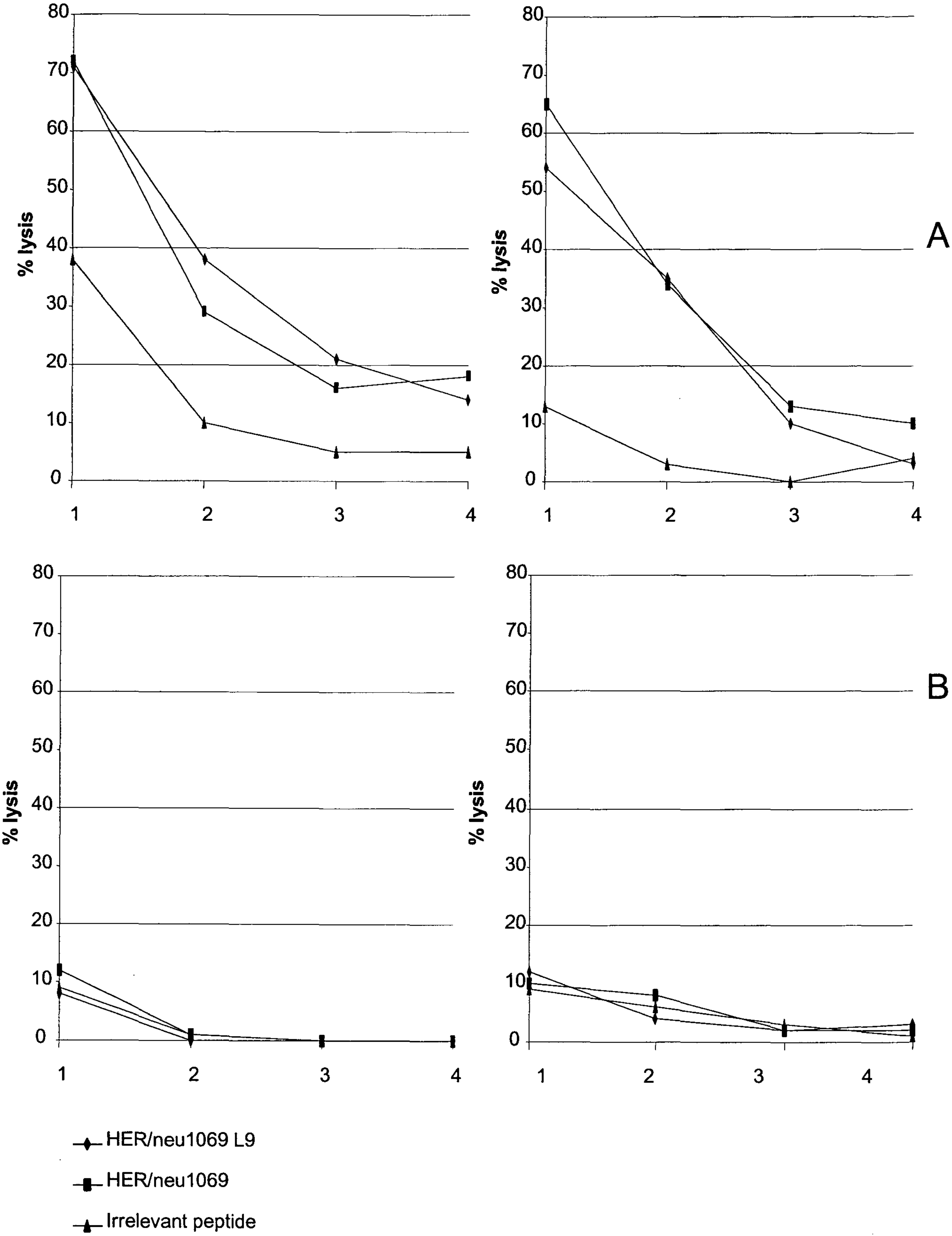
Figure 2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70
60
50
40
30
20
10
0
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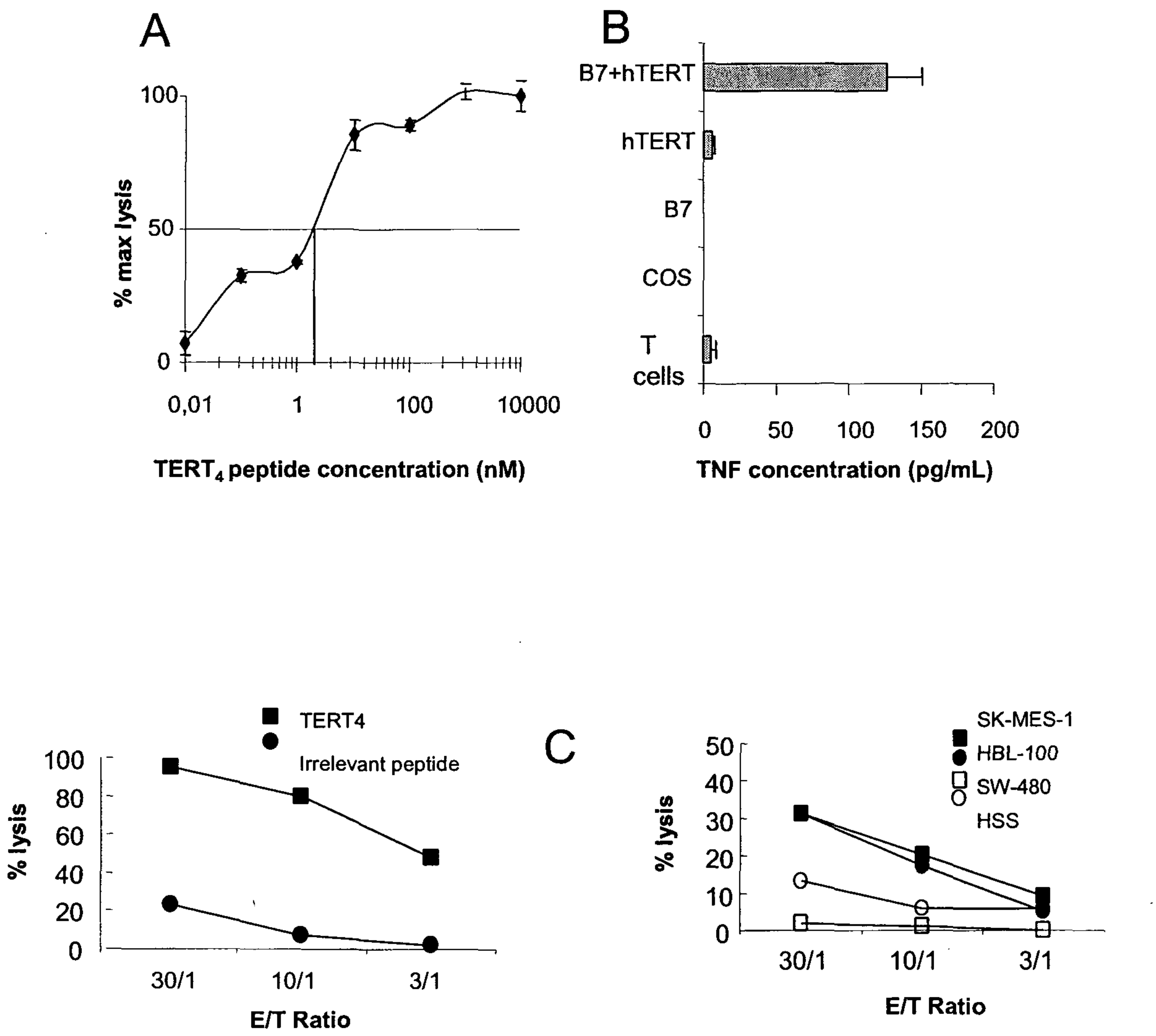
1
2
3
4
A
B
HER/neu1069 L9
HER/neu1069
Irrelevant peptide

Figure 3


A
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50
0
% max lysis
0,01
1
100
10000
TERT4 peptide concentration (nM)
B
B7+hTERT
hTERT
B7
COS
T
cells
0
50
100
150
200
TNF concentration (pg/mL)
TERT4
Irrelevant peptide
100
80
60
40
20
0
% lysis
30/1
10/1
3/1
E/T Ratio
C
SK-MES-1
HBL-100
SW-480
HSS
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40
30
20
10
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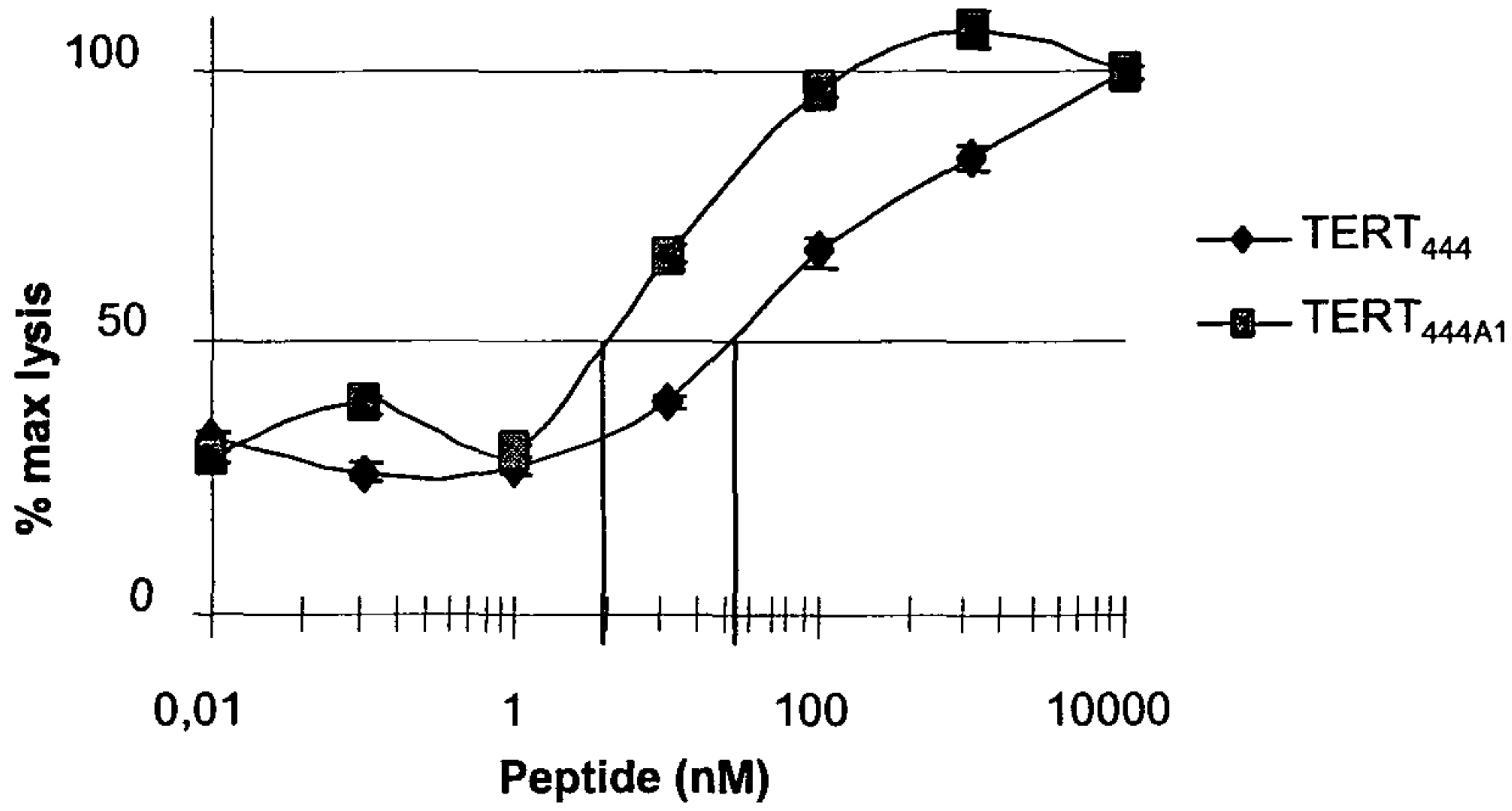
% lysis
30/1
10/1
3/1
E/T Ratio

Figure 4

A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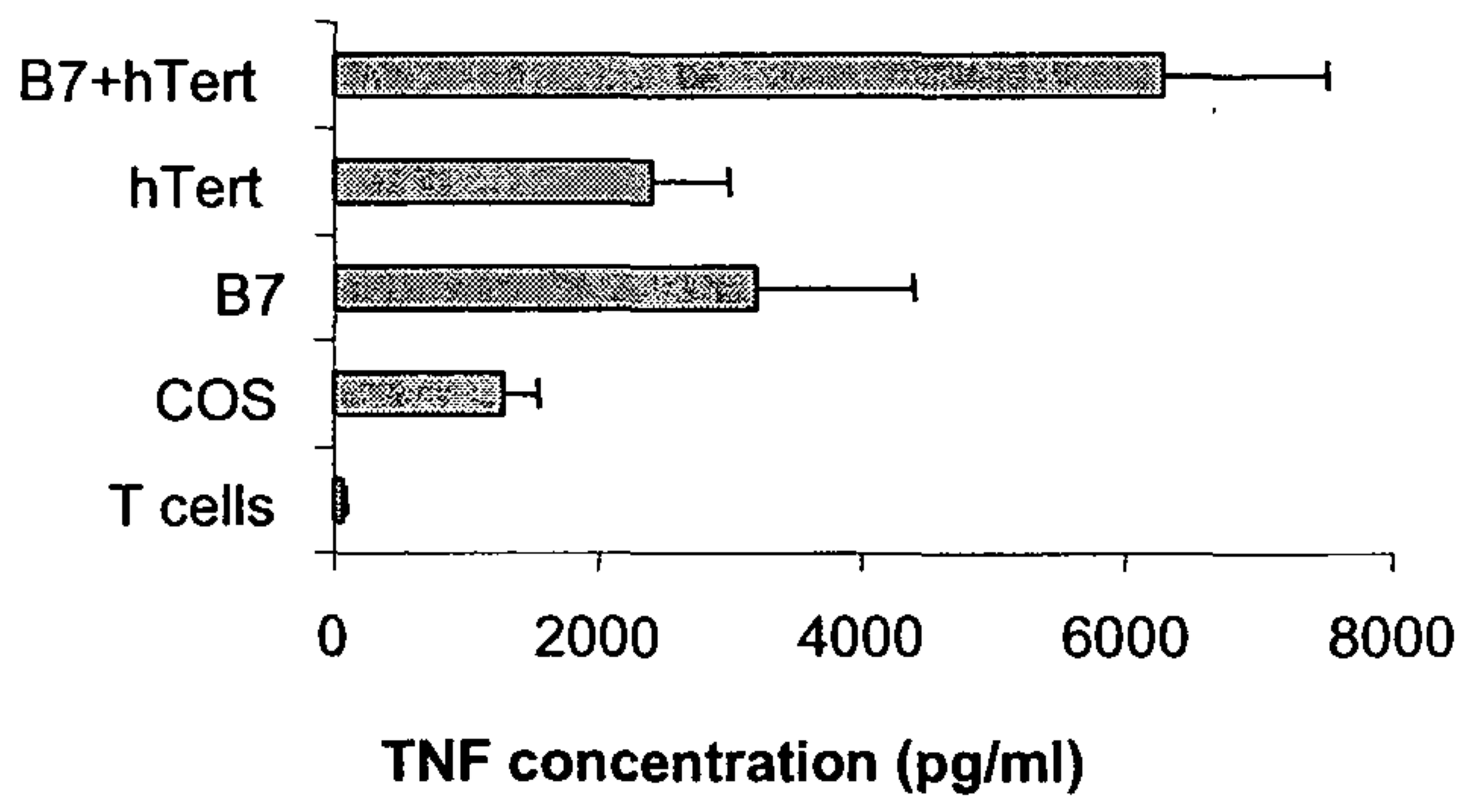
B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hTert
B7
COS
T cells
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2000
4000
6000
8000
TNF concentration (pg/ml)

Figure 5

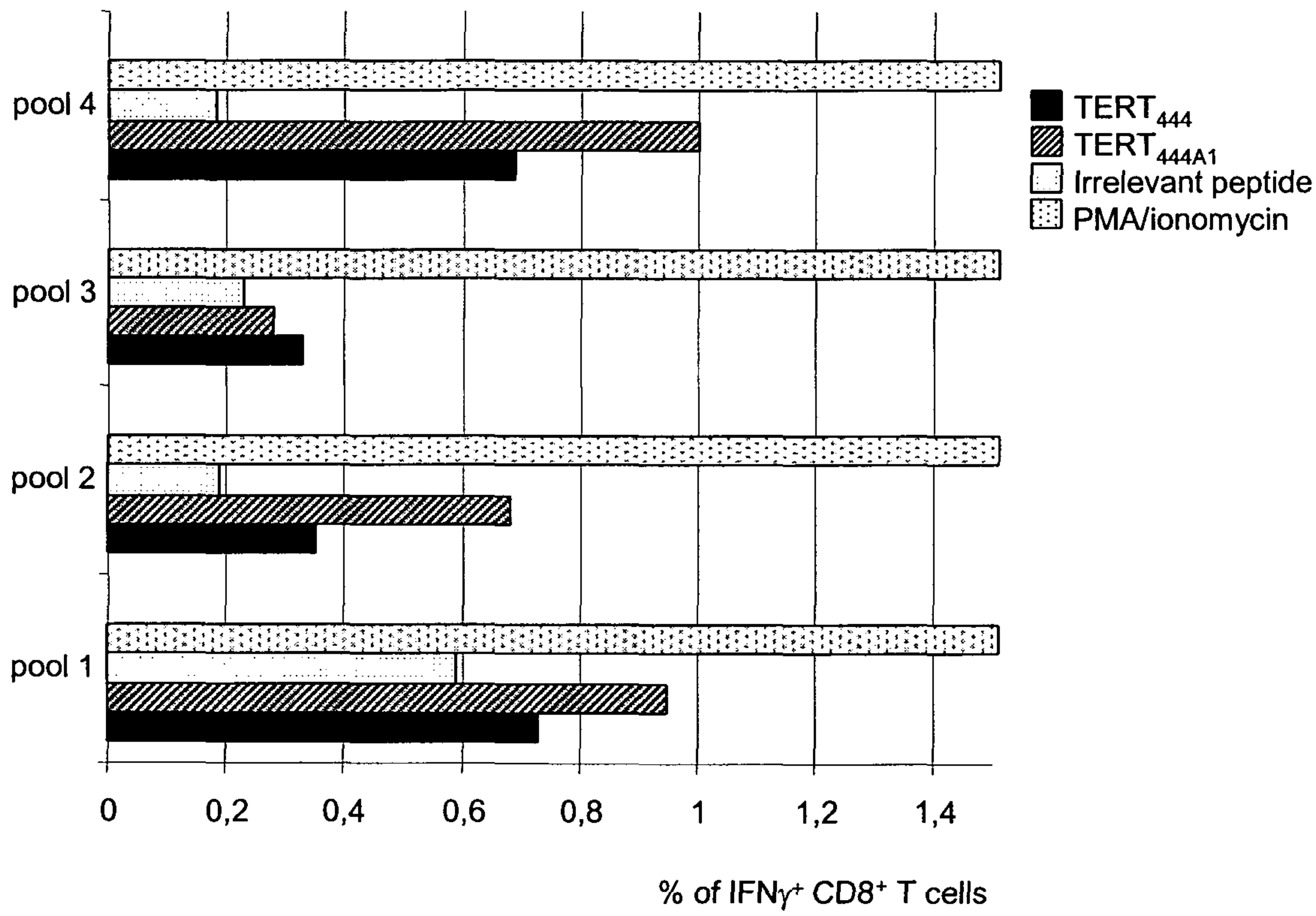
pool 4
pool 3
pool 2
pool 1
TERT444
TERT444A1
Irrelevant peptide
PMA/ionomycin
0
0,2
0,4
0,6
0,8
1
1,2
1,4
% of IFNγ+ CD8+ T cells

Figure 6