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More particularly, the invention provides immunogenic peptides capable of generating cytotoxic CD8 T-lymphocytes (CTLs) so that these cells eliminate undesirable cells such as SCLC cancerous cell, without reacting against non-cancerous such as neural cells.

The invention also provides nucleotides coding the immunogenic peptides and antibodies binding thereto.

The invention further relates to pharmaceutical compositions and to methods for inducing/stimulating of an immune response into a subject.

5 The invention also provides methods for purifying anti-Hu CTL cells and methods for treating a subject suffering of paraneoplastic neuropathy using these cells.

The invention is also concerned with methods for selecting therapeutic molecules capable of inducing an immune response *in vivo*.

10 An advantage of the present invention is that it allows to generate CTLs directed against HuD peptides, *in vivo* and *in vitro*. This is very interesting since CTLs that are potentially harmful to self are usually eliminated or tolerated.

Other objects and advantages of the present invention will be apparent upon reading the following non-restrictive detailed description, made with
15 reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B and 1C are graphs showing the induction of CTL against HuD and control FLU peptide in PBMC from normal blood donors. CD8 T cells from HLA A*0201 individuals were stimulated by autologous mDC pulsed with HuD-derived synthetic peptides as detailed in *materials and methods*. **Fig. 1A:** Results refer to effector cells from one donor immunized in vitro against p86. **Fig. 1B:** Results refer to effector cells from one donor immunized in vitro against p248.
20 **Fig. 1C:** Results refer to effector cells from one donor immunized in vitro against FLU peptide. X axis refers to Effector to Target ratio on an individual basis, Y axis to percentage of ⁵¹Cr release calculated as specified in materials and methods.
25

Figure 2: Shows the reading frame of the nucleotide sequence of HuD (SEQ ID NO:1; M62843) and the corresponding deduced amino acid sequence HuD (SEQ ID NO:2) from which HLA A2.1 peptides are obtained.
30

Figure 3: Shows the amino acid sequence of the HuD peptides (SEQ ID NOs:10-22) and, for each of these peptides, one of the many possible nucleotide sequences for encoding these peptides (SEQ ID NOs:30-42).
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DETAILED DESCRIPTION OF THE INVENTION

A) Definitions

In order to provide an even clearer and more consistent understanding of the specification and the claims, including the scope given herein to such terms,
5 the following definitions are provided:

The term “**analog**” as is generally understood and used herein, refers to a peptide that is substantially similar in function to the peptides of the invention.

Antibody : refers to a glycoprotein produced by lymphoid cells in response to a stimulation with an immunogen. Antibodies possess the ability to react *in vitro* and *in vivo* specifically and selectively with an antigenic determinant or
10 epitope eliciting their production or with an antigenic determinant closely related to the homologous antigen

As used herein, a protein/peptide is said to be a “**chemical derivative**” of another protein/peptide when it contains additional chemical moieties not
15 normally part of the protein/peptide, said moieties being added by using techniques well known in the art. Such moieties may improve the protein/peptide solubility, absorption, bioavailability, biological half life, and the like. Any undesirable toxicity and side-effects of the protein/peptide may be attenuated and even eliminated by using such moieties. For example, proteins/peptides can be
20 covalently coupled to biocompatible polymers (polyvinyl-alcohol, polyethylene-glycol, etc) in order to improve stability or to decrease/increase their antigenicity.

Derived: A protein/peptide is said to “derive” from a protein/peptide or from a fragment thereof when such protein/peptide comprises at least one portion, substantially similar in its sequence, to the native protein/peptide or to a fragment
25 thereof.

EL4S3 Rob mouse: Refers to mouse deposited at the CNCM under number I-258 (December 1,2000) which characteristics have been described in the J. exp. Med, vol. 185, number 12, June 16, 1997 2043-2051

Fragment: refers to a section of a molecule, such as protein/peptide or
30 nucleic acid, and is meant to refer to any portion of the amino acid or nucleotide sequence.

A “**functional derivative**”, as is generally understood and used herein, refers to a protein/peptide sequence that possesses a functional biological activity that is substantially similar to the biological activity of the whole protein/peptide
35 sequence. A functional derivative of a protein/peptide may or may not contain post-translational modifications such as covalently linked carbohydrate, if such

modification is not necessary for the performance of a specific function. The term "functional derivative" is intended to the "fragments", "segments", "variants", "analogs" or "chemical derivatives" of a protein/peptide.

5 **Fusion protein:** A protein formed by the expression of a hybrid gene made by combining two gene sequences. Typically, this is accomplished by cloning a cDNA into an expression vector in frame with an existing gene.

HHD mouse: Refers to a transgenic mouse deposited which characteristics have been described in the European Journal of Immunology (1999), 29:3112-3121.

10 **Immunogenic:** Refers to the property of a molecule or compound, such as a protein/peptide to induce *in vivo* or *in vitro* a cellular or humoral immune response.

Immune response: Refers to an *in vivo* or *in vitro* reaction in response to a challenge by an immunogen. An immune response is generally expressed by
15 an antibody production and/or a cell-mediated immunity or immunologic tolerance.

Isolated or Purified: Means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide naturally present in a living
20 organism is not "isolated", the same polynucleotide separated from the coexisting materials of its natural state, obtained by cloning, amplification and/or chemical synthesis is "isolated" as the term is employed herein. Moreover, a polynucleotide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said
25 organism.

Oligonucleotides means nucleic acid, either deoxyribonucleic acid (DNA), or ribonucleic acid (RNA), in single-stranded or double-stranded form and any chemical modifications thereof. Such modifications include, but are not limited to providing other chemical groups that incorporate additional charge,
30 polarizability, hydrogen bonding or electrostatic interaction to one or more of nucleic acid bases of the oligonucleotide. Examples of modifications are, but are not limited to, modifying the bases such as substitution of 5-bromouracil, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines, 2'-position sugar modifications, methylations, unusual
35 base-pairing combinations such as the isobases isocytidine and isoguanidine, backbone modifications, 3' and 5' modifications such as capping, and the like. Are

also compatible with the current invention, modifications that occur after each round of amplification in a reversible or irreversible manner.

The term "**peptide**" herein includes any natural or synthetic compounds containing two or more amino acids. Therefore, it comprises proteins, glycoproteins, and protein fragments derived from pathogenic organisms such as viruses, bacteria, parasites and the like, or proteins isolated from normal or pathogenic tissues, such as cancerous cells. It also includes proteins and fragments thereof produced through recombinant means that has been associated or not with other peptides coding for tumoral, viral, bacterial or fungic epitopes for forming a fusion protein.

Polynucleotide: Any DNA, RNA sequence or molecule having one nucleotide or more, including nucleotide sequences encoding a complete gene. The term is intended to encompass all nucleic acids whether occurring naturally or non-naturally in a particular cell, tissue or organism. This includes DNA and fragments thereof, RNA and fragments thereof, cDNAs and fragments thereof, expressed sequence tags, artificial sequences including randomized artificial sequences.

The term "**variant**" as is generally understood and used herein, refers to a protein that is substantially similar in structure and biological activity to either the protein or fragment thereof. Thus two proteins are considered variants if they possess a common activity and may substitute each other, even if the amino acid sequence, the secondary, tertiary, or quaternary structure of one of the proteins is not identical to that found in the other.

Vector: A self-replicating RNA or DNA molecule which can be used to transfer an RNA or DNA segment from one organism to another. Vectors are particularly useful for manipulating genetic constructs and different vectors may have properties particularly appropriate to express protein(s) in a recipient during cloning procedures and may comprise different selectable markers. Bacterial plasmids are commonly used vectors.

B) General overview of the invention

The present invention relates to Hu antigen-derived peptides. The peptides of the invention are useful for the prevention and treatment of certain types of cancers, and more particularly for the elimination of cancerous cells in vertebrates by inducing specific cytotoxic CD8 T-lymphocytes (CTLs). Advantageously, the induced specific CTLs according to the invention are

capable of eliminating, from a subject, specific cells such as SCLC cancerous cells, without reacting against non-cancerous cells such as neural cells.

The peptides of the invention are also useful for the treatment or the prevention of nervous system damage diseases.

5 The peptides of the invention may also be used for detecting an early CTL response against SCLC, for detecting an early CTL response against neuronal cells or for detecting neuronal damage in a subject.

10 The peptides of the present invention may be used in all members of the class Vertebrates. Preferably, the vertebrate is a mammalian subject including, without limitation, human and non-human primates, farm animals, domestic animals, laboratory animals.

C) Immunogenic Peptides and corresponding Nucleotides

15 In one aspect, the invention is directed to immunogenic peptides that derive from the Hu antigen. Advantageously, the peptides of the invention are capable of inducing an immune response against a cancerous cell of a subject without inducing an immune response against non-cancerous cells of said subject.

20 More particularly, the immunogenic peptides according to the invention can induce *in vitro*, *ex vivo* and/or *in vivo* specific cytotoxic CD8 T-lymphocytes (CTLs) capable of eliminating specifically small-cell lung cancers, neuroblastomas, sarcoma and prostate carcinoma. Preferably, the immunogenic peptides are nonameric or docameric peptides and more preferably, they are selected from the group consisting of SEQ ID NOs:20 to 31, and 32 (see Figure
25 3).

30 The peptides of the present invention may be prepared by any suitable process. Preferably, they are obtained by chemical synthesis, but they may also be obtained using biological processes using expression vectors comprising a polynucleotide sequence encoding for the peptide of interest. They may also be incorporated in polypeptides having a length varying from about 10 to about 50 amino acids, preferably 15 amino acids. According to a preferred embodiment, the peptides are incorporated in a tetrameric complex of HLA-A2.2 comprising a plurality of identical or different peptides/polypeptides according to the invention. According to another preferred embodiment, the peptides of the invention are
35 incorporated into a support comprising at least two peptide molecules. Examples

of suitable support include polymers, lipidic vesicles, microsphere, latex beads, polystyrene beads, proteins and the like.

The reading frame of the nucleotide sequence of HuD is known (GENBANK™ No. M62843, see SEQ ID NO:1 at Fig. 2), as well as the
5 corresponding deduced amino acid sequence HuD (see SEQ ID NO:2 at Fig. 2). Figure 3 shows the amino acid sequence of the HuD peptides of the invention (SEQ ID NOs:10-22) and, for each of these peptides, the corresponding nucleotide sequence encoding these peptides (SEQ ID NOs:30-42). However, since the genetic code is degenerated, it is clear that the nucleotide sequences
10 given in Figure 3 are, for each of these peptides, one specific example of the many possible examples of sequence for coding these peptides. A person skilled in the art will easily be capable of determining other nucleotide sequences coding for the peptides of the present invention.

In another aspect, the invention is directed to a method for producing, *in*
15 *vitro*, an immunogenic peptide, comprising: culturing *in vitro*, in a suitable culture medium, a cell incorporating an expression vector as described previously; and collecting in the culture medium immunogenic peptides produced by these cells. Therefore, the invention is also concerned with cells transformed for expressing the peptides of the invention. Methods for producing such cells and methods for
20 using these cells in the production of proteins/peptides are well known in the art and will no be described in detail herein.

The peptides, polypeptides and polynucleotides of the invention may also be used for producing polyclonal or monoclonal antibodies capable of recognizing and binding the same. Methods for producing such antibodies are well known in
25 the art. These antibodies may be used for the preparation of a medicine for the treatment of human SCLC or for the treatment of nervous system damages caused by anti-Hu.

D) Pharmaceutical Compositions

30 The peptides/polypeptides of the present invention, the polynucleotides coding the same, and polyclonal or monoclonal antibodies produced according to the invention, may be used in many ways as antitumoral agents, for the preparation of pharmaceutical compositions, for the preparation of an antitumoral vaccine, for the treatment or the prevention of HLA-A2.1 human SCLC and/or for
35 the treatment or the prevention of nervous system damage diseases.

Therefore, in another aspect, the invention is directed to pharmaceutical compositions comprising:

a) at least one component selected from the group consisting of:

- an immunogenic peptide/polypeptide and/or a polynucleotide and/or a fragment thereof and/or an antibody as defined previously; and
- Hu specific CD8 T-cells primed against an immunogenic peptide/polypeptide and/or a fragment thereof;

and

b) a pharmaceutically acceptable vehicle or carrier.

The compositions of the invention may be in a solid or liquid form or in any suitable form for a therapeutic use. They may be formulated for a rapid or slow release of its components and may further comprise compounds for stimulating/inhibiting the immune system. The compositions of the invention may be prepared according to conventional methods known in the art.

E) Stimulation of a Immune Response

In another aspect, the invention is directed to a method for priming *in vitro* immune cells of a eukaryotic subject, comprising the steps of:

a) isolating HLA-A2.1 lymphoid or myeloid cells from said subject; and

b) priming *in vitro* the cells isolated at step a) with at least one immunogenic peptide/polypeptide and/or polynucleotide as defined previously.

According to a preferred embodiment, the method further comprises the steps of:

c) isolating CD8+ T-cells from the subject; and

d) using the cells primed at step b) for priming *in vitro* the CD8+ T-cells isolated at step c).

In a further aspect, the invention is directed to a method for stimulating a subject immune response comprising:

- administering into a compatible subject HLA-A2.1 lymphoid or myeloid cells primed *in vitro* using the method defined hereinabove; and/or
- administering into a compatible subject CD8+ T-cells primed according to the method using the method defined hereinabove.

The invention also provides an *ex vivo* stimulation process of the human immune response. This method comprises the steps of: separating the autologous lymphoid or myeloid cells, then incubating *in vitro* of the separated

cells with an immunogenic peptide/polypeptide and or a polynucleotide as defined previously, these cells allowing the induction of a cytotoxic response *in vitro*.

In another aspect, the invention is directed to a method for detecting an early CTL response against SCLC, to a method for detecting an early CTL response against neuronal cells and to a method for detecting neuronal damage in a subject, comprising:

- providing a tetrameric complex of HLA-A2.2 as defined previously;
- incubating this complex with peripheral blood lymphocytes of a subject; and
- determining the presence of Hu specific CTL.

More specifically, for detecting an early CTL response against SCLC, the presence of SCLC Hu specific CTLs is determined, whereas for detecting an early CTL response against neuronal cells, the presence of neuronal Hu specific CTLs is determined. The presence of Hu specific CTLs may be determined by comparing cells from the subject with normal cells in culture using any suitable method such as FACS.

In another aspect, the invention is directed to a method for stimulating a human immune response against SCLC, comprising:

- isolating from a human SCLC Hu specific CTLs;
- amplifying *ex vivo* the isolated SCLC Hu specific CTLs; and
- injecting in a compatible human suffering of SCLC, the SCLC Hu specific CTLs.

F) Purification of anti-Hu CTL cells and Use thereof

In another aspect, the invention is directed to a method for purifying anti-Hu CTLs from blood of a subject, comprising the steps of:

- fixing on a column a tetrameric complex of HLA-A2.2 comprising at least one immunogenic peptide as defined previously;
- passing blood lymphocytes from said subject into the column ; and
- recovering anti-Hu CTLs retained by the column.

Such anti-Hu CTLs may be used for treating a subject suffering from paraneoplastic neuropathy by injecting thereto the purified anti-Hu CTLs.

G) Selection of Therapeutic molecules

In a further aspect, the invention provides a method for selecting therapeutic molecules capable of inducing an immune response *in vivo* against at least one of the peptides/polypeptides defined previously. The method comprises the steps of:

- a) administering EL4S3-Rob tumoral cells as defined previously to a first animal model having a genotype compatible with the tumoral cells and having beforehand been immunized with the therapeutic molecules to be selected; and
- 5 b) comparing the immune response of the first animal model with the immune response of a second animal model having beforehand been immunized with the therapeutic molecules to be selected.

By comparing the immune response between the two animal models, it is possible to select therapeutic molecules capable of inducing an immune response *in vivo* against at least one of the peptides/polypeptides defined previously. For instance, this method may help in evaluating peptidic sequence or nucleotide sequence capable of inducing an *in vivo* response against non modified tumoral epitopes, viral epitopes, bacterial epitopes, or fungic epitopes.

15

EXAMPLE

Cytotoxic T lymphocytes can be generated against Hu antigen-derived peptides: a new step in deciphering pathogenesis of anti-Hu syndrome.

The following example is illustrative of the wide range of applicability of the present invention and is not intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

25

A) Introduction

Paraneoplastic sensory neuronopathies, often associated with encephalomyelitis (PEM/SN), are commonly associated with circulating anti-Hu antibodies. More than 80% of patients with "anti-Hu syndrome" have a small-cell lung cancer (SCLC), which has limited extension in this particular setting (Dalmau, Medicine, 1992). Anti-Hu antibodies specifically react with a set of protein antigens, expressed by both neurons and SCLC cells (Dalmau, Am J Path, 1992). It is currently believed that expression of HuD protein by the tumor triggers an immune response misdirected against homologous proteins

expressed in the nervous system (Darnell, PNAS, 1996[rev]). It now seems clear, however, that anti-Hu antibodies do not cause neuronal damage by themselves (Hormigo, J Neuro immunol, 1994). In contrast, there are arguments suggesting a T-cell mediated autoimmune process, including : (i) the finding of T-cells in DRGs
5 (Wanschitz, Graus 1990, Walter 1994, Panegyres 1993), where CD8⁺ T-cells may be seen indented in neurons (Wanschitz) ; (ii) repertoire analysis showing similar oligoclonal T-cell populations, mainly CD8⁺, in inflamed nervous tissue and tumor (Voltz, 1998).

HuD is encoded in the genome and is in all respects a self-antigen (Szabo,
10 Cell, 1991 and Dalmau, Am J Pathol, 1992). Consequently, CD8⁺ T-cells with a receptor for MHC/HuD peptide complexes are expected to be eliminated during thymic negative selection, reducing the potential precursor T-cell repertoire and imposing limitations on their expansions on encounter with tumor cells in adult life. Additionally, stimulation by antigen in the absence of a second signal induces
15 clonal anergy (Schwartz, Science, 1990), further hampering the potential repertoire of T-cells directed against systemic self-antigens. However, clonal anergy may be irrelevant in CNS, due to absence of HLA class 1 expression in functional neurons. Eventually, the extent to which these events affect the normal adult repertoire is not known. Answering these questions is relevant to future
20 strategies of immune intervention targeted at tumor antigens, which are also self antigens, that should be designed to avoid triggering auto-immune destructive processes.

We addressed this question using two systems : first, we evaluated the immunogenic potential of the HuD peptides in H-2 class 1 knockout, HLA-
25 A*0201transgenic mice (HHD mice) (Pascolo, J exp Med, 1997). Expected results are entirely relevant in a murine model because potentially immunogenic HuD peptides are identical in both murine and human HuD protein. Second, we analyzed the ability of normal individuals to mount a CTL response in vitro against HuD peptides restricted by the HLA-A2 allele.

30 In that respect, we decided to test the prevalent hypothesis of HuD as immune target : (i) - in HHD mice by peptidic vaccination to obtain cytotoxic T-cell

clones directed at HuD peptides (ii) - by artificially generating human cytotoxic T cells directed against peptides derived from HuD sequence.

B) Materials and Methods

5 Synthetic peptides.

HuD synthetic peptides included 8 nonamers : p163 (¹⁶³ILVDQVTGV¹⁷¹), p362 (³⁶²RLGDRVQLQV³⁷⁰), p248 (²⁴⁸NLLNMAYGV²⁵⁶), p111 (¹¹¹RLQTKTIKV¹¹⁹), p315 (³¹⁵QLFGPFGAV³²³), p314 (³¹⁴WQLFGPFGA³²²), p251 (²⁵¹NMAYGVKRL²⁵⁹), p64 (⁶⁴SLFGSIGEI⁷²); 5 decamers : p86 (⁸⁶SLGYGFVNY⁹⁵), p162 (¹⁶²RILVDQVTGV¹⁷¹), p133 (¹³³NLYVSGLPKT¹⁴²), p303 (³⁰³NLSPDSDESV³¹²), p314 (³¹⁴WQLFGPFGAV³²³) and p280 (²⁸⁰GMTSLVGMNI²⁸⁹). These peptides were purchased from Syntem (Nîmes, France), as well as control peptide for MHC binding and stabilization assay, HIV-1 pol p589 (⁵⁸⁹IVGAETFYV⁵⁹⁷).

15 Peptide ¹²⁸TPPAYRPPNAPIL¹⁴⁰ of the hepatitis B core antigen, peptide ⁵⁷¹YLSGANLNL⁵⁷⁹ of carcinoembryonic antigen, TcyP9 of Trypanozoma cruzi and Peptide FLU were also purchased, but from a different source.

Peptides were dissolved in DMSO (5μl/mg peptide) and subsequently diluted in PBS or dH2O. HuD peptides were diluted at 4mg/ml, other peptides
20 were diluted at 2mg/ml.

Cell lines and antibodies

Cell lines :

H69 (HTB-119) is a A2.1 small cell lung cancer cell line obtained from the
25 American Type Culture Collection.

Antibodies :

Human anti-Hu serum was obtained from patients with anti-Hu syndrome from different hospitals.

Human blood cells

Peripheral Blood Mononuclear Cells (PBMC) were obtained from leukapheresis from 2 donors of HLA-A*0201 group. Autologous immature dendritic cells (iDC) were obtained from monocytes as follows : PBMC were
 5 incubated at 10^7 cells/mm³ in 6-well plaques for 2 hours. Non adherent cells were removed by successive washes in RPMI. Adherent cells were subsequently induced to differentiate into iDC in RPMI with 2% SAB, GM-CSF and IL-4 for 6 days (Sallusto, 1994). iDC were frozen in DMSO until use. At the time of
 10 induction, iDC were thawed and allowed to differentiate in mature dendritic cells (mDC) by a 48-hours-long incubation with TNF- α , IFN- γ , and PGE-2. CD8⁺ T-cells, obtained from frozen PBMC, were purified with immuno-magnetic beads, eliminating CD4⁺ T cells, B cells, CD56⁺ cells and monocytes (Milteny et al.) and TCR β ⁺ T-cells (Milteny et al.).

15 HLA A2.1 Binding/Stabilization Assay

Binding assay : T2 (TAP-, HLA-A2.1) cells were incubated overnight at 37°C (3×10^5 /ml) in FCS-free RPMI medium supplemented with 100 ng/ml human β 2microglobulin (Sigma, St Louis, MO) in the absence (negative control) or
 20 presence of either reference HIV-1 pol 589-597 or tested HuD peptides at various final concentrations (100, 10, 1 and 0.1 μ M). T2 cells were subsequently labeled for 30mn at 4°C with a saturating concentration of anti-HLA-A2.1 BB7.2 antibody, then washed three times in PBS. The cells were then incubated for 30 min at 4°C with a saturating concentration of FITC-conjugated goat anti-mouse IgG (H+L) (Caltag, South San Fransisco), washed three times and fixed in PBS/1%
 25 paraformaldehyde and analyzed using a COULTER XL™ cytofluorometer (Beckman-Coulter, Nyon, Switzerland). The mean intensity of fluorescence (MIF) observed for each peptide concentration (after subtraction of the MIF observed without peptide) was used as an estimate of peptide binding. For each peptide, the concentration needed to reach 20% of the maximal binding (as defined with
 30 100mg/ml of HIV pol 589 peptide) was calculated. RA is the ratio of the concentrations of tested and HIV pol 589 reference peptides needed to reach this value. The lower the RA, the stronger the binding.

Stabilization assay: Stabilization assays were performed similarly. Following initial overnight incubation with 50 μ l/2ml well of peptide at 100 μ M, cells were washed in RPMI complete medium to remove free peptides and incubated in the continuous presence of 10 μ g/ml brefeldine A for 2, 4, and 6 h. The amount of stable peptide-HLA-A2.1 complexes was estimated as described above by indirect immunofluorescence analysis at t0, t2, t4 and t6. The half-life of complexes is the time required for a 50% reduction of the t0 MIF value.

10 Mice

HHD mice express a transgenic monochain histocompatibility class 1 molecule in which the C terminus of the human β 2microglobulin is covalently linked to the N terminus of a chimeric heavy chain (HLA-A2.1 α 1- α 2, H-2D^b α 3 transmembrane and intracytoplasmic domains; Pascolo, J exp Med 1997). The H-2D^b and mouse β 2microglobulin genes of these mice have been disrupted by homologous recombination resulting in complete lack of serologically detectable cell surface expression of mouse histocompatibility class 1 molecules.

In vivo immunization, generation of CTL, and cytotoxic assay

Each mouse was injected s.c. at the base of the tail with 100 μ g of individual HuD peptide, and 50 μ g of helper peptide ¹²⁸TPPAYRPPNAPIL¹⁴⁰ of the hepatitis B core antigen, emulsified in incomplete Freund's adjuvant. After 2 weeks, half of the mice were restimulated, 2.5 $\times$ 10⁷ RBC-depleted splenocytes were restimulated in vitro in complete RPMI 1640 medium (10%FCS, 2mM L-glutamine, 5.10⁻⁵ β -mercaptoethanol, 50U/ml penicillin, 50 μ g/ml streptomycin, and 10% TCGF) and 40 μ g of corresponding HuD peptide.

After 5 days, responder mice were individually tested in a standard cytolytic 4-h ⁵¹Cr-release assay. Briefly, Target HHD-transfected TAP⁻ RMA-S cells were loaded with relevant or negative control peptide ⁵⁷¹YLSGANLNL⁵⁷⁹ of carcinoembryonic antigen (10 μ g/ml, 5 $\times$ 10⁶ cells/ml, in FCS-free RPMI medium,

2 hr at room temperature. Specific lysis was calculated as follows : (experimental release – spontaneous release)/(total release-spontaneous release) ×100.

Generation of cytotoxic T-cell clones

5 Spleen cells from primed mice were restimulated weekly using irradiated (5000 rads) peptide-loaded ($10\mu\text{g/ml}$, 5×10^6 cells/ml, in FCS-free RPMI medium, 2 hr at room temperature) HHD splenocytes, and grew in complete RPMI medium supplemented with 10%FCS, 2mM L-glutamine, $5 \cdot 10^{-5}$ β -mercaptoethanol, 50U/ml penicillin, 50 $\mu\text{g/ml}$ streptomycin, and 10% TCGF.

10 After 3 to 5 restimulations, the cytotoxic T cell lines were cloned in limiting dilution cultures at 1, 10 and 20 cells per well in the presence of irradiated, peptide-pulsed HHD splenocytes. After two weeks, the isolated clones were assayed for cytolytic activity against peptide-pulsed HHD transfected RMA-S cells. Cytotoxic clones were amplified and restimulated every week.

15

Adoptive transfer

Between 8 and 17×10^6 HuD or TCyP1 clones were purified from feeder cells by FYCOLL™ gradient separation, washed three times in PBS, and injected i.v. (retroorbital veins) in HHD mice. In some instances, mice had received 24h before 5×10^4 U/ml of murine recombinant γ -IFN administered i.v..

20

In vitro immunization of human precursor T cells, generation of CTL, and cytotoxic assay

Sorted CD8 T-cells were plated in 96-well plates at 10^6 cells /ml in RPMI medium supplemented with 10% human AB⁺ serum, L-glutamine, sodium pyruvate and antibiotics. mDC were pulsed for two hours at 37°C with saturating concentrations of peptides ($10\mu\text{g/ml}$), irradiated at 30 Gy, washed once, and added to CD8 T-cells at a responder to stimulator ratio (R :S) of 5 :1. IL-6 (10 ng/ml) and IL-12 (1 ng/ml) were added. After 3 days, IL-2 (25 U/ml) was added to the medium. Lymphocytes were restimulated weekly with peptide-pulsed irradiated autologous PBMC. IL-2 and IL-7 (10 ng/ml) were added to the cultures

25

30

the same day, and IL-2 alone after 3 days. Two rounds of restimulation were performed before the cytotoxicity assays.

The induction of CTL in human PBMC was monitored in a conventional ^{51}Cr -labeling release assay. Briefly, peptide-pulsed TAP-/HLA-A*0201 human T2 cells were incubated with 10 $\mu\text{g}/\text{ml}$ of HuD peptide or Flu control peptide for 120 min at 37°C followed by incubation with ^{51}Cr for 60 min. After washing, the target cells were added to serially diluted effectors in 96-well microplates. After a 4-h incubation at 37°C, supernatants were harvested and counted in a gamma counter. Results are expressed as the percentage of specific lysis and determined as follows : $[(\text{experimental cpm} - \text{spontaneous cpm}) / (\text{maximum cpm} - \text{spontaneous cpm})] \times 100$.

C) Results

Identification and Analysis of HLA – A*0201 – restricted HuD Peptides.

The amino acid sequence of HuD (Szabo Cell, 1991) was analyzed for 9- and 10mer peptides sequences containing known binding motifs for the HLA-A*0201 molecule (Ruppert, Sette, Cell 1993), a subtype encompassing 95% of the HLA A2 allele which is expressed in about 50% of the Caucasian population (Lee, 1990, Fernandez, 1992, Krausa, 1995). Peptides were identified by reverse genetics based on canonical anchor residues for HLA – A*0201 (Krausa, 1995), and by using the software of the Bioinformatics and Molecular Analysis Section (National Institute of Health, Washington, DC) available at http://bimas.dcrt.nih.gov/molbio/hla_bind/index.html which ranks 9- or 10-mer peptides on a predicted half-time dissociation coefficient from HLA class I molecules (Parker, JI, 1994). From an initial panel of thirty 9mer and thirty 10mer peptides, we retained 14 sequences, eight 9mer and six 10mer peptides (table 1).

Because the immunogenicity of MHC class-I-restricted peptides reflects to some degree their binding and stabilizing capacity for MHC class-I molecules (Vitiello, J exp Med 1991, Sette JI, 1994, van der Burg, JI 1996), we sought direct proof of the strength of interaction between the 14 HuD peptides and the HLA-A*0201 molecule in a binding/stabilization assay that uses the antigen-transporting deficient (TAP-) HLA-A*0201 human T2 cells. The RA was

calculated in reference to HIV-1 pol p589 peptide. Four to five tests were performed for each peptide. Mean values are indicated in Table 1. Stability of each peptide bound to HLA-A*0201 was measured as the half-life of the complex. Mean values of the 3 to 4 tests performed for each peptide are indicated in Table 1.

Table 1. MHC binding and stabilization of HuD peptides on HLA A*0201.

Sequence	Predicted score ⁽⁻⁾	RA ^{\$}	T1/2 [£]
¹⁶³ <u>ILVDQVTGV</u> ¹⁷¹	1115	0.20	8
³⁶² RLGDRVLQV ³⁷⁰	656	1.08	6
²⁴⁸ NLLNMAYGV ²⁵⁶	258	0.62	6
¹¹¹ RLQTKTIKV ¹¹⁹	70	1.60	4
³¹⁵ QLFGPFGAV ³²³	64	0.49	2
³¹⁴ WQLFGPFGA ³²²	50	0.34-0.4-0.63-2.3-*	2
²⁵¹ NMAYGVKRL ²⁵⁹	35	*/*/*	nd
⁶⁴ SLFGSIGEI ⁷²	12	2.2	6
⁸⁶ <u>SLGYGFVNYI</u> ⁹⁵	520	0.88	6
¹⁶² RILVDQVTGV ¹⁷¹	81	0.53	8
¹³³ NLYVSGLPKT ¹⁴²	50	* /*/*	nd
³⁰³ NLSPDSDES ³¹²	35	*/*/*	nd
³¹⁴ WQLFGPFGAV ³²³	29	0.62	4
²⁸⁰ GMTSLVGMNI ²⁸⁹	11	5.7-11.7-*-*	nd

⁽⁻⁾ : as defined by Parker et al (-). ^{\$} : RA is the ratio of the concentrations of tested and HIV pol589 reference peptides needed to reach this value. The lower the RA, the stronger the binding. Each value corresponds to the average result of 4 or 5 MHC binding assays. [£] : half-life of peptide-HLA-A2.1 complexe : time required for a 50% reduction of the t0 MIF value. 8 hours is the highest time tested. Each value corresponds to the average of 3 or 4 tests.

CTL response against HuD in HHD mice.

Whether peptides can serve as immunogens in vivo and elicit a CTL response depends on a variety of factors such as the mode of immunization, suitable activation of antigen presenting cells, the frequency of precursor cells, and binding and stabilization of MHC class I molecules by peptide. Also, whereas

the sole immunization with class I-restricted synthetic peptides of optimal size is sufficient for the induction of CTL responses in some cases (Vasilakos, JI 1993), the need for help has been documented in other circumstances (Ossendorp, J exp Med 1998). Therefore, the CTL responses of HHD mice which express H-2^b class II molecules were tested by co-injecting HuD peptides and the IA^b-restricted HBVc.128 peptide (Milich, PNAS, 1988). In these mice, the peripheral CD8 T-cell repertoire is essentially educated on the transgenic human molecule. Therefore, HHD mice are an excellent tool to assess at the pre-clinical level the ability of individual peptides to induce HLA-A*0201-restricted CTL responses in vivo (Firat, Eur J I 1999). 10/14 peptides were satisfying with regard to MHC binding status and were therefore injected into HHD mice (Table 2). 7/10 peptides elicited CTL responses in a proportion of injected mice. CTL lines were derived and then cloned by limit dilution method. Cytotoxic clones were obtained for p248(²⁴⁸NLLNMAYGV²⁵⁶), p86 (⁸⁶SLGYGFVNY¹⁹⁵), p111 (¹¹¹RLQTKTIKV¹¹⁹), p314 (³¹⁴WQLFGPFGAV³²³). Although overall correlation between the results of binding/stabilization of the HLA A*0201 molecule is expected (Firat), in this study, p163(¹⁶³ILVDQVTGV¹⁷¹)and p362 (³⁶²RLGDRV LQV³⁷⁰) were the most efficiently binding and stabilizing peptides, and yet elicited only 1/6 and 0/6 responses respectively.

Table 2. Induction of CTL against HuD in HLA-A2.1 transgenic mice.

HuD peptide	Nr. of responders	Percent lysis [£]
¹⁸³ <u>ILVDQVTGV</u> ¹⁷¹	1/6	62
³⁶² RLGDRV LQV ³⁷⁰	0/6	
²⁴⁸ NLLNMAYGV ²⁵⁶	3/6	44
¹¹¹ RLQTKTIKV ¹¹⁹	1/5	55
³¹⁵ QLFGPFGAV ³²³	0/5	
³¹⁴ WQLFGPFGA ³²²	0/5	
⁶⁴ SLFGSIGEI ⁷²	2/5	60
⁸⁶ <u>SLGYGFVNYI</u> ⁹⁵	2/6	15
¹⁶² RILVDQVTGV ¹⁷¹	3/6	16
³¹⁴ WQLFGPFGAV ³²³	1/5	57

[£] : Values of cytotoxicity refer to cytotoxicity of highest responder at an effector-to-target ratio of 60 :1.

5

CTL response against HuD in normal human individuals.

The presence of precursor T cells was tested for 8/10 HuD peptides, which gave results in HHD mice, plus p362, which bound and stabilized HLA-A*0201 very efficiently and yet elicited no response in HHD mice. Expansion on antigen stimulation was tested by using PBMC and autologous monocyte-derived dendritic cells of 2 HLA-A*0201 normal blood donors in an *in vitro* assay. T cells that lysed peptide-pulsed T2 cells as targets starting from the third round of peptide stimulation were generated in response to 2 peptides, p248 (²⁴⁸NLLNMAYGV²⁵⁶) and p86 (⁸⁶SLGYGFVNY⁹⁵). The same procedure was applied to generate CTL against FLU peptide to serve as irrelevant CTL against H69. Maximal specific lysis at E:S ratio 50:1 was 75% for p248 (Fig 1A), maximal specific lysis at E:S ratio 25:1 was 57% (Fig 1B). For one peptide, p111 (¹¹¹RLQTKTIKV¹¹⁹), there was a measurable nonspecific lysis (20% at E:S ratio 50:1), although the expanded population consisted of 90% of CD8 T cells (data not shown).

20

CTL response against H69 in normal human individuals.

Human CTL generated against p248 and p86, as well as control CTL generated against FLU peptide were tested against H69 HLA A*0201 SCLC cell line. H69 was incubated with 100U/ml of recombinant IFN-g for 48h before the ⁵¹Cr-labeling release assay to upregulate HLA class I expression (Doyle, J Exp Med 1985 and Traversari, J I Ther, 1997). No lysis was obtained against H69 (Fig1), although H69 pulsed according to the same protocol as T2 cells could be lysed by CTL against p86 (Fig 1A).

10 **E) References**

Throughout this application, reference is made to a number of articles of scientific literature that are listed below and incorporated herein by reference:

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While several embodiments of the invention have been described, it will be understood that the present invention is capable of further modifications, and this

application is intended to cover any variations, uses, or adaptations of the invention, following in general the principles of the invention and including such departures from the present disclosure as to come within knowledge or customary practice in the art to which the invention pertains, and as may be
5 applied to the essential features hereinbefore set forth and falling within the scope of the invention or the limits of the appended claims.

WHAT IS CLAIMED IS:

1. A purified immunogenic peptide characterized in that it derives from the Hu antigen.

5

2. The immunogenic peptide according to claim 1, characterized in that it is capable of inducing an immune response against a cancerous cell of a subject without inducing an immune response against non-cancerous cells of said subject.

10

3. The immunogenic peptide according to claim 1 or 2, characterized in that said cancerous cells are selected from the group consisting of small-cell lung cancers, neuroblastomas, sarcoma and prostate carcinoma.

15

4. The immunogenic peptide according to any one of claims 1 to 3, characterized in that said immune response is an *in vitro*, *ex vivo* and/or *in vivo* CTL response.

20

5. The immunogenic peptide according to any one of claims 1 to 4, characterized in that it is a nonamer or a decamer.

6. The immunogenic peptide according to any one of claims 1 to 5, characterized in that the Hu antigen comprises SEQ ID NO:2.

25

7. The immunogenic peptide according to any one of claims 1 to 6, characterized in that it is selected from the group consisting of SEQ ID NOs:10 to 22 and functional derivatives thereof.

30

8. The immunogenic peptide according to any one of claims 1 to 7, characterized in that its administration to an HLA-A2.1 human induces the activation of a specific CTL response against autologous or syngenic small-cell lung cancer (SCLC).

35

9. The immunogenic peptide according to any one of claims 1 to 8, characterized in that it is incorporated into a support comprising at least two molecules of said peptide.

10. The immunogenic peptide according to claim 9, characterized in that said support is selected from the group consisting of polymers, lipidic vesicles, microsphere, latex beads, polystyrene beads, and proteins.

5 11. A purified polypeptide characterized in that it comprises an immunogenic peptide according to any one of claims 1 to 10.

12. A polypeptide according to claim 11, characterized in that it has a length varying from about 10 to about 50 amino acids, and preferably 15 amino acid.

10

13. A purified polyclonal or monoclonal antibody characterized in that it recognizes and binds to at least one immunogenic peptide according to any one of claims 1 to 10, to a polypeptide according to claim 11 or 12, or to a fragment thereof.

15

14. A purified polynucleotide characterized in that it codes for at least one immunogenic peptide according to any one of claims 1 to 10, or for a fragment thereof.

20

15. The polynucleotide of claim 14, characterized in that it is selected from the group consisting of SEQ ID NOs:1, 30 to 41, and 42.

16. An expression vector, characterized in that it comprises a polynucleotidic sequence coding for a polynucleotide according to claim 14 or 15.

25

17. A cell incorporating the expression vector according to claim 16.

18. A method for producing *in vitro* an immunogenic peptide, comprising:

- culturing *in vitro* in a suitable culture medium a cell according to claim 17, and;
- 30 - collecting said peptide in the culture medium.

19. A pharmaceutical composition comprising:

a) at least one component selected from the group consisting of:

- an immunogenic peptide according to anyone of claims 1 to 10 or a functional derivative thereof;
- 35 - a polypeptide according to claim 11 or 12;
- an antibody according to claim 13;

- a polynucleotide according to claim 14 or 15, and
- Hu specific CD8 T-cells primed against an immunogenic peptide according to anyone of claims 1 to 10, against a polypeptide according to claim 11 or 12 and/or a functional derivative thereof;

5 and

b) a pharmaceutically acceptable vehicle or carrier.

20. Use of a pharmaceutical composition according to claim 19, as an antitumoral agent, for the preparation of an antitumoral vaccine, for the treatment
10 or the prevention of HLA-A2.1 human SCLC and/or for the treatment or the prevention of nervous system damage diseases.

21. A method for priming *in vitro* immune cells of a eukaryotic subject, comprising the steps of:

- 15 a) isolating HLA-A2.1 lymphoid or myeloid cells from said subject; and
- b) priming *in vitro* the cells isolated at step a) with at least one immunogenic peptide as defined in any one of claims 1 to 10, with at least one polypeptide as defined in claim 11 or 12, and/or with at least one the polynucleotide as defined in claim 14 or 15.

20

22. The method of claim 21, further comprising the steps of:

- c) isolating CD8+ T-cells from said subject; and
- d) using the cells primed at step b) for priming *in vitro* the CD8+ T-cells isolated at step c).

25

23. A method for stimulating a subject immune response comprising:

- administering into a compatible subject HLA-A2.1 lymphoid or myeloid cells primed according to the method of claim 21; and/or
- administering into a compatible subject CD8+ T-cells primed according to the
30 method of claim 22.

24. A tumoral cell characterized in that it is obtained by a double transfection of a EL4 S3–Rob mouse (β 2-microglobulin negative CNCM number I-2587) with:

- a human gene coding for a HHD molecule; and
- 35 - a human gene coding for at least one peptide according to anyone of claims 1 to 10.

25. A method for detecting an early CTL response against SCLC, for detecting an early CTL response against neuronal cells or for detecting neuronal damage in a subject comprising:

- providing a tetrameric complex of HLA-A2.2 comprising at least one immunogenic peptide according to any one of claims 1 to 10;
- incubating said complex with peripheral blood lymphocytes of said subject; and
- determining the presence of Hu specific CTL.

26. The method of claim 25, wherein for detecting an early CTL response against SCLC the presence of SCLC Hu specific CTL is determined, and wherein for detecting an early CTL response against neuronal cells the presence of neuronal Hu specific CTL is determined.

27. A method for stimulating a human immune response against SCLC, comprising:

- isolating from a human SCLC Hu specific CTLs;
- amplifying *ex vivo* said isolated SCLC Hu specific CTLs; and
- injecting to a compatible human suffering of SCLC, said SCLC Hu specific CTLs.

28. A method for purifying anti-Hu CTLs from blood of a subject, comprising the steps of:

- fixing on a column a tetrameric complex of HLA-A2.2 comprising at least one immunogenic peptide according to any one of claims 1 to 10;
- passing blood lymphocytes from said subject into said column ; and
- recovering anti-Hu CTLs retained by the column.

29. A method for treating a subject suffering from paraneoplastic neuropathy comprising the steps of:

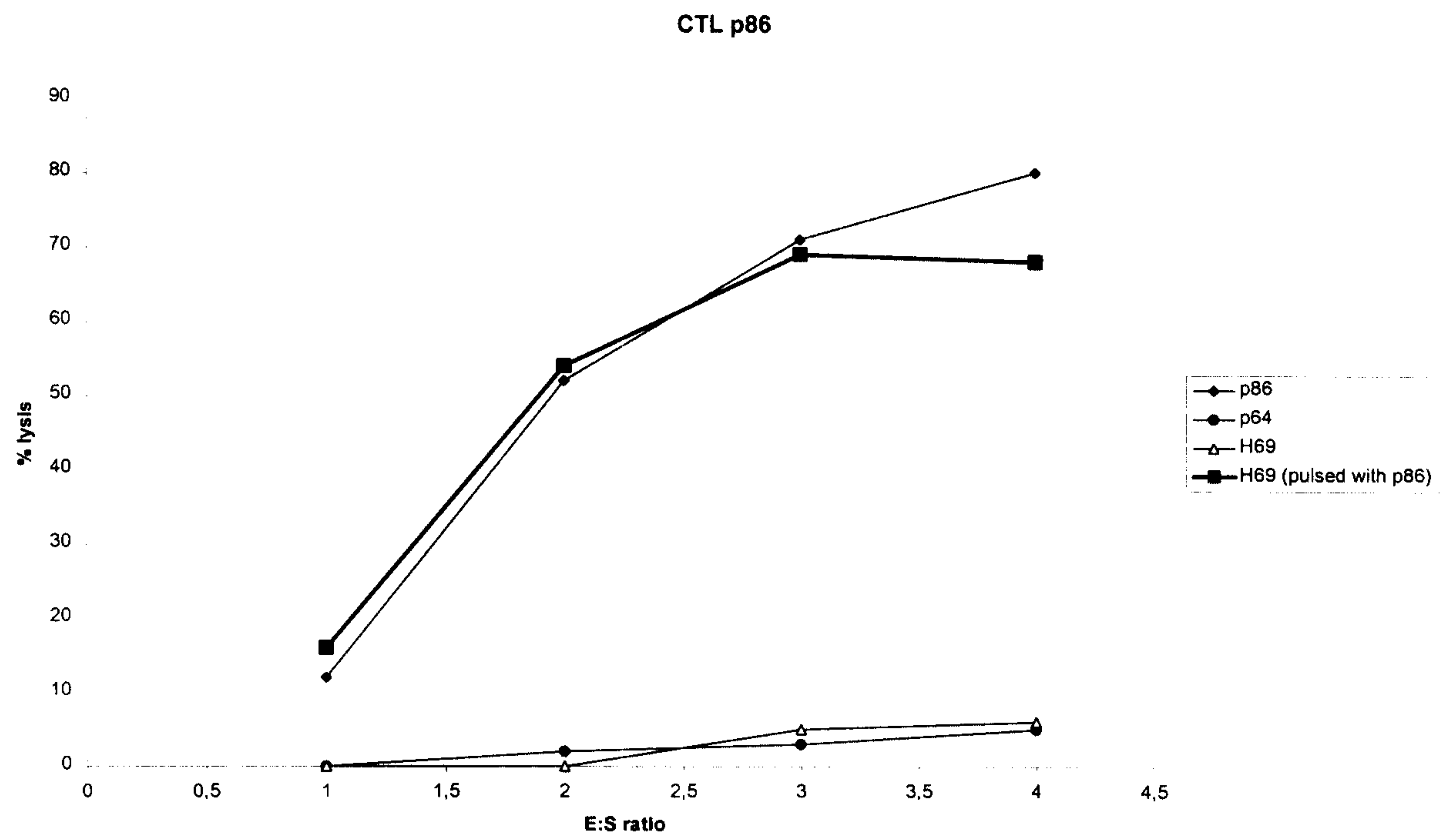
- purifying anti-Hu CTLs from blood of a subject according to the method of claim 28; and
- injecting in said purified anti-Hu CTLs in said subject suffering from paraneoplastic neuropathy.

30. A method for selecting therapeutic molecules capable of inducing an immune response *in vivo* against at least one of the peptides according to anyone of claims 1 to 11, comprising the steps of:

- 5 a) administering EL4S3-Rob tumoral cells according to claim 24 to a first animal model, said animal model having a genotype compatible with said tumoral cells and having beforehand been immunized with the therapeutic molecules to be selected; and
- 10 b) comparing the immune response of said first animal model with the immune response of a second animal model having beforehand been immunized with the therapeutic molecules to be selected.

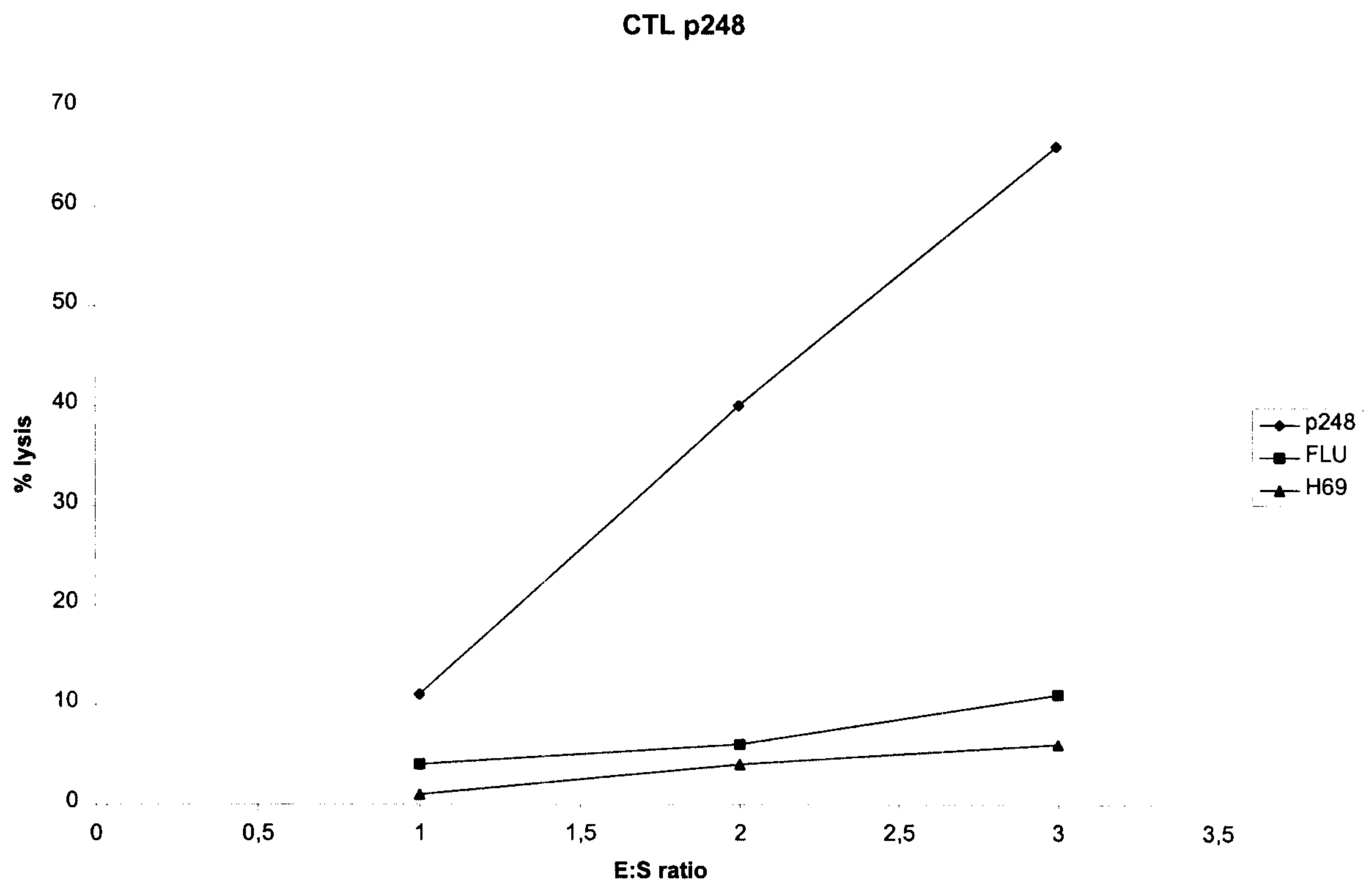
30

FIGURE 1A



31

FIGURE 1B



32

FIGURE 1C

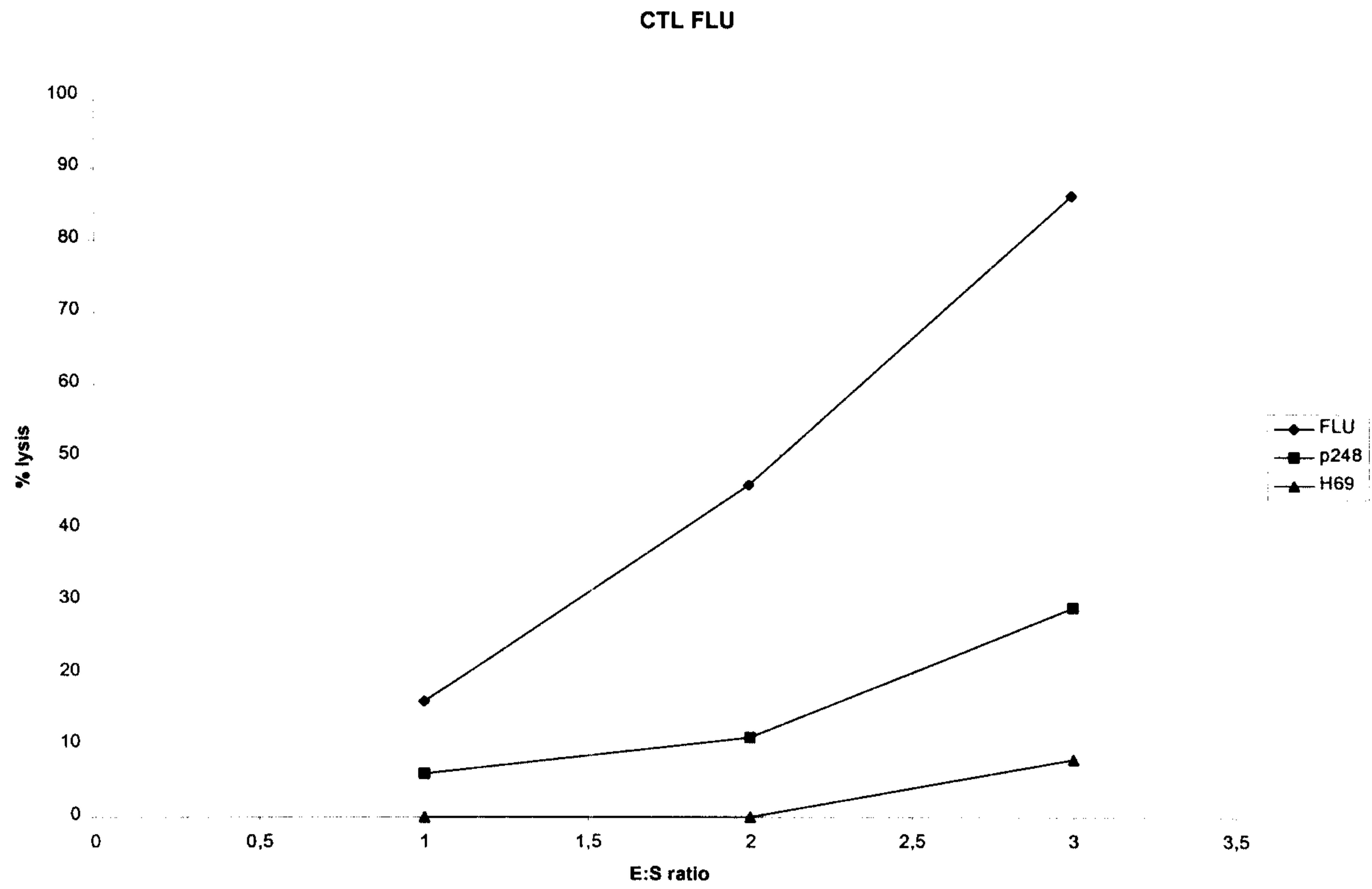


FIGURE 2:

95 atg gtt atg ata att agc acc atg gag cct cag gtg tca aat ggt
 5 M V M I I S T M E P Q V S N G

140 ccg aca tcc aat aca agc aat gga ccc tcc agc aac aac aga aac
 P T S N T S N G P S S N N R N

10 185 tgt cct tct ccc atg caa aca ggg gca acc aca gat gac agc aaa
 C P S P M Q T G A T T D D S K

230 acc aac ctc atc gtc aac tat tta ccc cag aat atg acc caa gaa
 T N L I V N Y L P Q N M T Q E

15 275 gaa ttc agg **agt ctc ttc ggg agc att ggt gaa ata** gaa tcc tgc
 E F R S L F G S I G E I E S C p64

320 aaa ctt gtg aga gac aaa att aca gga cag **agt tta ggg tat gga**
 20 K L V R D K I T G Q S L G Y G p86

365 ttt gtt **aac tat att** gat cca aag gat gca gag aaa gcc atc aac
 F V N Y I D P K D A E K A I N

25 410 act tta aat gga ctc **aga ctc cag acc aaa acc ata aag gtc tca**
 T L N G L R L Q T K T I K V S p111

34

455 tat gcc cgt ccg agc tct gcc tca atc agg gat gct aac ctc tat

Y A R P S S A S I R D A N L Y

p133

500 gtt agc ggc ctt ccc aaa acc atg acc cag aag gaa ctg gag caa

5 V S G L P K T M T Q K E L E Q

545 ctt ttc tcg caa tac ggc cgt atc atc acc tca cga atc ctg gtt

L F S Q Y G R I I T S R I L V

p162/p163

10 590 gat caa gtc aca gga gtg tcc aga ggg gtg gga ttc atc cgc ttt

D Q V T G V S R G V G F I R F

635 gat aag agg att gag gca gaa gaa gcc atc aaa ggg ctg aat ggc

D K R I E A E E A I K G L N G

15

680 cag aag ccc agc ggt gct acg gaa ccg att act gtg aag ttt gcc

Q K P S G A T E P I T V K F A

725 aac aac ccc agc cag aag tcc agc cag gcc ctg ctc tcc cag ctc

20 N N P S Q K S S Q A L L S Q L

770 tac cag tcc cct aac cgg cgc tac cca ggt cca ctt cac cac cag

Y Q S P N R R Y P G P L H H Q

25 815 gct cag agg ttc agg ctg gac aat ttg ctt aat atg gcc tat ggc

A Q R F R L D N L L N M A Y G

p248/p251

35

860 gta aag aga ctg atg tct gga cca gtc ccc cct tct gct tgt tcc
V K R L M S G P V P P S A C S

905 ccc agg ttc tcc cca att acc att gat gga atg aca agc ctt gtg
 5 P R F S P I T I D G M T S L V p280

950 gga atg aac atc cct ggt cac aca gga act ggg tgg tgc atc ttt
 G M N I P G H T G T G W C I F

10 995 gtc tac aac ctg tcc ccc gat tcc gat gag agt gtc ctc tgg cag
 V Y N L S P D S D E S V L W Q p303/p314

1040 ctc ttt ggc ccc ttt gga gca gtg aac aac gta aag gtg att cgt
L F G P F G A V N N V K V I R p315

15

1085 gac ttc aac acc aac aag tgc aag gga ttc ggc ttt gtc acc atg
 D F N T N K C K G F G F V T M

1130 acc aac tat gat gag gcg gcc atg gcc atc gcc agc ctc aac ggg
 20 T N Y D E A A M A I A S L N G

1175 tac cgc ctg gga gac aga gtg ttg caa gtt tcc ttt aaa acc aac
 Y R L G D R V L Q V S F K T N p362

25 1220 aaa gcc cac aag tcc tga
 K A H K S *

FIGURE 3:

p64 : SLFGS I G EI (SEQ ID NO:10)

agt ctc ttc ggg agc att ggt gaa ata (SEQ ID NO:30)

5

p86 : SL G Y G FVNYI (SEQ ID NO:11)

agt tta ggg tat gga ttt gtt aac tat att (SEQ ID NO:31)

p111 : RLQT KTI KV (SEQ ID NO:12)

10

aga ctc cag acc aaa acc ata aag gtc (SEQ ID NO:32)

p133 : NLYVSGLPKT (SEQ ID NO:13)

aac ctc tat gtt agc ggc ctt ccc aaa acc (SEQ ID NO:33)

15

p162 : RI LVDQVTGV (SEQ ID NO:14)

cga atc ctg gtt gat caa gtc aca gga gtg (SEQ ID NO:34)

p163 : I LVDQVTGV (SEQ ID NO:15)

atc ctg gtt gat caa gtc aca gga gtg (SEQ ID NO:35)

20

p248 : NLLNMAYGV (SEQ ID NO:16)

aat ttg ctt aat atg gcc tat ggc gta (SEQ ID NO:36)

p251 : NMAYGVKRL (SEQ ID NO:17)

25

aat atg gcc tat ggc gta aag aga ctg (SEQ ID NO:37)

p280 : MTSLVGGMN (SEQ ID NO:18)

gga atg aca agc ctt gtg gga atg aac (SEQ ID NO:38)

p303: NLSPDSDESV (SEQ ID NO:19)

aac ctg tcc ccc gat tcc gat gag agt gtc (SEQ ID NO:39)

5 **p314 :** WQLFGPFGAV (SEQ ID NO:20)

tgg cag ctc ttt ggc ccc ttt gga gca gtg (SEQ ID NO:40)

p315 : QLFGPFGAV (SEQ ID NO:21)

cag ctc ttt ggc ccc ttt gga gca gtg (SEQ ID NO:41)

10

p362 : RLGDRVVLQV (SEQ ID NO:22)

cgc ctg gga gac aga gtg ttg caa gtt (SEQ ID NO:42)