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(54) Title: NOVEL PEPTIDES AND COMBINATION OF PEPTIDES FOR USE IN IMMUNOTHERAPY AGAINST CLL AND OTHER CANCERS

(57) Abstract: The present invention relates to peptides, proteins, nucleic acids and cells for use in immunotherapeutic methods. In particular, the present invention relates to the immunotherapy of cancer. The present invention furthermore relates to tumor- associated T-cell peptide epitopes, alone or in combination with other tumor- associated peptides that can for example serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses, or to stimulate T cells ex vivo and transfer into patients. Peptides bound to molecules of the major histocompatibility complex (MHC), or peptides as such, can also be targets of antibodies, soluble T-cell receptors, and other binding molecules.

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Novel peptides and combination of peptides for use in immunotherapy against CLL and other cancers

The present invention relates to peptides, proteins, nucleic acids and cells for use in immunotherapeutic methods. In particular, the present invention relates to the immunotherapy of cancer. The present invention furthermore relates to tumor-associated T-cell peptide epitopes, alone or in combination with other tumor-associated peptides that can for example serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses, or to stimulate T cells ex vivo and transfer into patients. Peptides bound to molecules of the major histocompatibility complex (MHC), or peptides as such, can also be targets of antibodies, soluble T-cell receptors, and other binding molecules.

The present invention relates to several novel peptide sequences and their variants derived from HLA class I molecules of human tumor cells that can be used in vaccine compositions for eliciting anti-tumor immune responses, or as targets for the development of pharmaceutically/immunologically active compounds and cells.

BACKGROUND OF THE INVENTION

Chronic lymphocytic leukemia (CLL) is a B-cell neoplasm with morphologically mature, immunologically not completely matured B lymphocytes. CLL is diagnosed when at least 5000 B lymphocytes/µl are present in peripheral blood, whereof up to 55% are immature. Abnormal B cells show a characteristic phenotype expressing CD19, dim CD20, dim CD5, CD23, CD79a and dim IgM or dim IgD (Gribben, 2010).

Two different staging systems are used to classify CLL: The Rai system and the Binet system. The Rai staging system comprises five stages (0-IV) that classify the disease according to the progressive accumulation of abnormal cells. The Binet system uses three stages (A, B, C) to rank the disease according to the number of involved sites (http://www.cancer.gov/cancertopics/pdq/treatment/CLL/Patient).

CLL is primarily a disease of the elderly. The mean age at diagnosis is 72 years for sporadic cases and 58 years for familial cases. The incidence of CLL is higher in males than in females, with a male:female ratio of about 2:1 (Cartwright et al., 2002).

CLL is the most common leukemia in the Western world where it comprises about 1/3rd of all leukemias. Incidence rates are similar in the US and Europe, and estimated new cases are about 16,000 per year. CLL is more common in Caucasians than in Africans, rarer in Hispanics and Native Americans and seldom in Asians. In people of Asian origin, CLL incidence rates are 3-fold lower than in Caucasians (Gunawardana et al., 2008).

The five-year overall survival for patients with CLL is about 79% (http://www.cancer.net/cancer-types/leukemia-chronic-lymphocytic-cll/statistics). The prognosis for individual patients depends on the stage at the time of diagnosis and the occurrence of several prognostic factors. Following the Rai system, patients with stage 0 show a median survival of 10 years or more, while patients at stage I/II have a median survival of 7 years and patients at stage III/IV 0.75-4 years (Gribben, 2010). Several factors are associated with poor prognosis in CLL. These include high expression levels of ZAP-70 or CD38, an IgVH unmutated status and the cytogenetic aberrations del 17p or del 11q (Gribben, 2010).

While CLL is not curable at present, many patients show only slow progression of the disease or worsening of symptoms. As patients do not benefit from an early onset of treatment, the initial approach is “watch and wait” (Richards et al., 1999). For patients with symptomatic or rapidly progressing disease, several treatment options are available. These include chemotherapy, targeted therapy, immune-based therapies like monoclonal antibodies, CARs and active immunotherapy, and stem cell transplants.

Chemotherapeutic drugs used for CLL treatment are mostly alkylating agents like chlorambucil and cyclophosphamide or purine analogues like fludarabine. The German CLL Study Group (GCLLSG) CCL4 demonstrated that fludarabine/cyclophosphamide combinational therapy is superior to sole fludarabine treatment (complete remission (CR) of 24% vs.7%) (Eichhorst et al., 2006).

Ibrutinib and idelalisib are kinase inhibitors that target molecules in the B-cell receptor signaling cascade. Ibrutinib inhibits Bruton's tyrosine kinase (BTK), a src-related cytoplasmic tyrosine kinase important for B-cell maturation, and is used for initial or second-line therapy (Byrd et al., 2013; O'Brien et al., 2014). Idelalisib is a PI3K-delta inhibitor used in combination with rituximab in refractory CLL (Furman et al., 2014).

Hematopoietic stem cell transplants (HSCTs) can be considered for patients with poor prognosis, e.g. patients with del 17p or p53 mutations. HSCTs can either be allogeneic, where the transplanted cells are donated from an HLA-matched person, or autologous, where the patients' own stem cells are re-infused after chemotherapy (Schetelig et al., 2008).

Monoclonal antibodies are widely used in hematologic malignancies. This is due to the knowledge of suitable antigens based on the good characterization of immune cell surface molecules and the accessibility of tumor cells in blood or bone marrow. Common monoclonal antibodies used in CLL therapy target either CD20 or CD52. Rituximab, the first monoclonal anti-CD20 antibody originally approved by the FDA for treatment of NHLs, is now widely used in CLL therapy. Combinational treatment with rituximab/fludarabine/cyclophosphamide leads to higher CR rates and improved overall survival (OS) than the combination fludarabine/cyclophosphamide and has become the preferred treatment option (GCLLSG CLL8) (Hallek et al., 2008). Ofatumomab targets CD20 and is used for therapy of refractory CLL patients (Wierda et al., 2011). Obinutuzumab is another monoclonal anti-CD20 antibody used in first-line treatment in combination with chlorambucil (Goede et al., 2014).

Alemtuzumab is an anti-CD52 antibody used for treatment of patients with chemotherapy-resistant disease or patients with poor prognostic factors as del 17p or p53 mutations (Parikh et al., 2011). Novel monoclonal antibodies target CD37 (otlertuzumab, BI 836826, IMGN529 and (177)Lu-tetulomab) or CD40 (dacetuzumab and lucatumumab) and are tested in pre-clinical settings (Robak and Robak, 2014).

Several completed and ongoing trials are based on engineered autologous chimeric antigen receptor (CAR)-modified T cells with CD19 specificity (Maus et al., 2014). So far, only the minority of patients showed detectable or persistent CARs. One partial response (PR) and two complete responses (CR) have been detected in the CAR T-cell trials by Porter et al. and Kalos et al. (Kalos et al., 2011; Porter et al., 2011).

Active immunotherapy includes the following strategies: gene therapy, whole modified tumor cell vaccines, DC-based vaccines and TAA-derived peptide vaccines.

Approaches in gene therapy make use of autologous genetically modified tumor cells. These B-CLL cells are transfected with immuno-(co-)stimulatory genes like IL-2, IL-12, TNF-alpha, GM-CSF, CD80, CD40L, LFA-3 and ICAM-1 to improve antigen presentation and T cell activation (Carballido et al., 2012). While specific T-cell responses and reduction in tumor cells are readily observed, immune responses are only transient.

Several studies have used autologous DCs as antigen presenting cells to elicit anti-tumor responses. DCs have been loaded ex vivo with tumor associated peptides, whole tumor cell lysate, tumor-derived RNA or DNA. Another strategy uses whole tumor cells for fusion with DCs and generation of DC-B-CLL-cell hybrids. Transfected DCs initiated both CD4+ and CD8+ T-cell responses (Muller et al., 2004). Fusion hybrids and DCs loaded with tumor cell lysate or apoptotic bodies increased tumor-specific CD8+ T-cell responses. Patients that showed a clinical response had increased IL-12 serum levels and reduced numbers of Tregs (Palma et al., 2008).

Different approaches use altered tumor cells to initiate or increase CLL-specific immune responses. An example for this strategy is the generation of trioma cells: B-CLL cells are fused to anti-Fc receptor expressing hybridoma cells that have anti-APC specificity. Trioma cells induced CLL-specific T-cell responses in vitro (Kronenberger et al., 2008). Another strategy makes use of irradiated autologous CLL cells with Bacillus Calmette-Guérin as an adjuvant as a vaccine. Several patients showed a reduction in leukocyte levels or stable disease (Hus et al., 2008). Besides isolated CLL cells, whole blood from CLL patients has been used as a vaccine after preparation in a blood treatment unit. The vaccine elicited CLL-specific T-cell responses and led to partial clinical responses or stable disease in several patients (Spaner et al., 2005).

Several TAAs are overexpressed in CLL and are suitable for vaccinations. These include fibromodulin (Mayr et al., 2005), RHAMM/CD168 (Giannopoulos et al., 2006), MDM2 (Mayr et al., 2006), hTERT (Counter et al., 1995), the oncofetal antigen-immature laminin receptor protein (OFAiLRP) (Siegel et al., 2003), adipophilin (Schmidt et al., 2004), survivin (Granziero et al., 2001), KW1 to KW14 (Krackhardt et al., 2002) and the tumor-derived IgVHCDR3 region (Harig et al., 2001; Carballido et al., 2012).
A phase I clinical trial was conducted using the RHAMM-derived R3 peptide as a vaccine. 5 of 6 patients had detectable R3-specific CD8+ T-cell responses (Giannopoulos et al., 2010).

Considering the severe side-effects and expense associated with treating cancer, there is a need to identify factors that can be used in the treatment of cancer in general and CLL in particular. There is also a need to identify factors representing biomarkers for cancer in general and CLL in particular, leading to better diagnosis of cancer, assessment of prognosis, and prediction of treatment success.

Immunotherapy of cancer represents an option of specific targeting of cancer cells while minimizing side effects. Cancer immunotherapy makes use of the existence of tumor associated antigens.

The current classification of tumor associated antigens (TAAs) comprises the following major groups:

a) Cancer-testis antigens: The first TAAs ever identified that can be recognized by T cells belong to this class, which was originally called cancer-testis (CT) antigens because of the expression of its members in histologically different human tumors and, among normal tissues, only in spermatocytes/spermatogonia of testis and, occasionally, in placenta. Since the cells of testis do not express class I and II HLA molecules, these antigens cannot be recognized by T cells in normal tissues and can therefore be considered as immunologically tumor-specific. Well-known examples for CT antigens are the MAGE family members and NY-ESO-1.

b) Differentiation antigens: These TAAs are shared between tumors and the normal tissue from which the tumor arose. Most of the known differentiation antigens are found in melanomas and normal melanocytes. Many of these melanocyte lineage-related proteins are involved in biosynthesis of melanin and are therefore not tumor specific but nevertheless are widely used for cancer immunotherapy. Examples include, but are not limited to, tyrosinase and Melan-A/MART-1 for melanoma or PSA for prostate cancer.

c) Over-expressed TAAs: Genes encoding widely expressed TAAs have been detected in histologically different types of tumors as well as in many normal tissues, generally with lower expression levels. It is possible that many of the epitopes processed and potentially presented by normal tissues are below the threshold level for T-cell recognition, while their over-expression in tumor cells can trigger an anticancer response by breaking previously established tolerance. Prominent examples for this class of TAAs are Her-2/neu, survivin, telomerase, or WT1.

d) Tumor-specific antigens: These unique TAAs arise from mutations of normal genes (such as β-catenin, CDK4, etc.). Some of these molecular changes are associated with neoplastic transformation and/or progression. Tumor-specific antigens are generally able to induce strong immune responses without bearing the risk for autoimmune reactions against normal tissues. On the other hand, these TAAs are in most cases only relevant to the exact tumor on which they were identified and are usually not shared between many individual tumors. Tumor-specificity (or -association) of a peptide may also arise if the peptide originates from a tumor- (-associated) exon in case of proteins with tumor-specific (-associated) isoforms.

e) TAAs arising from abnormal post-translational modifications: Such TAAs may arise from proteins which are neither specific nor overexpressed in tumors but nevertheless become tumor associated by posttranslational processes primarily active in tumors. Examples for this class arise from altered glycosylation patterns leading to novel epitopes in tumors as for MUC1 or events like protein splicing during degradation which may or may not be tumor specific.

f) Oncoviral proteins: These TAAs are viral proteins that may play a critical role in the oncogenic process and, because they are foreign (not of human origin), they can evoke a T-cell response. Examples of such proteins are the human papilloma type 16 virus proteins, E6 and E7, which are expressed in cervical carcinoma.

T-cell based immunotherapy targets peptide epitopes derived from tumor-associated or tumor-specific proteins, which are presented by molecules of the major histocompatibility complex (MHC). The antigens that are recognized by the tumor specific T lymphocytes, that is, the epitopes thereof, can be molecules derived from all protein classes, such as enzymes, receptors, transcription factors, etc. which are expressed and, as compared to unaltered cells of the same origin, usually up-regulated in cells of the respective tumor.

There are two classes of MHC-molecules, MHC class I and MHC class II. MHC class I molecules are composed of an alpha heavy chain and beta-2-microglobulin, MHC class II molecules of an alpha and a beta chain. Their three-dimensional conformation results in a binding groove, which is used for non-covalent interaction with peptides.

MHC class I molecules can be found on most nucleated cells. They present peptides that result from proteolytic cleavage of predominantly endogenous proteins, defective ribosomal products (DRIPs) and larger peptides. However, peptides derived from endosomal compartments or exogenous sources are also frequently found on MHC class I molecules. This non-classical way of class I presentation is referred to as cross-presentation in the literature (Brossart and Bevan, 1997; Rock et al., 1990). MHC class II molecules can be found predominantly on professional antigen presenting cells (APCs), and primarily present peptides of exogenous or transmembrane proteins that are taken up by APCs e.g. during endocytosis, and are subsequently processed.

Complexes of peptide and MHC class I are recognized by CD8-positive T cells bearing the appropriate T-cell receptor (TCR), whereas complexes of peptide and MHC class II molecules are recognized by CD4-positive-helper-T cells bearing the appropriate TCR. It is well known that the TCR, the peptide and the MHC are thereby present in a stoichiometric amount of 1:1:1.

CD4-positive helper T cells play an important role in inducing and sustaining effective responses by CD8-positive cytotoxic T cells. The identification of CD4-positive T-cell epitopes derived from tumor associated antigens (TAA) is of great importance for the development of pharmaceutical products for triggering anti-tumor immune responses (Gnjatic et al., 2003). At the tumor site, T helper cells, support a cytotoxic T cell- (CTL-) friendly cytokine milieu (Mortara et al., 2006) and attract effector cells, e.g. CTLs, natural killer (NK) cells, macrophages, and granulocytes (Hwang et al., 2007).

In the absence of inflammation, expression of MHC class II molecules is mainly restricted to cells of the immune system, especially professional antigen-presenting cells (APC), e.g., monocytes, monocyte-derived cells, macrophages, dendritic cells. In cancer patients, cells of the tumor have been found to express MHC class II molecules (Dengjel et al., 2006).

Elongated (longer) peptides of the invention can act as MHC class II active epitopes.

T-helper cells, activated by MHC class II epitopes, play an important role in orchestrating the effector function of CTLs in anti-tumor immunity. T-helper cell epitopes that trigger a T-helper cell response of the TH1 type support effector functions of CD8-positive killer T cells, which include cytotoxic functions directed against tumor cells displaying tumor-associated peptide/MHC complexes on their cell surfaces. In this way tumor-associated T-helper cell peptide epitopes, alone or in combination with other tumor-associated peptides, can serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses.

It was shown in mammalian animal models, e.g., mice, that even in the absence of CD8-positive T lymphocytes, CD4-positive T cells are sufficient for inhibiting manifestation of tumors via inhibition of angiogenesis by secretion of interferon-gamma (IFNγ) (Beatty and Paterson, 2001; Mumberg et al., 1999). There is evidence for CD4 T cells as direct anti-tumor effectors (Braumuller et al., 2013; Tran et al., 2014).

Since the constitutive expression of HLA class II molecules is usually limited to immune cells, the possibility of isolating class II peptides directly from primary tumors was previously not considered possible. However, Dengjel et al. were successful in identifying a number of MHC Class II epitopes directly from tumors (WO 2007/028574, EP 1 760 088 B1).

Since both types of response, CD8 and CD4 dependent, contribute jointly and synergistically to the anti-tumor effect, the identification and characterization of tumor-associated antigens recognized by either CD8+ T cells (ligand: MHC class I molecule + peptide epitope) or by CD4-positive T-helper cells (ligand: MHC class II molecule + peptide epitope) is important in the development of tumor vaccines.

For an MHC class I peptide to trigger (elicit) a cellular immune response, it also must bind to an MHC-molecule. This process is dependent on the allele of the MHC-molecule and specific polymorphisms of the amino acid sequence of the peptide. MHC-class-I-binding peptides are usually 8-12 amino acid residues in length and usually contain two conserved residues ("anchors") in their sequence that interact with the corresponding binding groove of the MHC-molecule. In this way, each MHC allele has a “binding motif” determining which peptides can bind specifically to the binding groove.

In the MHC class I dependent immune reaction, peptides not only have to be able to bind to certain MHC class I molecules expressed by tumor cells, they subsequently also have to be recognized by T cells bearing specific T cell receptors (TCR).

For proteins to be recognized by T-lymphocytes as tumor-specific or -associated antigens, and to be used in a therapy, particular prerequisites must be fulfilled. The antigen should be expressed mainly by tumor cells and not, or in comparably small amounts, by normal healthy tissues. In a preferred embodiment, the peptide should be over-presented by tumor cells as compared to normal healthy tissues. It is furthermore desirable that the respective antigen is not only present in a type of tumor, but also in high concentrations (i.e. copy numbers of the respective peptide per cell). Tumor-specific and tumor-associated antigens are often derived from proteins directly involved in transformation of a normal cell to a tumor cell due to their function, e.g. in cell cycle control or suppression of apoptosis. Additionally, downstream targets of the proteins directly causative for a transformation may be up-regulated und thus may be indirectly tumor-associated. Such indirect tumor-associated antigens may also be targets of a vaccination approach (Singh-Jasuja et al., 2004). It is essential that epitopes are present in the amino acid sequence of the antigen, in order to ensure that such a peptide ("immunogenic peptide"), being derived from a tumor associated antigen, leads to an *in vitro* or *in vivo* T-cell-response.

Basically, any peptide able to bind an MHC molecule may function as a T-cell epitope. A prerequisite for the induction of an *in vitro* or *in vivo* T-cell-response is the presence of a T cell having a corresponding TCR and the absence of immunological tolerance for this particular epitope.

Therefore, TAAs are a starting point for the development of a T cell based therapy including but not limited to tumor vaccines. The methods for identifying and characterizing the TAAs are usually based on the use of T-cells that can be isolated from patients or healthy subjects, or they are based on the generation of differential transcription profiles or differential peptide expression patterns between tumors and normal tissues. However, the identification of genes over-expressed in tumor tissues or human tumor cell lines, or selectively expressed in such tissues or cell lines, does not provide precise information as to the use of the antigens being transcribed from these genes in an immune therapy. This is because only an individual subpopulation of epitopes of these antigens are suitable for such an application since a T cell with a corresponding TCR has to be present and the immunological tolerance for this particular epitope needs to be absent or minimal. In a very preferred embodiment of the invention it is therefore important to select only those over- or selectively presented peptides against which a functional and/or a proliferating T cell can be found. Such a functional T cell is defined as a T cell, which upon stimulation with a specific antigen can be clonally expanded and is able to execute effector functions ("effector T cell").

In case of targeting peptide-MHC by specific TCRs (e.g. soluble TCRs) and antibodies or other binding molecules (scaffolds) according to the invention, the immunogenicity of the underlying peptides is secondary. In these cases, the presentation is the determining factor.

In a first aspect of the present invention, the present invention relates to a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 385 or a variant sequence thereof which is at least 77%, preferably at least 88%, homologous (preferably at least 77% or at least 88% identical) to SEQ ID NO: 1 to SEQ ID NO: 385, wherein said variant binds to MHC and/or induces T cells cross-reacting with said peptide, or a pharmaceutical acceptable salt thereof, wherein said peptide is not the underlying full-length polypeptide.

The present invention further relates to a peptide of the present invention comprising a sequence that is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 385 or a variant thereof, which is at least 77%, preferably at least 88%, homologous (preferably at least 77% or at least 88% identical) to SEQ ID NO: 1 to SEQ ID NO: 385, wherein said peptide or variant thereof has an overall length of between 8 and 100, preferably between 8 and 30, and most preferred of between 8 and 14 amino acids.

The following tables show the peptides according to the present invention, their respective SEQ ID NOs, and the prospective source (underlying) genes for these peptides. All peptides in Table 1 and Table 2 bind to HLA-A*02. The peptides in Table 2 have been disclosed before in large listings as results of high-throughput screenings with high error rates or calculated using algorithms, but have not been associated with cancer at all before. The peptides in Table 3 are additional peptides that may be useful in combination with the other peptides of the invention. The peptides in Table 4 are furthermore useful in the diagnosis and/or treatment of various other malignancies that involve an over-expression or over-presentation of the respective underlying polypeptide.

Table 1: Peptides according to the present invention.
J = phospho-serine

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
1	AIPPSFASIFL	3507	IGHM
2	ALHRPDVYL	3507	IGHM
3	VIAELPPKV	3507	IGHM
4	VIAELPPKVSV	3507	IGHM
5	ALIFKIASA	4214	MAP3K1
6	ALDTLEDDMTI	2222	FDFT1
7	ALLERTGYTL	10236,10492	HNRNPR, SYNCRIP
8	ALAASALPALV	6124	RPL4
9	ALCDTLITV	27033	ZBTB32
10	FVYGESVEL	27033	ZBTB32
11	ALFTFJPLTV	54665	RSBN1
12	ALGEDEITL	25909,285116	AHCTF1, AHCTF1P1
13	VVDGMPPGV	2969,2970	GTF2I, GTF2IP1
14	ALLRLLPGL	25920	COBRA1
15	ALPEVSVEA	79649	MAP7D3
16	ALPGGAAVAAV	79697	C14orf169
17	ALTKTNLQL	9750	FAM65B
18	LLGEFSIKM	9750	FAM65B
19	QVMEKLAAV	9750	FAM65B
20	ALVDPGPDFVV	546	ATRX
21	ALWAGLLTL	2208	FCER2
22	ALWDPVIEL	27340	UTP20
23	ALYLTEVFL	55024	BANK1

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
24	AMAGDVVYA	160365	CLECL1
25	ATYSGLESQSV	3185	HNRNPF
26	AVLLVLPLV	11322	TMC6
27	AVLGLVWLL	11322	TMC6
28	AVLHLLLSV	1535	CYBA
29	AVLQAVTAV	58155	PTBP2
30	ELLEGSEIYL	147007,23098	TMEM199, SARM1
31	ELMHGVAGV	165631	PARP15
32	FIDKFTPPV	3117,3122	HLA-DQA1, HLA-DRA
33	FIINSSNIFL	253959	RALGAPA1
34	YLPYIFPNI	253959	RALGAPA1
35	FILPSSLYL	81539	SLC38A1
36	FILTHVDQL	257106	ARHGAP30
37	FIMEGGAMVL	4174	MCM5
38	FIMEGGAMV	4174	MCM5
39	FLDALLTDV	79915	ATAD5
40	FLDEDDMSL	23141	ANKLE2
41	FLDPSLDPLL	58508,85318	MLL3, BAGE3
42	FLEEGGVVTV	5810	RAD1
43	FLLGPEALSFA	55183	RIF1
44	FLLSINDFL	157680	VPS13B
45	FLPELPADLEA	11064	CNTRL
46	YIIDSAQAV	11064	CNTRL
47	FLSDQPEPYL	160518,23258	DENND5B, DENND5A
48	FLSPQQPPLL	8621	CDK13
49	FLTDLFAQL	55671	SMEK1
50	FLFEPVVKAFL	55671	SMEK1
51	FLVEAPHDWDL	9790	BMS1
52	FLVETGFLHV	100268168,114987,4602	WDR31, MYB
53	FLWQHVELVL	55181	SMG8
54	FLYPFPLALF	440026	TMEM41B
55	FMEPTLLML	23	ABCF1
56	FVFEAPYTL	139818	DOCK11
57	GLSEISLRL	139818	DOCK11
58	YIQQGIFSV	139818	DOCK11
59	FVFGDENGTVSL	79084	WDR77
60	FVLDHEDGLNL	4926	NUMA1
61	FVYFIVREV	285527	FRYL
62	GIIDGSPRL	80183	KIAA0226L
63	SLAHVAGCEL	80183	KIAA0226L

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
64	KLLESVASA	80183	KIAA0226L
65	GLDDMKANL	5079	PAX5
66	SLAGGLDDMKA	5079	PAX5
67	GLDDVTVEV	51019	CCDC53
68	GLDQQFAGLDL	1654	DDX3X
69	GLHQREIFL	9922	IQSEC1
70	FVPDTPVGV	9922	IQSEC1
71	GLKHDIARV	388512	CLEC17A
72	GLLDAGKMYV	911	CD1C
73	GLLEVISALQL	9816	URB2
74	GLLRASFLL	11344,54106	TWF2, TLR9
75	GLSIFAQDLRL	11344,54106	TWF2, TLR9
76	GLLRIIPYL	23352	UBR4
77	GLLRLTWFL	11214	AKAP13
78	GLPSFLTEV	100532732,4439	MSH5-SAPCD1, MSH5
79	GLQAKIQEA	23224	SYNE2
80	VLIEDELEEL	23224	SYNE2
81	WLVGQEFEL	23224	SYNE2
82	GLQSGVDIGV	1265	CNN2
83	GQGEVLVYV	2316	FLNA
84	GVMDVNTAL	391370,6206	RPS12P4, RPS12
85	HLMLHTAAL	80012	PHC3
86	HLYPGAVTI	55103	RALGPS2
87	HQIEAVDGEEL	10567	RABAC1
88	ILDEIGADVQA	29920	PYCR2
89	ILDFGTFQL	54832	VPS13C
90	VIADLGLIRV	54832	VPS13C
91	ILDLNTYNV	5336	PLCG2
92	ILEPLNPLL	9330	GTF3C3
93	ILFNTQINI	64801	ARV1
94	ILFPLRFTL	9675	TTI1
95	ILGYMAHEHKV	6530	SLC6A2
96	ILIDKTSFV	951	CD37
97	LLFATQITL	951	CD37
98	SLIKYFLFV	951	CD37
99	ILIFHSVAL	84636	GPR174
100	ILNNEVFAI	26999	CYFIP2
101	ILVVIEPLL	23451	SF3B1
102	IQDRAVPSL	3930	LBR
103	KLGGTPAPA	5218	CDK14

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
104	KLILLDTPLFL	253190,94009	SERHL2, SERHL
105	KLMNDIADI	472	ATM
106	FMASHLDYL	472	ATM
107	ILYNLYDLL	472	ATM
108	VIYTLIHYI	472	ATM
109	KLWEGLTELV	10235	RASGRP2
110	LLFDHLEPMEL	10235	RASGRP2
111	KLWNVAAPLYL	92105	INTS4
112	KTLDVDATYEI	3978	LIG1
113	KVPAEEVLVAV	91782	CHMP7
114	LIPEGPPQV	64224	HERPUD2
115	LLFDKLYLL	25885	POLR1A
116	LLIGATMQV	23225	NUP210
117	LLILENILL	23225	NUP210
118	RLLILENILL	23225	NUP210
119	VLPAEFFEV	23225	NUP210
120	AIDAALTSV	23225	NUP210
121	RLYEITIEV	23225	NUP210
122	LLIPVVPGV	55178	RNMTL1
123	LLLAEAELLTL	57724	EPG5
124	LLLEETEKQAV	92154,9788	MTSS1L, MTSS1
125	LLLEIGEVGKLFV	23158	TBC1D9
126	LLPEGGITAI	9904	RBM19
127	LLPTAPTTV	55729	ATF7IP
128	LLSEEEYHL	9793	CKAP5
129	LLVGTLDVV	23061,23158	TBC1D9B, TBC1D9
130	LLVLIPVYL	23344	ESYT1
131	LQALEVLKI	27304	MOCS3
132	LVYEAIIMV	27250	PDCD4
133	YLLSGDISEA	27250	PDCD4
134	MLLEHGITLV	200576	PIKFYVE
135	MTAGFSTIAGSV	64078	SLC28A3
136	NLDKLWTLV	389435,6157	RPL27AP6, RPL27A
137	NLIKTVIKL	25816	TNFAIP8
138	NLLDIDAPVTV	5530	PPP3CA
139	NLTDVVEKL	120892	LRRK2
140	QIAELPATSV	401145	CCSER1
141	QILSEIVEA	10724	MGEA5
142	QLDEPAPQV	387032,80317	ZKSCAN4, ZKSCAN3
143	QLLDTYFTL	7405	UVRAG

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
144	QLPPFPREL	8498	RANBP3
145	SALDTITTV	3832	KIF11
146	SIIEGPIIKL	23347	SMCHD1
147	SILETVATL	253714	MMS22L
148	SIVASLITV	221188	GPR114
149	SLDNGGYYI	4067	LYN
150	SLFDQPLSII	117289	TAGAP
151	SLFDSAYGA	2313	FLI1
152	SLIRILQTI	160518	DENND5B
153	SLLAELHVL	115352	FCRL3
154	SLLAELHVLTV	115352	FCRL3
155	SLMLEVPAL	1756	DMD
156	SLNIGDVQL	973	CD79A
157	SLNIRDFTM	147694,7565	ZNF548, ZNF17
158	SLPEAPLDV	29123	ANKRD11
159	SLQEEKLIYV	84668	FAM126A
160	SLSFLVPSL	9631	NUP155
161	SMDDGMINV	7011	TEP1
162	SMKDDLENV	3688	ITGB1
163	SQLDISEPYKV	3394	IRF8
164	SVHKGFAFV	101060301,3183,343069,440563,649330	HNRNPC, HNRNPCL1
165	TLDDDLDTV	51092	SIDT2
166	TLDPNQVSL	85464	SSH2
167	TLDTSKLYV	5923	RASGRF1
168	YLLDQSFVM	5923	RASGRF1
169	TLLLGLTEV	55423	SIRPG
170	TLTFRVETV	51379	CRLF3
171	TLVPPAALISI	6907	TBL1X
172	TLYDMLASI	10973	ASCC3
173	TVIENIHTI	23358	USP24
174	VLAELPIIVV	8295	TRRAP
175	FTVPRVVAV	8295	TRRAP
176	VLAEQNIIPSA	54855	FAM46C
177	VLDDRELLL	23178	PASK
178	VLFFNVQEV	57536	KIAA1328
179	VLLGLEMTL	1606	DGKA
180	LLKDGPEIGL	1606	DGKA
181	VLLSIPFVSV	94103	ORMDL3
182	VLLSVPGPPV	2264	FGFR4

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
183	VLMPTVYQQGV	54472	TOLLIP
184	VLSHNLYTV	50619	DEF6
185	VMDDQRDLI	972	CD74
186	VMDPTKILI	255967	PAN3
187	VMDTHLVNI	9779	TBC1D5
188	VMGDIPAAV	4678	NASP
189	VMLEMTPEL	3112,6891	HLA-DOB, TAP2
190	VVMGTVPRL	1729	DIAPH1
191	YIFDGSDGGV	5287	PIK3C2B
192	YIQEYLTLL	57674	RNF213
193	YLDLSNNRL	81793	TLR10
194	YLDNVLAEL	1840,23220	DTX1, DTX4
195	YLGGFALSV	6850	SYK
196	YLLLQTYVL	51259	TMEM216
197	YLQEVPILTL	1633	DCK
198	YLTFLPAEV	64135	IFIH1
199	YLVELSSLL	80279	CDK5RAP3
200	YMFEEVPIVI	8667	EIF3H
201	YQLELHGIEL	5698	PSMB9
202	YVDDVFLRV	10347	ABCA7
203	ALLSSQLAL	8925	HERC1
204	GLLQINDKIAL	5000	ORC4
205	GLSQANFTL	10288,10859,10990,11024,11025,11026,11027,23547,79166,79168	LILRB2, LILRB1, LILRB5, LILRA1, LILRB3, LILRA3, LILRA2, LILRA4, LILRP2, LILRA6
206	HMQDVRVLL	84824	FCRLA
207	IIADLDTTIMFA	7094,83660	TLN1, TLN2
208	ILLKTEGINL	166379	BBS12
209	ILQAELPSL	57508	INTS2
210	KLLVQDFFL	8564	KMO
211	LIDVKPLGV	134510	UBLCP1
212	NIIEAINELLV	8892	EIF2B2
213	RLLYQLVFL	3566	IL4R
214	RLQELTEKL	255394	TCP11L2
215	VMQDIVYKL	84759	PCGF1
216	WLAGDVPAA	24148	PRPF6
217	ALDEPPYLTV	51742	ARID4B
218	ALGEEWKGYVV	6194,728131	RPS6
219	ALLNLLESA	5788	PTPRC

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
220	ALPEILFAKV	643	CXCR5
221	ALVSTIIMV	80228	ORAI2
222	ALWELSLKI	2889	RAPGEF1
223	ALWVSQPPEI	259197	NCR3
224	AMEALVVEV	1660	DHX9
225	ILQERELLPV	1660	DHX9
226	AMNISVPQV	23387	SIK3
227	FLAEASVMTQL	1445	CSK
228	FLGGLSPGV	2968	GTF2H4
229	FLLNLQNCHL	55608	ANKRD10
230	FLQDSKVIFV	4772	NFATC1
231	FLYIRQLAI	26155,401010	NOC2L
232	FMHQIIDQV	79789	CLMN
233	GIIDINVRL	51163	DBR1
234	GLDDAEYAL	7376	NR1H2
235	GLDDLLLFL	8914	TIMELESS
236	GLLESGRHYL	9744	ACAP1
237	GLQENLDVVV	8881	CDC16
238	GLVETELQL	55690	PACS1
239	ILAGEMLSV	7203	CCT3
240	ILARDILEI	5714	PSMD8
241	ILGDILLKV	79831	KDM8
242	ILLGIQELL	7329	UBE2I
243	ILPTLEKELFL	79886	CAAP1
244	ILQALAVHL	54936	ADPRHL2
245	KIMDYSLLLGV	23396	PIP5K1C
246	KLDETGVAL	7155	TOP2B
247	KLKDRLPSI	81606	LBH
248	KTVEPPISQV	57466	SCAF4
249	LLPTGVFQV	51131	PHF11
250	LLVQEPDGLMV	404734,54882	ANKHD1-EIF4EBP3, ANKHD1
251	LLYDNVPGA	11262	SP140
252	NLLDPGSSYLL	5074	PAWR
253	NLWSVDGEVTV	92017	SNX29
254	QLIPKLIFL	57705	WDFY4
255	YLFEEAISM	57705	WDFY4
256	QLLPVSNVVSV	55374	TMCO6
257	RIINGIIISV	84329	HVCN1
258	RLDYITAEI	10471	PFDN6
259	RLLDEQFAVL	9026	HIP1R

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
260	SLDDVEGMSV	80196	RNF34
261	SLVEAQGWLV	161176	SYNE3
262	SLWNAGTSV	3495	IGHD
263	SQWEDIHVV	55843	ARHGAP15
264	TILDYINVV	89790,89858	SIGLEC10, SIGLEC12
265	TLLADDLEIKL	2124	EVI2B
266	TLLDQLDTQL	8678	BECN1
267	TLLDWQDSL	8289	ARID1A
268	TLLQVFHLL	63892	THADA
269	TLTDEQFLV	738	VPS51
270	TVLPVPPLSV	5430	POLR2A
271	VIRNIVEAA	100128168,100996747,389472,441502,6231,643003,728937,729188	RPS26P39, RPS26P32, RPS26P11, RPS26, RPS26P28, RPS26P25, RPS26P58
272	VLDELPPLI	55832,91689	CAND1, C22orf32
273	VLGEYSYLL	23431	AP4E1
274	VLLEYHIAYL	8087	FXR1
275	VLLFIEHSV	134265,7805	AFAP1L1, LAPTM5
276	VLNDGAPNV	117246	FTSJ3
277	VMILKLPFL	25929	GEMIN5
278	YLDDLLPKL	89910	UBE3B
279	YMAPEVVEA	2872	MKNK2
280	YTLDSLYWSV	81887	LAS1L

KIAA0226L (also known as C13orf18) encodes KIAA0226-like and is located on chromosome 13q14.13 (RefSeq, 2002). KIAA0226L is thought to be a tumor suppressor gene and is hyper-methylated in cervical cancer. Re-activation of KIAA0226L leads to decreased cell growth, viability, and colony formation (Huisman et al., 2015; Eijsink et al., 2012; Huisman et al., 2013). The methylation pattern of KIAA0226L can be used to differ between precursor lesions and normal cervix cancer (Milutin et al., 2015). The methylation pattern of KIAA0226L cannot be used as specific biomarker for cervical cancer (Sohrabi et al., 2014). Re-activation of KIAA0226L partially de-methylates its promotor region and also decreases repressive histone methylations (Huisman et al., 2013).

FCRL3, also known as FCRH3 or IRTA3, encodes Fc receptor like 3, one of several FC receptor-like glycoproteins of the immunoglobulin receptor superfamily which may play a role in regulation of the immune system (RefSeq, 2002). FCRL3 is up-regulated on CD4+CD26- T cells in Sézary syndrome patients with a high tumor burden, suggesting that FCRL3 expression correlates with a high circulating tumor burden (Wysocka et al., 2014). FCRL3 is up-regulated in chronic lymphocytic leukemia (Polson et al., 2006). FCRL3 is a potential marker with prognostic relevance in chronic lymphocytic leukemia (Zucchetto et al., 2011)

Table 2: Additional peptides according to the present invention with no prior known cancer association.

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
281	NLLDDRGMTAL	146206	RLTPR
282	LLRDGIELV	152926	PPM1K
283	ILQPMDIHV	54832	VPS13C
284	LLSAAEPVPA	974	CD79B
285	GVATAGCVNEV	3394	IRF8
286	FLLEDLSQKL	2177	FANCD2
287	FLWEEKFNSL	10750	GRAP
288	GLAESTGLLAV	50855,84552	PARD6A, PARD6G
289	ILEEQPMDMLL	84301	DDI2
290	LANPHELSL	84301	DDI2
291	ILLNEDDLVTI	57724	EPG5
292	AAALIIHHV	25942	SIN3A
293	ALDIMIPMV	4217	MAP3K5
294	ALLDQLHTLL	8202	NCOA3
295	ALLQKLQQL	122618	PLD4
296	FIAPTGHSL	23157,55752	SEPT6, SEPT11
297	FLVEPQEDTRL	8237	USP11
298	IILPVEVEV	23505	TMEM131
299	ILEENIPVL	55653	BCAS4
300	ILLNPAYDVYL	23225	NUP210
301	AASPIITLV	23225	NUP210
302	ILQDLTFVHL	2889	RAPGEF1
303	ILSQPTPSL	29028	ATAD2
304	LAIVPVNTL	23132	RAD54L2
305	LLFPQIEGIKI	26263	FBXO22

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
306	VVAEELENV	26263	FBXO22
307	LLLTKPTEA	5336	PLCG2
308	SLYDVSRMYV	5336	PLCG2
309	ILYGTQFVL	5336	PLCG2
310	LLSTLHLLV	64098	PARVG
311	LLVDVEPKV	100129492,6632	SNRPD1
312	LLYNSTDPTL	64005	MYO1G
313	LMADLEGLHL	8546	AP3B1
314	LMKDCEAEV	5518	PPP2R1A
315	LVYEAPETV	57448	BIRC6
316	MLLEHGITL	200576	PIKFYVE
317	NLLAHIWAL	58513	EPS15L1
318	NLQVTQPTV	100505503,402057,442216,6218	RPS17L, RPS17P16, RPS17P5, RPS17
319	QVIPQLQTV	6671	SP4
320	TIAPVTVAV	6671	SP4
321	RLLEFELAQL	6093	ROCK1
322	SLASIHVPL	23163	GGA3
323	SLDLFNCEV	10541,23520,646791,647020,723972,8125	ANP32B, ANP32C, ANP32BP1, ANP32AP1, ANP32A
324	SLYSALQQA	11190	CEP250
325	TLENGVPCV	23240	KIAA0922
326	VLAFLVHEL	11176	BAZ2A
327	VLIKWFPEV	54509	RHOF
328	VLLPQETAEIHL	5292	PIM1
329	VLMDGSVKL	11184	MAP4K1
330	VLMWEIYSL	695	BTK
331	VLWELAHLPTL	23358	USP24
332	VMIQHVENL	4033	LRMP
333	VTLEFPQLIRV	126382	NR2C2AP
334	YLLEEKIASL	51199	NIN
335	YLYQEQYFI	10668	CGRRF1
336	YMAVTTQEV	79840	NHEJ1
337	YMYEKESEL	22806	IKZF3
338	FLDMTNWNL	64771	C6orf106
339	GLWGTVVNI	25914	RTTN
340	KLLEEICNL	10075	HUWE1
341	LLAELPASVHA	2319	FLOT2
342	SLITPLQAV	5591	PRKDC
343	TLLEALDCI	158078,1915	EEF1A1P5, EEF1A1

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
344	VLAFENPQV	25852	ARMC8
345	YLIEPDVELQRI	25852	ARMC8
346	VLVQVSPSL	23404	EXOSC2
347	YLGPVSPSL	4154	MBNL1
348	ALAKPPVVSV	57634	EP400
349	ALATHILSL	11215	AKAP11
350	ALEDRVWEL	285025	CCDC141
351	ALSEKLARL	115106	HAUS1
352	ALVFELHYV	57705	WDFY4
353	ATPMPTPSV	55206	SBNO1
354	FIMDDPAGNSYL	8882	ZNF259
355	FIWPMLIHI	11325	DDX42
356	FLHDHQAEL	259197	NCR3
357	FLIQEIKTL	23352	UBR4
358	FLTDYLNDL	54880	BCOR
359	FMQDPMEVFV	10212	DDX39A
360	HLIDTNKIQL	55619	DOCK10
361	ILQEFESKL	2074,267004	ERCC6, PGBD3
362	ILTELGGFEV	51366	UBR5
363	ITTEVVNELYV	57222	ERGIC1
364	KMDWIFHTI	5290	PIK3CA
365	LISPLLLPV	144132	DNHD1
366	LLSETCVTI	23469	PHF3
367	NLWSLVAKV	9889	ZBED4
368	QLQPTDALLCV	125950	RAVER1
369	RLLDLENSLLGL	54899	PXK
370	SIFASPESV	10285	SMNDC1
371	SLADDSVLERL	23195	MDN1
372	SLFGPLPGPGPALV	9595	CYTIP
373	TLLADQGEIRV	9219	MTA2
374	VLSVITEEL	55621	TRMT1
375	VLWFKPVEL	55720	TSR1
376	VLYNQRVEEI	6730	SRP68
377	VVDGTCVAV	57589	KIAA1432
378	YILGKFFAL	8914	TIMELESS
379	YLAELVTPIL	1981	EIF4G1
380	YLDRKLLTL	6850	SYK
381	YLLEENKIKL	139818	DOCK11
382	YLLPLLQRL	55794	DDX28
383	YLLREWVNL	23019	CNOT1

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
384	YMIGSEVGNYL	6598	SMARCB1
385	YTIPLAIKL	128869	PIGU

Table 3: Peptides useful for e.g. personalized cancer therapies.

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
386	FLGDYVENL	54832	VPS13C
387	YLILSSHQL	1269	CNR2
388	GLLSALENV	1269	CNR2
389	GLAALAVHL	2175	FANCA
390	GLEERLYTA	29933	GPR132
391	FLLEREQLL	165055	CCDC138
392	FLWERPTLLV	79922	MRM1
393	FVMEGEPPKL	348654	GEN1
394	ILSTEIFGV	79703	C11orf80
395	ALYGKLLKL	157680	VPS13B
396	TLLGKQVTL	157680	VPS13B
397	FLPPEHTIVYI	9896	FIG4
398	FLAELPGSLSL	5326	PLAGL2
399	ALAAPDIVPAL	79886	CAAP1
400	ALFQPHLINV	10097	ACTR2
401	AMADKMDMSL	10189	ALYREF
402	LLVSNLDFGV	10189	ALYREF
403	FIMPATVADATAV	23352	UBR4
404	FLQPDLDSL	10514	MYBBP1A
405	FLVEKQPPQV	6778	STAT6
406	FLWPKEVEL	146206	RLTPR
407	FMIDASVHPTL	221960,51622	CCZ1B, CCZ1
408	FMVDNEAIYDI	10376,112714,113457,51807,7277,7278,7846,84790	TUBA1B, TUBA3E, TUBA3D, TUBA8, TUBA4A, TUBA3C, TUBA1A, TUBA1C
409	GLDAATATV	9869	SETDB1
410	GLFDGVPTTA	122618	PLD4
411	GLFEDVTQPGILL	23140	ZZEF1
412	ILGTEDLIVEV	79719	AAGAB
413	ILLEHGADPNL	22852	ANKRD26
414	ILSVVNSQL	80183	KIAA0226L
415	ILVTSIFFL	643	CXCR5
416	IMEDIILTL	1656	DDX6
417	IQIGEETVITV	2316	FLNA

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
418	IVTEIISEI	64151	NCAPG
419	LLNEILEQV	64151	NCAPG
420	KLIDDQDISISL	80208	SPG11
421	KLWTGGLDNTV	7088,7090,7091	TLE1, TLE3, TLE4
422	KQFEGTVEI	675	BRCA2
423	LLIGTDVSL	9730	VPRBP
424	LLPPLESLATV	5518	PPP2R1A
425	LLSDVRFVL	53339	BTBD1
426	LLWGNLPEI	653820,729533	FAM72B, FAM72A
427	LLYDAVHIV	2899	GRIK3
428	LMYPYIYHV	54954	FAM120C
429	NLLETKLQL	219988	PATL1
430	QLIEKNWLL	56992	KIF15
431	RMVAEIQNV	11262	SP140
432	SAVDFIRTL	9263	STK17A
433	SILDRDDIFV	8237	USP11
434	SIQQSIERLLV	4809	NHP2L1
435	SLFNQEVQI	100528032,22914,8302	KLRK1, KLRC4
436	SLFSSLEPQIQPV	23029	RBM34
437	SLEPQIQPV	23029	RBM34
438	SLFIGEKAVLL	23029	RBM34
439	SLLDLHTKV	27340	UTP20
440	SLLEQGKEPWMV	147949,163087,342892,374899,84503	ZNF583, ZNF383, ZNF850, ZNF829, ZNF527
441	SLLEVNEASSV	149628	PYHIN1
442	SLNDLEKDVMLL	6597	SMARCA4
443	SLTIDGIYYV	1659	DHX8
444	SMSGYDQVL	3187,3188	HNRNPH1, HNRNPH2
445	VIIKGLEEITV	3832	KIF11
446	VILTSSPFL	10800	CYSLTR1
447	VLDDSIYLV	57565	KLHL14
448	VMNDRLYAI	57565	KLHL14
449	LLDAMNYHL	57565	KLHL14
450	VLGPGPPPL	254359	ZDHHC24
451	VLIEYNFSI	55215	FANCI
452	VLLSLLEKV	1130	LYST
453	SLLPLVWKI	1130	LYST
454	VLMDEGAVLTL	54596	L1TD1
455	VLPDPEVLEAV	57326	PBXIP1

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
456	YAAPGGLIGV	1968,255308	EIF2S3
457	YIMEPSIFNTL	51497	TH1L
458	YIQEHLLQI	10625	IVNS1ABP
459	YLDFSNNRL	51284	TLR7
460	YLEDGFAYV	5558	PRIM2
461	YLLNLNHLGL	23471	TRAM1
462	YVLPKLYVKL	100128168,100996747,441502,6231,643003,644166,644928,644934,646753,728937,729188	RPS26P39, RPS26P11, RPS26, RPS26P28, RPS26P20, RPS26P15, RPS26P50, RPS26P2, RPS26P25, RPS26P58
463	ALMAVVSGL	55103	RALGPS2
464	ALSDETKNNWEV	5591	PRKDC
465	FLYDEIEAEVNL	23191,26999	CYFIP1, CYFIP2
466	FLYDEIEAEV	23191,26999	CYFIP1, CYFIP2
467	IMQDFPAEIFL	25914	RTTN
468	KIQEILTQV	10643	IGF2BP3
469	SLDGTELQL	284114	TMEM102
470	TLTNIIHNL	94101	ORMDL1
471	VLLAAGPSAA	23225	NUP210
472	YLFESEGLVL	283431	GAS2L3
473	ALADDDFLTV	4173	MCM4
474	ALADLIEKELSV	4850	CNOT4
475	ALDDMISTL	7203	CCT3
476	ALITEVVRL	26005	C2CD3
477	ALLDQTKTLAESAL	7094	TLN1
478	AMFESSQNVLL	64328	XPO4
479	YLAHFIEGL	64328	XPO4
480	FLAEDPKVTL	60489	APOBEC3G
481	FLIDTSASM	203522,26512	DDX26B, INTS6
482	FLTDLEDLTL	26151	NAT9
483	FLTEMVHFI	93517	SDR42E1
484	FLVDGPRVQL	90204	ZSWIM1
485	GLLDCPIFL	2177	FANCD2
486	GLLDLPFRVGV	23347	SMCHD1
487	GLSDGNPSL	79684	MSANTD2
488	GVYDGEEHSV	4113	MAGEB2
489	HLANIVERL	117854,445372,53840	TRIM6, TRIM6-TRIM34, TRIM34
490	ILDEKPVII	23460	ABCA6
491	ILFSEDSTKLFV	84916	CIRH1A

SEQ ID No.	Sequence	GeneID(s)	Official Gene Symbol(s)
492	ILLDDNMQIRL	5261	PHKG2
493	LIIDQADIYL	27042	DIEXF
494	LLGDSSFFL	283254	HARBI1
495	LLLDEEGTFSL	27013	CNPPD1
496	LLVEQPPLAGV	4773	NFATC2
497	LLWDVPAPSL	1388,7148	ATF6B, TNXB
498	LTAPPEALLMV	79050	NOC4L
499	NLMEMVAQL	55636	CHD7
500	QIITSVVSV	5378	PMS1
501	QLALKVEGV	25896	INTS7
502	QLLDETSAITL	10915	TCERG1
503	QLYEEPDTKL	10270	AKAP8
504	RLYSGISGLEL	84172	POLR1B
505	RVLEVGALQAV	25885	POLR1A
506	SLLPLDDIVRV	3708,3709	ITPR1, ITPR2
507	SLLTEQDLWTV	90806	ANGEL2
508	SVDSAPAAV	10635	RAD51AP1
509	TLQEVVTGV	55750	AGK
510	TVDVATPSV	8924	HERC2
511	VLAYFLPEA	4171	MCM2
512	VLISVLQAI	26999	CYFIP2
513	YLIPFTGIVGL	26001	RNF167
514	YLLDDGTLVV	8872	CDC123
515	YLMPGFIHL	168400,55510	DDX53, DDX43
516	YLPDIIKDQKA	5496	PPM1G
517	YLQLTQSEL	283237	TTC9C
518	YLTEVFLHVV	55024	BANK1
519	YLVEGRFSV	55125	CEP192
520	YLVYILNEL	51202	DDX47
521	YLYGQTTTYL	7153	TOP2A
522	YVLTQPPSV	28796,28815,28831,3537,3538	IGLV3-21, IGLV2-14, IGLJ3, IGLC1, IGLC2

The present invention furthermore generally relates to the peptides according to the present invention for use in the treatment of proliferative diseases, such as, for example, acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer.

Particularly preferred are the peptides – alone or in combination - according to the present invention selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 385. More preferred are the peptides – alone or in combination - selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 202 (see Table 1), and their uses in the immunotherapy of CLL, acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer, and preferably CLL.

As shown in the following Tables 4A and B, many of the peptides according to the present invention are also found on other tumor types and can, thus, also be used in the immunotherapy of other indications. Also, refer to Figure 1 and Example 1.

Table 4A: Peptides according to the present invention and their specific uses in other proliferative diseases, especially in other cancerous diseases. The table shows for selected peptides on which additional tumor types they were found and either over-presented on more than 5% of the measured tumor samples, or presented on more than 5% of the measured tumor samples with a ratio of geometric means tumor vs normal tissues being larger than 3. Over-presentation is defined as higher presentation on the tumor sample as compared to the normal sample with highest presentation. Normal tissues against which over-presentation was tested were: adipose tissue, adrenal gland, blood cells, blood vessel, bone marrow, brain, breast, esophagus, eye, gallbladder, heart, kidney, large intestine, liver, lung, lymph node, nerve, pancreas, parathyroid gland, peritoneum, pituitary gland, pleura, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, thyroid gland, trachea, ureter, and urinary bladder.

SEQ ID No.	Sequence	Other relevant organs/diseases
1	AIPPSFASIFL	NHL
2	ALHRPDVYL	NHL
3	VIAELPPKV	NHL
5	ALIFKIASA	AML, BRCA
7	ALLERTGYTL	HCC, NHL
8	ALAASALPALV	AML
9	ALCDTLITV	NHL
10	FVYGESVEL	NHL
12	ALGEDEITL	NHL, AML, Melanoma
13	VVDGMPPGV	NHL
14	ALLRLLPGL	NHL, AML
15	ALPEVSVEA	NHL, AML, Melanoma
19	QVMEKLAAV	NHL
20	ALVDPGPDFVV	NHL
21	ALWAGLLTL	NHL
22	ALWDPVIEL	NHL
24	AMAGDVVYA	NHL
25	ATYSGLESQSV	Melanoma
26	AVLLVLPLV	NHL
27	AVLGLVWLL	NSCLC, NHL, Uterine Cancer
28	AVLHLLLSV	NHL, BRCA
29	AVLQAVTAV	SCLC, NHL, AML, Melanoma, OC, Gallbladder Cancer, Bile Duct Cancer
30	ELLEGSEIYL	NHL
32	FIDKFTPPV	NHL
33	FIINSSNIFL	NHL
35	FILPSSLYL	RCC, NHL, AML, Gallbladder Cancer, Bile Duct Cancer
37	FIMEGGAMVL	AML
38	FIMEGGAMV	NHL
39	FLDALLTDV	SCLC, NHL, AML, Melanoma
40	FLDEDDMSL	SCLC, NHL, Urinary bladder cancer
42	FLEEGGVVTV	NHL, Melanoma
43	FLLGPEALSFA	NHL, AML
44	FLLSINDFL	Melanoma
45	FLPELPADLEA	NHL, Melanoma
46	YIIDSAQAV	NHL
48	FLSPQQPPLL	NHL
49	FLTDLFAQL	NHL
52	FLVETGFLHV	Melanoma
55	FMEPTLLML	NHL, Urinary bladder cancer
56	FVFEAPYTL	NHL, AML

SEQ ID No.	Sequence	Other relevant organs/diseases
57	GLSEISLRL	NHL, AML, Melanoma
58	YIQQGIFSV	NHL
59	FVFGDENGTVSL	NHL, Esophageal Cancer, Urinary bladder cancer, Uterine Cancer
60	FVLDHEDGLNL	Melanoma, Esophageal Cancer
62	GIIDGSPRL	NHL
63	SLAHVAGCEL	NHL
64	KLLESVASA	NHL
66	SLAGGLDDMKA	NHL
67	GLDDVTVEV	HCC, NHL, Melanoma, Esophageal Cancer, OC, Gallbladder Cancer, Bile Duct Cancer
68	GLDQQFAGLDL	RCC, HCC, NHL, Melanoma
69	GLHQREIFL	NHL
70	FVPDTPVGV	NHL
71	GLKHDIARV	NHL
72	GLLDAGKMYV	NHL, Melanoma
74	GLLRASFLL	NHL, AML, BRCA
75	GLSIFAQDLRL	NHL
76	GLLRIIPYL	NHL
78	GLPSFLTEV	HCC, NHL, AML, Melanoma
79	GLQAKIQEA	NHL, Esophageal Cancer, Uterine Cancer
80	VLIEDELEEL	NHL
81	WLVGQEFEL	NHL
82	GLQSGVDIGV	NHL
83	GQGEVLVYV	SCLC, GC, Esophageal Cancer
86	HLYPGAVTI	HCC, NHL
87	HQIEAVDGEEL	GC, NHL, Esophageal Cancer
88	ILDEIGADVQA	PC, NHL
89	ILDFGTFQL	NHL
91	ILDLNTYNV	NHL
92	ILEPLNPLL	NHL
93	ILFNTQINI	SCLC, NHL, AML, Melanoma, OC
94	ILFPLRFTL	NSCLC, RCC, CRC, NHL, Melanoma, OC
96	ILIDKTSFV	NHL
97	LLFATQITL	NHL
98	SLIKYFLFV	NHL
99	ILIFHSVAL	NHL, AML
100	ILNNEVFAI	NHL, AML
101	ILVVIEPLL	NHL
102	IQDRAVPSL	AML
103	KLGGTPAPA	NHL

SEQ ID No.	Sequence	Other relevant organs/diseases
105	KLMNDIADI	NHL
109	KLWEGLTELV	AML
110	LLFDHLEPMEL	NHL
111	KLWNVAAPLYL	OC
112	KTLDVDATYEI	HCC, NHL, Melanoma, Esophageal Cancer, OC
113	KVPAEEVLVAV	NHL
116	LLIGATMQV	SCLC, NHL, AML
117	LLILENILL	NHL, AML, Melanoma
118	RLLILENILL	NHL
119	VLPAEFFEV	NHL, AML, Urinary bladder cancer, Uterine Cancer
120	AIDAALTSV	NHL, AML
121	RLYEITIEV	SCLC, NHL, AML, BRCA, OC
122	LLIPVVPGV	NSCLC, NHL
124	LLLEETEKQAV	RCC, HCC
125	LLLEIGEVGKLFV	NHL
126	LLPEGGITAI	NHL
127	LLPTAPTTV	NHL
130	LLVLIPVYL	NHL, Melanoma
133	YLLSGDISEA	NHL
136	NLDKLWTLV	GC, Esophageal Cancer
137	NLIKTVIKL	NHL
138	NLLDIDAPVTV	SCLC, NHL
139	NLTDVVEKL	RCC, NHL
141	QILSEIVEA	NHL, Melanoma
145	SALDTITTV	NHL
146	SIIEGPIIKL	NHL, Urinary bladder cancer
147	SILETVATL	NHL, AML
148	SIVASLITV	NHL, AML
149	SLDNGGYYI	NHL
150	SLFDQPLSII	NHL
151	SLFDSAYGA	AML
152	SLIRILQTI	HCC
153	SLLAELHVL	NHL
154	SLLAELHVLTV	NHL
155	SLMLEVPAL	HCC, NHL
156	SLNIGDVQL	NHL
157	SLNIRDFTM	AML
158	SLPEAPLDV	NHL, AML, Uterine Cancer
159	SLQEEKLIYV	Brain Cancer, NHL, MCC, Melanoma, Esophageal Cancer, Urinary bladder cancer
160	SLSFLVPSL	NHL, AML, Urinary bladder cancer

SEQ ID No.	Sequence	Other relevant organs/diseases
161	SMDDGMINV	CRC, NHL, Gallbladder Cancer, Bile Duct Cancer
162	SMKDDLENV	NSCLC, SCLC, RCC, HCC, NHL, BRCA, Melanoma, Esophageal Cancer, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer
163	SQLDISEPYKV	NHL
164	SVHKGFAFV	NSCLC, RCC, Brain Cancer, GC, AML, Esophageal Cancer, Urinary bladder cancer, Uterine Cancer
165	TLDDDLDTV	NHL
166	TLDPNQVSL	AML
167	TLDTSKLYV	NHL
168	YLLDQSFVM	NHL
169	TLLLGLTEV	NHL, Melanoma
170	TLTFRVETV	NHL, Melanoma
172	TLYDMLASI	CRC, NHL, AML, Melanoma
175	FTVPRVVAV	NHL
177	VLDDRELLL	NHL
178	VLFFNVQEV	SCLC, NHL
179	VLLGLEMTL	NHL, Esophageal Cancer
180	LLKDGPEIGL	NHL
181	VLLSIPFVSV	HCC, NHL
183	VLMPTVYQQGV	AML
185	VMDDQRDLI	AML
186	VMDPTKILI	AML, Urinary bladder cancer
187	VMDTHLVNI	NHL, Urinary bladder cancer
188	VMGDIPAAV	NHL, AML, Gallbladder Cancer, Bile Duct Cancer
189	VMLEMTPEL	SCLC, PC, NHL, BRCA, Melanoma, Gallbladder Cancer, Bile Duct Cancer
190	VVMGTVPRL	NHL, AML
192	YIQEYLTLL	NHL
193	YLDLSNNRL	NHL
194	YLDNVLAEL	NHL
195	YLGGFALSV	NHL, AML
196	YLLLQTYVL	NHL
197	YLQEVPILTL	NHL, Melanoma
199	YLVELSSLL	HCC, NHL, AML
200	YMFEEVPIVI	NSCLC, SCLC, CRC, HCC, NHL, Melanoma, Esophageal Cancer, OC, Urinary bladder cancer, Gallbladder Cancer, Bile Duct Cancer
201	YQLELHGIEL	Esophageal Cancer
202	YVDDVFLRV	Urinary bladder cancer
203	ALLSSQLAL	NHL
204	GLLQINDKIAL	SCLC, RCC, HCC, Melanoma, OC, Urinary bladder

SEQ ID No.	Sequence	Other relevant organs/diseases
		cancer
205	GLSQANFTL	NHL
207	IIADLDTTIMFA	SCLC, NHL, Melanoma, OC
208	ILLKTEGINL	NHL, BRCA, OC, Urinary bladder cancer
209	ILQAELPSL	NHL
210	KLLVQDFFL	HCC, NHL
211	LIDVKPLGV	NHL, BRCA, Melanoma
212	NIIEAINELLV	NHL, BRCA, Melanoma
213	RLLYQLVFL	NHL, Esophageal Cancer, Urinary bladder cancer, Gallbladder Cancer, Bile Duct Cancer
214	RLQELTEKL	NHL, AML
215	VMQDIVYKL	NHL, Esophageal Cancer, Urinary bladder cancer
216	WLAGDVPAA	SCLC, NHL
217	ALDEPPYLTV	NHL, Melanoma
218	ALGEEWKGYVV	Esophageal Cancer
219	ALLNLLESA	NHL, AML, OC
220	ALPEILFAKV	NHL
221	ALVSTIIMV	NHL, AML
222	ALWELSLKI	NHL
223	ALWVSQPPEI	NHL
224	AMEALVVEV	NHL
225	ILQERELLPV	NHL, Melanoma, OC, Urinary bladder cancer
227	FLAEASVMTQL	NHL
228	FLGGLSPGV	NHL, Esophageal Cancer
229	FLLNLQNCHL	NHL
230	FLQDSKVIFV	NHL
231	FLYIRQLAI	AML
232	FMHQIIDQV	RCC, CRC, Melanoma
233	GIIDINVRL	PC, NHL, Melanoma, Esophageal Cancer, Urinary bladder cancer, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer
234	GLDDAEYAL	NHL, AML, Melanoma, Urinary bladder cancer
235	GLDDLLLFL	SCLC, PC, NHL, AML, Urinary bladder cancer, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer
236	GLLESGRHYL	NHL, AML
237	GLQENLDVVV	SCLC, NHL, Melanoma, Uterine Cancer
238	GLVETELQL	NHL, AML
239	ILAGEMLSV	NHL, AML, Melanoma, Gallbladder Cancer, Bile Duct Cancer
240	ILARDILEI	NHL, Urinary bladder cancer
241	ILGDILLKV	AML
242	ILLGIQELL	NHL, AML

SEQ ID No.	Sequence	Other relevant organs/diseases
243	ILPTLEKELFL	AML
244	ILQALAVHL	NHL, AML, OC
245	KIMDYSLLLGV	SCLC, RCC, OC
246	KLDETGVAL	HCC, NHL, AML, Melanoma
247	KLKDRLPSI	NHL
248	KTVEPPISQV	NHL
249	LLPTGVFQV	Urinary bladder cancer
250	LLVQEPDGLMV	SCLC, HCC, Melanoma, Urinary bladder cancer
251	LLYDNVPGA	NHL
252	NLLDPGSSYLL	Esophageal Cancer
253	NLWSVDGEVTV	NHL
254	QLIPKLIFL	NHL, Melanoma
255	YLFEEAISM	NHL
257	RIINGIIISV	NHL
258	RLDYITAEI	RCC, NHL, AML, Melanoma
260	SLDDVEGMSV	NHL, Gallbladder Cancer, Bile Duct Cancer
261	SLVEAQGWLV	NHL
263	SQWEDIHVV	NHL, AML
264	TILDYINVV	Brain Cancer, NHL, AML, BRCA, OC, Uterine Cancer
265	TLLADDLEIKL	Esophageal Cancer
266	TLLDQLDTQL	NHL, OC, Urinary bladder cancer
267	TLLDWQDSL	NHL, AML, Melanoma
268	TLLQVFHLL	NHL
269	TLTDEQFLV	NHL, AML
271	VIRNIVEAA	GC, CRC, PC, AML, Esophageal Cancer
272	VLDELPPLI	SCLC, NHL
273	VLGEYSYLL	NHL
274	VLLEYHIAYL	NSCLC, SCLC, HCC, NHL, Urinary bladder cancer
275	VLLFIEHSV	NHL
276	VLNDGAPNV	NSCLC, SCLC, NHL, Melanoma, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer
278	YLDDLLPKL	PC, NHL
280	YTLDSLYWSV	NHL
281	NLLDDRGMTAL	NHL, AML
282	LLRDGIELV	NHL, Melanoma, OC
283	ILQPMDIHV	NHL
284	LLSAAEPVPA	NHL
286	FLLEDLSQKL	NHL
288	GLAESTGLLAV	NHL
290	LANPHELSL	AML
291	ILLNEDDLVTI	SCLC, NHL, Melanoma

SEQ ID No.	Sequence	Other relevant organs/diseases
292	AAALIIHHV	Urinary bladder cancer
294	ALLDQLHTLL	NHL
295	ALLQKLQQL	NHL
296	FIAPTGHSL	SCLC, NHL, AML, BRCA
297	FLVEPQEDTRL	NHL, Melanoma
298	IILPVEVEV	SCLC, NHL, AML, Urinary bladder cancer
299	ILEENIPVL	NHL
300	ILLNPAYDVYL	NHL, Melanoma
303	ILSQPTPSL	NHL, AML, Melanoma, OC
304	LAIVPVNTL	AML
305	LLFPQIEGIKI	NHL, Melanoma
306	VVAEELENV	NHL, Melanoma, OC
307	LLLTKPTEA	NHL
308	SLYDVSRMYV	NHL
309	ILYGTQFVL	NHL
310	LLSTLHLLV	NHL
311	LLVDVEPKV	NSCLC, SCLC, RCC, HCC, PC, NHL, AML, Melanoma
312	LLYNSTDPTL	NHL
313	LMADLEGLHL	Melanoma, Urinary bladder cancer
314	LMKDCEAEV	NHL
316	MLLEHGITL	NHL
317	NLLAHIWAL	AML
320	TIAPVTVAV	NHL
321	RLLEFELAQL	SCLC, CRC, NHL, OC
322	SLASIHVPL	NHL
323	SLDLFNCEV	NHL
324	SLYSALQQA	NHL
325	TLENGVPCV	NHL
326	VLAFLVHEL	Esophageal Cancer
327	VLIKWFPEV	NHL
328	VLLPQETAEIHL	NHL, BRCA, Melanoma, OC, Urinary bladder cancer
329	VLMDGSVKL	NHL
330	VLMWEIYSL	NHL
332	VMIQHVENL	NHL
333	VTLEFPQLIRV	SCLC, HCC, NHL
334	YLLEEKIASL	NHL
335	YLYQEQYFI	SCLC, NHL, AML, Urinary bladder cancer
337	YMYEKESEL	NHL
338	FLDMTNWNL	HCC, NHL, Melanoma, OC, Urinary bladder cancer
339	GLWGTVVNI	NHL, AML, Melanoma, Uterine Cancer

SEQ ID No.	Sequence	Other relevant organs/diseases
341	LLAELPASVHA	HCC, NHL, Urinary bladder cancer
342	SLITPLQAV	NHL, AML, Urinary bladder cancer, Gallbladder Cancer, Bile Duct Cancer
343	TLLEALDCI	NHL, AML, Melanoma, Esophageal Cancer
344	VLAFENPQV	Esophageal Cancer
346	VLVQVSPSL	RCC, NHL, AML, Gallbladder Cancer, Bile Duct Cancer
347	YLGPVSPSL	NHL
348	ALAKPPVVSV	SCLC, HCC, Melanoma, Esophageal Cancer
349	ALATHILSL	NHL, Urinary bladder cancer
350	ALEDRVWEL	NHL, Esophageal Cancer
351	ALSEKLARL	HCC, NHL, AML, Urinary bladder cancer
352	ALVFELHYV	NHL
353	ATPMPTPSV	SCLC, NHL
354	FIMDDPAGNSYL	CRC, NHL, AML, Melanoma, Esophageal Cancer, Urinary bladder cancer, Gallbladder Cancer, Bile Duct Cancer
357	FLIQEIKTL	NHL, OC, Urinary bladder cancer
358	FLTDYLNDL	NHL
359	FMQDPMEVFV	Melanoma
360	HLIDTNKIQL	NHL
362	ILTELGGFEV	NHL
363	ITTEVVNELYV	NHL, Melanoma
364	KMDWIFHTI	Urinary bladder cancer
365	LISPLLLPV	NHL, AML, Melanoma, Urinary bladder cancer
367	NLWSLVAKV	NSCLC, NHL, AML, Melanoma, OC
369	RLLDLENSLLGL	AML
370	SIFASPESV	Melanoma, Esophageal Cancer, Urinary bladder cancer
371	SLADDSVLERL	RCC, HCC, NHL, AML, Esophageal Cancer, Urinary bladder cancer
373	TLLADQGEIRV	NHL, Melanoma
374	VLSVITEEL	NHL, AML
375	VLWFKPVEL	NSCLC, HCC, NHL, Melanoma, Esophageal Cancer
376	VLYNQRVEEI	Urinary bladder cancer
377	VVDGTCVAV	NHL, Melanoma
378	YILGKFFAL	NHL, AML
379	YLAELVTPIL	NSCLC, SCLC, NHL, Melanoma
380	YLDRKLLTL	NHL
381	YLLEENKIKL	NHL
382	YLLPLLQRL	NHL, AML
383	YLLREWVNL	HCC, NHL
384	YMIGSEVGNYL	NHL, Urinary bladder cancer
385	YTIPLAIKL	NHL, AML, BRCA, Esophageal Cancer

NSCLC= non-small cell lung cancer, SCLC= small cell lung cancer, RCC= kidney cancer, CRC= colon or rectum cancer, GC= stomach cancer, HCC= liver cancer, PC= pancreatic cancer, PrC= prostate cancer, leukemia, BRCA=breast cancer, MCC= Merkel cell carcinoma, NHL = Non Hodgkin lymphoma

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 1, 2, 3, 7, 9, 10, 13, 14, 15, 19, 20, 21, 22, 24, 26, 27, 28, 29, 30, 32, 33, 35, 38, 39, 40, 42, 43, 45, 46, 48, 49, 55, 56, 57, 58, 59, 62, 63, 64, 66, 67, 68, 69, 70, 71, 72, 74, 75, 76, 78, 79, 80, 81, 82, 86, 87, 88, 89, 91, 92, 93, 94, 96, 97, 98, 99, 100, 101, 103, 105, 110, 112, 113, 116, 117, 118, 119, 120, 121, 122, 125, 126, 127, 130, 133, 137, 138, 139, 141, 145, 146, 147, 148, 149, 150, 153, 154, 155, 156, 158, 159, 160, 161, 162, 163, 165, 167, 168, 169, 170, 172, 175, 177, 178, 179, 180, 181, 187, 188, 189, 190, 192, 193, 194, 195, 196, 197, 199, 200, 203, 205, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 219, 220, 221, 222, 223, 224, 225, 227, 228, 229, 230, 233, 234, 235, 236, 237, 238, 239, 240, 242, 244, 246, 247, 248, 251, 253, 254, 255, 257, 258, 260, 261, 263, 264, 266, 267, 268, 269, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 286, 288, 290, 291, 294, 295, 296, 297, 298, 299, 300, 303, 305, 306, 307, 308, 309, 310, 311, 312, 314, 316, 320, 321, 322, 323, 324, 325, 327, 328, 329, 330, 332, 333, 334, 335, 337, 338, 339, 341, 342, 343, 346, 347, 349, 350, 351, 352, 353, 354, 357, 358, 360, 362, 363, 365, 367, 371, 373, 374, 375, 377, 378, 379, 380, 381, 382, 383, 384, and 385 for the – in one preferred embodiment combined - treatment of NHL.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 5, 8, 12, 14, 15, 29, 35, 37, 39, 43, 56, 57, 74, 78, 93, 99, 100, 102, 109, 116, 117, 119, 120, 121, 147, 148, 151, 157, 158, 160, 164, 166, 172, 183, 185, 186, 188, 190, 195, 199, 214, 219, 221, 231, 234, 235, 236, 238, 239, 241, 242, 243, 244, 246, 258, 263, 264, 267, 269, 271, 281, 290, 296, 298, 303, 304, 311, 317, 335, 339, 342, 343, 346, 351, 354, 365, 367, 369, 374, 378, 382, and 385 for the – in one preferred embodiment combined - treatment of AML.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 5, 28, 74, 121, 162, 208, 211, 212, 264, 296, 328, and 385 for the – in one preferred embodiment combined - treatment of BRCA.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 7, 67, 68, 78, 86, 112, 124, 152, 155, 162, 181, 199, 200, 204, 210, 246, 250, 274, 311, 333, 338, 341, 348, 351, 371, 375, and 383 for the – in one preferred embodiment combined - treatment of HCC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 12, 15, 25, 29, 39, 42, 44, 45, 52, 57, 60, 67, 68, 72, 78, 93, 94, 112, 117, 130, 141, 159, 162, 169, 170, 172, 189, 197, 200, 204, 207, 211, 212, 217, 225, 232, 233, 234, 237, 239, 246, 250, 254, 258, 267, 276, 282, 291, 297, 300, 303, 305, 306, 311, 313, 328, 338, 339, 343, 348, 354, 359, 363, 365, 367, 370, 373, 375, 377, and 379 for the – in one preferred embodiment combined - treatment of melanoma.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 27, 94, 122, 162, 164, 200, 274, 276, 311, 367, 375, and 379 for the – in one preferred embodiment combined - treatment of NSCLC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 27, 59, 79, 119, 158, 162, 164, 233, 235, 237, 264, 276, and 339 for the – in one preferred embodiment combined - treatment of uterine cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 29, 67, 93, 94, 111, 112, 200, 204, 207, 208, 219, 225, 244, 245, 264, 266, 282, 303, 306, 321, 328, 338, 357, and 367 for the – in one preferred embodiment combined - treatment of ovarian cancer (OC).

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 29, 35, 67, 161, 162, 188, 189, 200, 213, 233, 235, 239, 260, 276, 342, 346, and 354 for the – in one preferred embodiment combined - treatment of gallbladder cancer and/or bile duct cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 35, 68, 94, 124, 139, 162, 164, 197, 204, 232, 245, 258, 311, 346, and 371 for the – in one preferred embodiment combined - treatment of RCC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 39, 40, 83, 93, 121, 138, 162, 178, 189, 200, 204, 207, 216, 235, 237, 245, 250, 272, 274, 276, 291, 296, 298, 311, 321, 333, 335, 348, 353, and 379 for the – in one preferred embodiment combined - treatment of SCLC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 40, 55, 59, 119, 146, 159, 160, 164, 186, 187, 200, 202, 204, 208, 213, 215, 225, 233, 234, 235, 240, 249, 250, 266, 274, 292, 298, 313, 328, 335, 338, 341, 342, 349, 351, 354, 357, 364, 365, 370, 371, 376, and 384 for the – in one preferred embodiment combined - treatment of urinary bladder cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 59, 60, 67, 79, 83, 87, 112, 136, 159, 162, 164, 179, 200, 201, 213, 215, 218, 228, 233, 252, 265, 271, 326, 343, 344, 348, 350, 354, 370, 371, 375, and 385 for the – in one preferred embodiment combined - treatment of esophageal cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 83, 87, 136, 164, and 271 for the – in one preferred embodiment combined - treatment of gastric cancer (GC).

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 88, 189, 233, 235, 271, 278, and 311 for the – in one preferred embodiment combined - treatment of pancreatic cancer (PC).

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 94, 161, 172, 200, 232, 271, 321, and 354 for the – in one preferred embodiment combined - treatment of colorectal cancer (CRC).

Thus, another aspect of the present invention relates to the use of the peptide according to the present invention according to SEQ ID No. 159 for the treatment of MCC.

Thus, another aspect of the present invention relates to the use of the peptides according to the present invention for the - preferably combined - treatment of a proliferative disease selected from the group of CLL, acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer.

Table 4B: Peptides according to the present invention and their specific uses in other proliferative diseases, especially in other cancerous diseases. The table shows, like Table 4A, for selected peptides on which additional tumour types they were found showing over-presentation (including specific presentation) on more than 5% of the measured tumour samples, or presentation on more than 5% of the measured tumour samples with a ratio of geometric means tumour vs normal tissues being larger than 3. Over-presentation is defined as higher presentation on the tumour sample as compared to the normal sample with highest presentation. Normal tissues against which over-presentation was tested were: adipose tissue, adrenal gland, artery, bone marrow, brain, central nerve, colon, duodenum, oesophagus, eye, gallbladder, heart, kidney, liver, lung, lymph node, mononuclear white blood cells, pancreas, parathyroid gland, peripheral nerve, peritoneum, pituitary, pleura, rectum, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thyroid gland, trachea, ureter, urinary bladder, vein.

SEQ ID NO.	Sequence	Additional Entities
5	ALIFKIASA	Uterine Cancer
7	ALLERTGYTL	Uterine Cancer, AML
8	ALAASALPALV	AML, HNSCC
11	ALFTFSPLTV	AML, HNSCC, NHL, NSCLC, OC, SCLC
13	VVDGMPPGV	BRCA, Uterine Cancer
14	ALLRLLPGL	BRCA, Melanoma, HNSCC
15	ALPEVSVEA	Esophageal Cancer
25	ATYSGLESQSV	AML
26	AVLLVLPLV	NHL, NSCLC
27	AVLGLVWLL	HNSCC
29	AVLQAVTAV	Uterine Cancer
30	ELLEGSEIYL	CRC, BRCA, Melanoma, Uterine Cancer
35	FILPSSLYL	SCLC, Uterine Cancer
37	FIMEGGAMVL	NHL
38	FIMEGGAMV	HNSCC
40	FLDEDDMSL	HNSCC

41	FLDPSLDPLL	Uterine Cancer
42	FLEEGGVVTV	SCLC, AML
44	FLLSINDFL	RCC, Uterine Cancer, AML, NHL
45	FLPELPADLEA	NSCLC, Uterine Cancer, OC
48	FLSPQQPPLL	HNSCC
49	FLTDLFAQL	NHL, Melanoma
51	FLVEAPHDWDL	Uterine Cancer
55	FMEPTLLML	HNSCC
58	YIQQGIFSV	NHL, NSCLC
59	FVFGDENGTVSL	SCLC, HNSCC
67	GLDDVTVEV	AML
68	GLDQQFAGLDL	BRCA, OC, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer, HNSCC
76	GLLRIIPYL	RCC
82	GLQSGVDIGV	AML, Esophageal Cancer, PC
83	GQGEVLVYV	NHL
84	GVMDVNTAL	AML, HCC, HNSCC, NHL, NSCLC, GC, RCC
86	HLYPGAVTI	AML
87	HQIEAVDGEEL	PC
89	ILDFGTFQL	BRCA
90	VIADLGLIRV	Melanoma
91	ILDLNTYNV	AML
93	ILFNTQINI	Uterine Cancer, HNSCC
94	ILFPLRFTL	Gallbladder Cancer, Bile Duct Cancer, AML, HNSCC
101	ILVVIEPLL	Uterine Cancer
107	ILYNLYDLL	Melanoma
108	VIYTLIHYI	BRCA, Melanoma
111	KLWNVAAPLYL	Melanoma
112	KTLDVDATYEI	Uterine Cancer
113	KVPAEEVLVAV	NHL, NSCLC
114	LIPEGPPQV	AML, HNSCC
115	LLFDKLYLL	Uterine Cancer
118	RLLILENILL	HNSCC
119	VLPAEFFEV	SCLC
124	LLLEETEKQAV	HNSCC
125	LLLEIGEVGKLFV	BRCA
126	LLPEGGITAI	SCLC, HCC, Melanoma, Uterine Cancer, HNSCC
127	LLPTAPTTV	SCLC

128	LLSEEEYHL	Urinary Bladder Cancer, NHL, HNSCC
130	LLVLIPVYL	Uterine Cancer
136	NLDKLWTLV	AML, CRC, Melanoma, NSCLC, Brain Cancer
138	NLLDIDAPVTV	Uterine Cancer, AML
141	QILSEIVEA	Uterine Cancer
145	SALDTITTV	NSCLC, RCC, GC, HCC, AML
146	SIIEGPIIKL	HNSCC
147	SILETVATL	Melanoma
152	SLIRILQTI	SCLC
156	SLNIGDVQL	AML
159	SLQEEKLIYV	HNSCC
160	SLSFLVPSL	Uterine Cancer
161	SMDDGMINV	SCLC, Melanoma, Uterine Cancer, AML, HNSCC
162	SMKDDLENV	PC, OC, HNSCC
164	SVHKGFAFV	CRC, Melanoma, HNSCC
172	TLYDMLASI	SCLC, Urinary Bladder Cancer, Uterine Cancer
173	TVIENIHTI	CRC, HCC, Melanoma, NHL, NSCLC, OC, Esophageal Cancer, GC, RCC
174	VLAELPIIVV	NSCLC, OC
176	VLAEQNIIPSA	NSCLC, SCLC, GC, Melanoma, NHL, HNSCC
179	VLLGLEMTL	HNSCC
181	VLLSIPFVSV	Melanoma, Gallbladder Cancer, Bile Duct Cancer
184	VLSHNLYTV	NHL
187	VMDTHLVNI	CRC, Melanoma, Uterine Cancer
188	VMGDIPAAV	Urinary Bladder Cancer, HNSCC
189	VMLEMTPEL	HNSCC
190	VVMGTVPRL	Melanoma
191	YIFDGSDGGV	Uterine Cancer
192	YIQEYLTLL	SCLC, Urinary Bladder Cancer, AML, HNSCC
197	YLQEVPILTL	Uterine Cancer
198	YLTFLPAEV	BRCA, Uterine Cancer
199	YLVELSSLL	Melanoma
200	YMFEEVPIVI	BRCA, HNSCC
201	YQLELHGIEL	NHL
202	YVDDVFLRV	AML, HNSCC
203	ALLSSQLAL	NHL, AML, NSCLC
204	GLLQINDKIAL	BRCA, Uterine Cancer, AML
208	ILLKTEGINL	Melanoma, HNSCC
211	LIDVKPLGV	RCC, Brain Cancer, HCC, Esophageal Cancer, HNSCC

212	NIIEAINELLV	Uterine Cancer, AML, HNSCC
215	VMQDIVYKL	HNSCC
216	WLAGDVPAA	AML, HNSCC
217	ALDEPPYLTV	AML
218	ALGEEWKGYVV	AML
221	ALVSTIIMV	Brain Cancer
224	AMEALVVEV	AML
226	AMNISVPQV	AML, PrC, Brain Cancer, Gallbladder Cancer , CCC, HNSCC, NHL, NSCLC, OC, SCLC, Uterine Cancer
228	FLGGLSPGV	SCLC, BRCA, Uterine Cancer, AML, HNSCC
231	FLYIRQLAI	Uterine Cancer
232	FMHQIIDQV	Uterine Cancer
234	GLDDAEYAL	Uterine Cancer, HNSCC
235	GLDDLLLFL	BRCA, HNSCC
237	GLQENLDVVV	AML
239	ILAGEMLSV	SCLC, HCC
240	ILARDILEI	Melanoma
242	ILLGIQELL	BRCA
247	KLKDRLPSI	HCC
257	RIINGIIISV	AML
258	RLDYITAEI	GC, Uterine Cancer
260	SLDDVEGMSV	SCLC, HNSCC
261	SLVEAQGWLV	AML
264	TILDYINVV	HNSCC
266	TLLDQLDTQL	CRC, BRCA, Uterine Cancer
268	TLLQVFHLL	Uterine Cancer
269	TLTDEQFLV	BRCA
272	VLDELPPLI	BRCA, Uterine Cancer, HNSCC
273	VLGEYSYLL	NHL, HNSCC
274	VLLEYHIAYL	Melanoma, HNSCC
275	VLLFIEHSV	Melanoma
279	YMAPEVVEA	HNSCC, NHL, NSCLC, SCLC, Uterine Cancer
280	YTLDSLYWSV	SCLC, BRCA, Melanoma, HNSCC
286	FLLEDLSQKL	Uterine Cancer
289	ILEEQPMDMLL	Uterine Cancer
293	ALDIMIPMV	AML, CRC, Brain Cancer, Gallbladder Cancer , CCC, HNSCC, Melanoma, NHL, NSCLC, OC, Brain Cancer, RCC, SCLC, Urinary Bladder Cancer, Uterine Cancer

294	ALLDQLHTLL	AML
298	IILPVEVEV	CRC, HNSCC
299	ILEENIPVL	Uterine Cancer, AML
303	ILSQPTPSL	Uterine Cancer
305	LLFPQIEGIKI	Urinary Bladder Cancer, HNSCC
306	VVAEELENV	RCC
311	LLVDVEPKV	Uterine Cancer, HNSCC
312	LLYNSTDPTL	Urinary Bladder Cancer
313	LMADLEGLHL	Uterine Cancer, AML
317	NLLAHIWAL	NHL
321	RLLEFELAQL	Brain Cancer, PrC, Uterine Cancer, AML, HNSCC
323	SLDLFNCEV	Uterine Cancer, AML
324	SLYSALQQA	SCLC, Uterine Cancer, AML
326	VLAFLVHEL	Esophageal Cancer, NSCLC, RCC
327	VLIKWFPEV	Uterine Cancer
333	VTLEFPQLIRV	BRCA
334	YLLEEKIASL	AML
335	YLYQEQYFI	CRC, Brain Cancer, Gallbladder Cancer , CCC, NSCLC, UterineCancer
336	YMAVTTQEV	Uterine Cancer
339	GLWGTVVNI	HNSCC
340	KLLEEICNL	Melanoma, Uterine Cancer, AML, NHL
342	SLITPLQAV	HNSCC
343	TLLEALDCI	GC, Uterine Cancer, HNSCC
344	VLAFENPQV	HNSCC
346	VLVQVSPSL	HNSCC
348	ALAKPPVVSV	NSCLC, Urinary Bladder Cancer, NHL, HNSCC
349	ALATHILSL	HCC, Melanoma
351	ALSEKLARL	CRC, HNSCC, Melanoma, NSCLC, OC, PC, Brain Cancer, RCC, Uterine Cancer
353	ATPMPTPSV	BRCA, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer, AML, HNSCC
354	FIMDDPAGNSYL	Brain Cancer, HNSCC
355	FIWPMLIHI	Melanoma, Uterine Cancer
357	FLIQEIKTL	HCC
358	FLTDYLNDL	AML
359	FMQDPMEVFV	Melanoma, NHL, NSCLC, OC
360	HLIDTNKIQL	NHL, NSCLC
361	ILQEFESKL	AML, NHL

362	ILTELGGFEV	AML, PrC, BRCA, PC, CRC, HCC, HNSCC, Melanoma, NSCLC, OC, Esophageal Cancer, GC, RCC, SCLC, Urinary Bladder Cancer, Uterine Cancer
363	ITTEVVNELYV	BRCA
365	LISPLLLPV	SCLC, Uterine Cancer, HNSCC
367	NLWSLVAKV	Uterine Cancer
368	QLQPTDALLCV	Uterine Cancer
370	SIFASPESV	Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer, AML, HNSCC
374	VLSVITEEL	SCLC, HCC, PrC, Urinary Bladder Cancer, Uterine Cancer
375	VLWFKPVEL	BRCA, OC, Uterine Cancer, Gallbladder Cancer, Bile Duct Cancer, HNSCC
377	VVDGTCVAV	BRCA, Uterine Cancer, HNSCC
378	YILGKFFAL	Melanoma, Esophageal Cancer
379	YLAELVTPIL	HCC, BRCA, Uterine Cancer, AML, HNSCC
383	YLLREWVNL	Melanoma, Uterine Cancer
384	YMIGSEVGNYL	SCLC, Uterine Cancer
385	YTIPLAIKL	CRC

NSCLC= non-small cell lung cancer, SCLC= small cell lung cancer, RCC= kidney cancer, CRC= colon or rectum cancer, GC= stomach cancer, HCC= liver cancer, PC= pancreatic cancer, PrC= prostate cancer, BRCA=breast cancer, NHL= non-Hodgkin lymphoma, AML= acute myeloid leukemia, OC= ovarian cancer, HNSCC= head and neck squamous cell carcinoma, CCC = cholangiocarcinoma.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 5, 7, 13, 29, 30, 35, 41, 44, 45, 51, 68, 93, 101, 112, 115, 126, 130, 138, 141, 160, 161, 172, 187, 191, 197, 198, 204, 212, 226, 228, 231, 232, 234, 258, 266, 268, 272, 279, 286, 289, 293, 299, 303, 311, 313, 321, 323, 324, 327, 335, 336, 340, 343, 351, 353, 355, 362, 365, 367, 368, 370, 374, 375, 377, 379, 383, and 384 for the – in one preferred embodiment combined - treatment of uterine cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 7, 8, 11, 25, 42, 44, 67, 82, 84, 86, 91, 94, 114, 136, 138, 145, 156, 161, 192, 202, 203, 204, 212, 216, 217, 218, 224, 226, 237, 257, 261, 293, 294, 299, 313, 321, 323, 324, 334, 340, 353, 358, 361, 362, 370, and 379 for the – in one preferred embodiment combined - treatment of AML.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 8, 11, 14, 27, 38, 40, 48, 55, 59, 68, 84, 93, 94, 114, 118, 124, 126, 128, 146, 159, 161, 162, 164, 176, 179, 188, 189, 192, 200, 202, 208, 211, 212, 215, 216, 226, 228, 234, 235, 260, 264, 272, 273, 274, 279, 280, 293, 298, 305, 311, 321, 339, 342, 343, 344, 346, 348, 351, 353, 354, 362, 365, 370, 375, 377, and 379 for the – in one preferred embodiment combined - treatment of HNSCC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 11, 26, 37, 44, 49, 58, 83, 84, 113, 128, 173, 176, 184, 201, 203, 226, 273, 279, 293, 317, 340, 348, 359, 360, and 361 for the – in one preferred embodiment combined - treatment of NHL.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 11, 26, 45, 58, 84, 113, 136, 145, 173, 174, 176, 203, 226, 279, 293, 326, 335, 348, 351, 359, 360, and 362 for the – in one preferred embodiment combined - treatment of NSCLC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 11, 45, 68, 162, 173, 174, 226, 293, 351, 359, 362, and 375 for the – in one preferred embodiment combined - treatment of OC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 11, 35, 42, 59, 119, 126, 127, 152, 161, 172, 176, 192, 226, 228, 239, 260, 280, 293, 324, 362, 365, 374, and 384 for the – in one preferred embodiment combined - treatment of SCLC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 13, 14, 30, 68, 89, 108, 125, 198, 200, 204, 228, 235, 242, 266, 269, 272, 280, 333, 353, 362, 363, 375, 377, and 379 for the – in one preferred embodiment combined - treatment of BRCA.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 14, 30, 49, 90, 107, 108, 111, 126, 136, 147, 161, 164, 173, 176, 181, 187, 190, 199, 208, 240, 274, 275, 280, 293, 340, 349, 351, 355, 359, 362, 378, and 383 for the – in one preferred embodiment combined - treatment of melanoma.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 15, 82, 173, 211, 326, 362, and 378 for the – in one preferred embodiment combined - treatment of esophageal cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 30, 136, 164, 173, 187, 266, 293, 298, 335, 351, 362, 363, and 385 for the – in one preferred embodiment combined - treatment of CRC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 44, 76, 84, 145, 173, 211, 293, 306, 326, 351, and 362 for the – in one preferred embodiment combined - treatment of RCC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 68, 94, 181, 226, 293, 335, 353, 370, and 375 for the – in one preferred embodiment combined - treatment of gallbladder cancer and/or bile duct cancer and/or cholangiocarcinoma.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 82, 87, 162, 351, and 362 for the – in one preferred embodiment combined - treatment of PC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 84, 145, 173, 176, 258, 343, and 362 for the – in one preferred embodiment combined - treatment of GC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 84, 126, 145, 173, 211, 239, 247, 349, 357, 362, 374, and 379 for the – in one preferred embodiment combined - treatment of HCC.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 136, 211, 221, 226, 293, 321, 335, 351, and 354 for the – in one preferred embodiment combined - treatment of brain cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 128, 172, 188, 192, 293, 305, 312, 321, 348, 362, and 374 for the – in one preferred embodiment combined - treatment of urinary bladder cancer.

Thus, another aspect of the present invention relates to the use of at least one peptide according to the present invention according to any one of SEQ ID No. 226, 321, 362, and 374 for the – in one preferred embodiment combined - treatment of PrC.

The present invention furthermore relates to peptides according to the present invention that have the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I or - in an elongated form, such as a length-variant - MHC class -II.

The present invention further relates to the peptides according to the present invention wherein said peptides (each) consist or consist essentially of an amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 385.

The present invention further relates to the peptides according to the present invention, wherein said peptide is modified and/or includes non-peptide bonds.

The present invention further relates to the peptides according to the present invention, wherein said peptide is part of a fusion protein, in particular fused to the N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii), or fused to (or into the sequence of) an antibody, such as, for example, an antibody that is specific for dendritic cells.

The present invention further relates to a nucleic acid, encoding the peptides according to the present invention. The present invention further relates to the nucleic acid according to the present invention that is DNA, cDNA, PNA, RNA or combinations thereof.

The present invention further relates to an expression vector capable of expressing and/or expressing a nucleic acid according to the present invention.

The present invention further relates to a peptide according to the present invention, a nucleic acid according to the present invention or an expression vector according to the present invention for use in the treatment of diseases and in medicine, in particular in the treatment of cancer.

The present invention further relates to antibodies that are specific against the peptides according to the present invention or complexes of said peptides according to the present invention with MHC, and methods of making these.

The present invention further relates to T-cell receptors (TCRs), in particular soluble TCR (sTCRs) and cloned TCRs engineered into autologous or allogeneic T cells, and methods of making these, as well as NK cells or other cells bearing said TCR or cross-reacting with said TCRs.

The antibodies and TCRs are additional embodiments of the immunotherapeutic use of the peptides according to the invention at hand.

The present invention further relates to a host cell comprising a nucleic acid according to the present invention or an expression vector as described before. The present invention further relates to the host cell according to the present invention that is an antigen presenting cell, and preferably is a dendritic cell.

The present invention further relates to a method for producing a peptide according to the present invention, said method comprising culturing the host cell according to the present invention, and isolating the peptide from said host cell or its culture medium.

The present invention further relates to said method according to the present invention, wherein the antigen is loaded onto class I or II MHC molecules expressed on the surface of a suitable antigen-presenting cell or artificial antigen-presenting cell by contacting a sufficient amount of the antigen with an antigen-presenting cell.

The present invention further relates to the method according to the present invention, wherein the antigen-presenting cell comprises an expression vector capable of expressing or expressing said peptide containing SEQ ID No. 1 to SEQ ID No.: 385, preferably containing SEQ ID No. 1 to SEQ ID No. 202, or a variant amino acid sequence.

The present invention further relates to activated T cells, produced by the method according to the present invention, wherein said T cell selectively recognizes a cell which expresses a polypeptide comprising an amino acid sequence according to the present invention.

The present invention further relates to a method of killing target cells in a patient which target cells aberrantly express a polypeptide comprising any amino acid sequence according to the present invention, the method comprising administering to the patient an effective number of T cells as produced according to the present invention.

The present invention further relates to the use of any peptide as described, the nucleic acid according to the present invention, the expression vector according to the present invention, the cell according to the present invention, the activated T lymphocyte, the T cell receptor or the antibody or other peptide- and/or peptide-MHC-binding molecules according to the present invention as a medicament or in the manufacture of a medicament. Preferably, said medicament is active against cancer.

Preferably, said medicament is a cellular therapy, a vaccine or a protein based on a soluble TCR or antibody.

The present invention further relates to a use according to the present invention, wherein said cancer cells are CLL, acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer, and preferably CLL cells.

The present invention further relates to biomarkers based on the peptides according to the present invention, herein called “targets”, that can be used in the diagnosis of cancer, preferably CLL. The marker can be over-presentation of the peptide(s) themselves, or over-expression of the corresponding gene(s). The markers may also be used to predict the probability of success of a treatment, preferably an immunotherapy, and most preferred an immunotherapy targeting the same target that is identified by the biomarker. For example, an antibody or soluble TCR can be used to stain sections of the tumor to detect the presence of a peptide of interest in complex with MHC.

Optionally the antibody carries a further effector function such as an immune stimulating domain or toxin. The present invention also relates to the use of these novel targets in the context of cancer treatment.

Stimulation of an immune response is dependent upon the presence of antigens recognized as foreign by the host immune system. The discovery of the existence of tumor associated antigens has raised the possibility of using a host's immune system to intervene in tumor growth. Various mechanisms of harnessing both the humoral and cellular arms of the immune system are currently being explored for cancer immunotherapy.

Specific elements of the cellular immune response are capable of specifically recognizing and destroying tumor cells. The isolation of T-cells from tumor-infiltrating cell populations or from peripheral blood suggests that such cells play an important role in natural immune defense against cancer. CD8-positive T-cells in particular, which recognize class I molecules of the major histocompatibility complex (MHC)-bearing peptides of usually 8 to 10 amino acid residues derived from proteins or defect ribosomal products (DRIPS) located in the cytosol, play an important role in this response. The MHC-molecules of the human are also designated as human leukocyte-antigens (HLA).

As used herein and except as noted otherwise all terms are defined as given below.

The term “T-cell response” means the specific proliferation and activation of effector functions induced by a peptide *in vitro* or *in vivo*. For MHC class I restricted cytotoxic T cells, effector functions may be lysis of peptide-pulsed, peptide-precursor pulsed or naturally peptide-presenting target cells, secretion of cytokines, preferably Interferon-gamma, TNF-alpha, or IL-2 induced by peptide, secretion of effector molecules, preferably granzymes or perforins induced by peptide, or degranulation.

The term “peptide” is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The peptides are preferably 9 amino acids in length, but can be as short as 8 amino acids in length, and as long as 10, 11, 12, 13, or 14 or longer, and in case of MHC class II peptides (elongated variants of the peptides of the invention) they can be as long as 15, 16, 17, 18, 19 or 20 or more amino acids in length.

Furthermore, the term “peptide” shall include salts of a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. Preferably, the salts are pharmaceutical acceptable salts of the peptides, such as, for example, the chloride or acetate (trifluoroacetate) salts. It has to be noted that the salts of the peptides according to the present invention differ substantially from the peptides in their state(s) *in vivo*, as the peptides are not salts *in vivo*.

The term “peptide” shall also include “oligopeptide”. The term “oligopeptide” is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The length of the oligopeptide is not critical to the invention, as long as the correct epitope or epitopes are maintained therein. The oligopeptides are typically less than about 30 amino acid residues in length, and greater than about 15 amino acids in length.

The term “polypeptide” designates a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The length of the polypeptide is not critical to the invention as long as the correct epitopes are maintained. In contrast to the terms peptide or oligopeptide, the term polypeptide is meant to refer to molecules containing more than about 30 amino acid residues.

The term “full length polypeptide” means a complete protein (amino acid chain) or complete subunit (amino acid chain) of a multimeric protein.

A peptide, oligopeptide, protein or polynucleotide coding for such a molecule is “immunogenic” (and thus is an “immunogen” within the present invention), if it is capable of inducing an immune response. In the case of the present invention, immunogenicity is more specifically defined as the ability to induce a T-cell response. Thus, an “immunogen” would be a molecule that is capable of inducing an immune response, and in the case of the present invention, a molecule capable of inducing a T-cell response. In another aspect, the immunogen can be the peptide, the complex of the peptide with MHC, oligopeptide, and/or protein that is used to raise specific antibodies or TCRs against it.

A class I T cell “epitope” requires a short peptide that is bound to a class I MHC receptor, forming a ternary complex (MHC class I alpha chain, beta-2-microglobulin, and peptide) that can be recognized by a T cell bearing a matching T-cell receptor binding to the MHC/peptide complex with appropriate affinity. Peptides binding to MHC class I molecules are typically 8-14 amino acids in length, and most typically 9 amino acids in length.

In humans, there are three different genetic loci that encode MHC class I molecules (the MHC-molecules of the human are also designated human leukocyte antigens (HLA)): HLA-A, HLA-B, and HLA-C. HLA-A*01, HLA-A*02, and HLA-B*07 are examples of different MHC class I alleles that can be expressed from these loci.

Table 5: Expression frequencies F of HLA-A*02 and HLA-A*24 and the most frequent HLA-DR serotypes. Frequencies are deduced from haplotype frequencies Gf within the American population adapted from Mori et al. (Mori et al., 1997) employing the Hardy-Weinberg formula $F = 1 - (1-Gf)^2$. Combinations of A*02 or A*24 with certain HLA-DR alleles might be enriched or less frequent than expected from their single frequencies due to linkage disequilibrium. For details refer to Chanock et al. (Chanock et al., 2004).

Allele	Population	Calculated phenotype from allele frequency
A*02	Caucasian (North America)	49.1%
A*02	African American (North America)	34.1%
A*02	Asian American (North America)	43.2%
A*02	Latin American (North American)	48.3%
DR1	Caucasian (North America)	19.4%
DR2	Caucasian (North America)	28.2%
DR3	Caucasian (North America)	20.6%
DR4	Caucasian (North America)	30.7%
DR5	Caucasian (North America)	23.3%
DR6	Caucasian (North America)	26.7%
DR7	Caucasian (North America)	24.8%
DR8	Caucasian (North America)	5.7%
DR9	Caucasian (North America)	2.1%
DR1	African (North) American	13.20%
DR2	African (North) American	29.80%
DR3	African (North) American	24.80%
DR4	African (North) American	11.10%
DR5	African (North) American	31.10%
DR6	African (North) American	33.70%
DR7	African (North) American	19.20%
DR8	African (North) American	12.10%
DR9	African (North) American	5.80%
DR1	Asian (North) American	6.80%
DR2	Asian (North) American	33.80%

Allele	Population	Calculated phenotype from allele frequency
DR3	Asian (North) American	9.20%
DR4	Asian (North) American	28.60%
DR5	Asian (North) American	30.00%
DR6	Asian (North) American	25.10%
DR7	Asian (North) American	13.40%
DR8	Asian (North) American	12.70%
DR9	Asian (North) American	18.60%
DR1	Latin (North) American	15.30%
DR2	Latin (North) American	21.20%
DR3	Latin (North) American	15.20%
DR4	Latin (North) American	36.80%
DR5	Latin (North) American	20.00%
DR6	Latin (North) American	31.10%
DR7	Latin (North) American	20.20%
DR8	Latin (North) American	18.60%
DR9	Latin (North) American	2.10%
A*24	Philippines	65%
A*24	Russia Nenets	61%
A*24:02	Japan	59%
A*24	Malaysia	58%
A*24:02	Philippines	54%
A*24	India	47%
A*24	South Korea	40%
A*24	Sri Lanka	37%
A*24	China	32%
A*24:02	India	29%
A*24	Australia West	22%
A*24	USA	22%
A*24	Russia Samara	20%
A*24	South America	20%
A*24	Europe	18%

The peptides of the invention, preferably when included into a vaccine of the invention as described herein bind to A*02. A vaccine may also include pan-binding MHC class II peptides. Therefore, the vaccine of the invention can be used to treat cancer in patients that are A*02 positive, whereas no selection for MHC class II allotypes is necessary due to the pan-binding nature of these peptides.

If A*02 peptides of the invention are combined with peptides binding to another allele, for example A*24, a higher percentage of any patient population can be treated compared with addressing either MHC class I allele alone. While in most populations less than 50% of patients could be addressed by either allele alone, a vaccine comprising HLA-A*24 and HLA-A*02 epitopes can treat at least 60% of patients in any relevant population. Specifically, the following percentages of patients will be positive for at least one of these alleles in various regions: USA 61%, Western Europe 62%, China 75%, South Korea 77%, Japan 86% (calculated from www.allelefrequencies.net).

In a preferred embodiment, the term “nucleotide sequence” refers to a heteropolymer of deoxyribonucleotides.

The nucleotide sequence coding for a particular peptide, oligopeptide, or polypeptide may be naturally occurring or they may be synthetically constructed. Generally, DNA segments encoding the peptides, polypeptides, and proteins of this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene that is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

As used herein the term “a nucleotide coding for (or encoding) a peptide” refers to a nucleotide sequence coding for the peptide including artificial (man-made) start and stop codons compatible for the biological system the sequence is to be expressed by, for example, a dendritic cell or another cell system useful for the production of TCRs.

As used herein, reference to a nucleic acid sequence includes both single stranded and double stranded nucleic acid. Thus, for example for DNA, the specific sequence, unless the context indicates otherwise, refers to the single strand DNA of such sequence, the duplex of such sequence with its complement (double stranded DNA) and the complement of such sequence.

The term “coding region” refers to that portion of a gene which either naturally or normally codes for the expression product of that gene in its natural genomic environment, i.e., the region coding *in vivo* for the native expression product of the gene.

The coding region can be derived from a non-mutated (“normal”), mutated or altered gene, or can even be derived from a DNA sequence, or gene, wholly synthesized in the laboratory using methods well known to those of skill in the art of DNA synthesis.

The term “expression product” means the polypeptide or protein that is the natural translation product of the gene and any nucleic acid sequence coding equivalents resulting from genetic code degeneracy and thus coding for the same amino acid(s).

The term “fragment”, when referring to a coding sequence, means a portion of DNA comprising less than the complete coding region, whose expression product retains essentially the same biological function or activity as the expression product of the complete coding region.

The term “DNA segment” refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the segment and its component nucleotide sequences by standard biochemical methods, for example, by using a cloning vector. Such segments are provided in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, which are typically present in eukaryotic genes. Sequences of non-translated DNA may be present downstream from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

The term “primer” means a short nucleic acid sequence that can be paired with one strand of DNA and provides a free 3'-OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

The term “promoter” means a region of DNA involved in binding of RNA polymerase to initiate transcription.

The term “isolated” means that the material is removed from its original environment (e.g., the natural environment, if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The polynucleotides, and recombinant or immunogenic polypeptides, disclosed in accordance with the present invention may also be in “purified” form. The term “purified” does not require absolute purity; rather, it is intended as a relative definition, and can include preparations that are highly purified or preparations that are only partially purified, as those terms are understood by those of skill in the relevant art. For example, individual clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Furthermore, a claimed polypeptide which has a purity of preferably 99.999%, or at least 99.99% or 99.9%; and even desirably 99% by weight or greater is expressly encompassed.

The nucleic acids and polypeptide expression products disclosed according to the present invention, as well as expression vectors containing such nucleic acids and/or such polypeptides, may be in “enriched form”. As used herein, the term “enriched” means that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously 0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated. The sequences, constructs, vectors, clones, and other materials comprising the present invention can advantageously be in enriched or isolated form. The term “active fragment” means a fragment, usually of a peptide, polypeptide or nucleic acid sequence, that generates an immune response (i.e., has immunogenic activity) when administered, alone or optionally with a suitable adjuvant or in a vector, to an animal, such as a mammal, for example, a rabbit or a mouse, and also including a human, such immune response taking the form of stimulating a T-cell response within the recipient animal, such as a human. Alternatively, the "active fragment" may also be used to induce a T-cell response *in vitro*.

As used herein, the terms “portion”, “segment“ and “fragment“, when used in relation to polypeptides, refer to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin or chymotrypsin, the oligopeptides resulting from such treatment would represent portions, segments or fragments of the starting polypeptide. When used in relation to polynucleotides, these terms refer to the products produced by treatment of said polynucleotides with any of the endonucleases.

In accordance with the present invention, the term “percent identity” or “percent identical”, when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the “Compared Sequence”) with the described or claimed sequence (the “Reference Sequence”). The percent identity is then determined according to the following formula:
percent identity = 100 [1 -(C/R)]
wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence, wherein

(i) each base or amino acid in the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and
(ii) each gap in the Reference Sequence and
(iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, constitutes a difference and
(iiii) the alignment has to start at position 1 of the aligned sequences;
and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

If an alignment exists between the Compared Sequence and the Reference Sequence for which the percent identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum percent identity to the Reference Sequence even though alignments may exist in which the herein above calculated percent identity is less than the specified percent identity.

As mentioned above, the present invention thus provides a peptide comprising a sequence that is selected from the group of consisting of SEQ ID NO: 1 to SEQ ID NO: 385 or a variant thereof which is 88% homologous to SEQ ID NO: 1 to SEQ ID NO: 385, or a variant thereof that will induce T cells cross-reacting with said peptide. The peptides of the invention have the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I or elongated versions of said peptides to class II.

In the present invention, the term “homologous” refers to the degree of identity (see percent identity above) between sequences of two amino acid sequences, i.e. peptide or polypeptide sequences. The aforementioned “homology” is determined by comparing two sequences aligned under optimal conditions over the sequences to be compared. Such a sequence homology can be calculated by creating an alignment using, for example, the ClustalW algorithm. Commonly available sequence analysis software, more specifically, Vector NTI, GENETYX or other tools are provided by public databases.

A person skilled in the art will be able to assess, whether T cells induced by a variant of a specific peptide will be able to cross-react with the peptide itself (Appay et al., 2006; Colombetti et al., 2006; Fong et al., 2001; Zaremba et al., 1997).

By a "variant" of the given amino acid sequence the inventors mean that the side chains of, for example, one or two of the amino acid residues are altered (for example by replacing them with the side chain of another naturally occurring amino acid residue or some other side chain) such that the peptide is still able to bind to an HLA molecule in substantially the same way as a peptide consisting of the given amino acid sequence in consisting of SEQ ID NO: 1 to SEQ ID NO: 385. For example, a peptide may be modified so that it at least maintains, if not improves, the ability to interact with and bind to the binding groove of a suitable MHC molecule, such as HLA-A*02 or -DR, and in that way it at least maintains, if not improves, the ability to bind to the TCR of activated T cells.

These T cells can subsequently cross-react with cells and kill cells that express a polypeptide that contains the natural amino acid sequence of the cognate peptide as defined in the aspects of the invention. As can be derived from the scientific literature and databases (Rammensee et al., 1999; Godkin et al., 1997), certain positions of HLA binding peptides are typically anchor residues forming a core sequence fitting to the binding motif of the HLA receptor, which is defined by polar, electrophysical, hydrophobic and spatial properties of the polypeptide chains constituting the binding groove. Thus, one skilled in the art would be able to modify the amino acid sequences set forth in SEQ ID NO: 1 to SEQ ID NO 385, by maintaining the known anchor residues, and would be able to determine whether such variants maintain the ability to bind MHC class I or II molecules. The variants of the present invention retain the ability to bind to the TCR of activated T cells, which can subsequently cross-react with and kill cells that express a polypeptide containing the natural amino acid sequence of the cognate peptide as defined in the aspects of the invention.

The original (unmodified) peptides as disclosed herein can be modified by the substitution of one or more residues at different, possibly selective, sites within the peptide chain, if not otherwise stated. Preferably those substitutions are located at the end of the amino acid chain. Such substitutions may be of a conservative nature, for example, where one amino acid is replaced by an amino acid of similar structure and characteristics, such as where a hydrophobic amino acid is replaced by another hydrophobic amino acid. Even more conservative would be replacement of amino acids of the same or similar size and chemical nature, such as where leucine is replaced by isoleucine. In studies of sequence variations in families of naturally occurring homologous proteins, certain amino acid substitutions are more often tolerated than others, and these are often show correlation with similarities in size, charge, polarity, and hydrophobicity between the original amino acid and its replacement, and such is the basis for defining ”conservative substitutions.”

Conservative substitutions are herein defined as exchanges within one of the following five groups: Group 1-small aliphatic, nonpolar or slightly polar residues (Ala, Ser, Thr, Pro, Gly); Group 2-polar, negatively charged residues and their amides (Asp, Asn, Glu, Gln); Group 3-polar, positively charged residues (His, Arg, Lys); Group 4-large, aliphatic, nonpolar residues (Met, Leu, Ile, Val, Cys); and Group 5-large, aromatic residues (Phe, Tyr, Trp).

Less conservative substitutions might involve the replacement of one amino acid by another that has similar characteristics but is somewhat different in size, such as replacement of an alanine by an isoleucine residue. Highly non-conservative replacements might involve substituting an acidic amino acid for one that is polar, or even for one that is basic in character. Such “radical” substitutions cannot, however, be dismissed as potentially ineffective since chemical effects are not totally predictable and radical substitutions might well give rise to serendipitous effects not otherwise predictable from simple chemical principles.

Of course, such substitutions may involve structures other than the common L-amino acids. Thus, D-amino acids might be substituted for the L-amino acids commonly found in the antigenic peptides of the invention and yet still be encompassed by the disclosure herein. In addition, non-standard amino acids (i.e., other than the common naturally occurring proteinogenic amino acids) may also be used for substitution purposes to produce immunogens and immunogenic polypeptides according to the present invention.

If substitutions at more than one position are found to result in a peptide with substantially equivalent or greater antigenic activity as defined below, then combinations of those substitutions will be tested to determine if the combined substitutions result in additive or synergistic effects on the antigenicity of the peptide. At most, no more than 4 positions within the peptide would be simultaneously substituted.

A peptide consisting essentially of the amino acid sequence as indicated herein can have one or two non-anchor amino acids (see below regarding the anchor motif) exchanged without that the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I or –II is substantially changed or is negatively affected, when compared to the non-modified peptide. In another embodiment, in a peptide consisting essentially of the amino acid sequence as indicated herein, one or two amino acids can be exchanged with their conservative exchange partners (see herein below) without that the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I or –II is substantially changed, or is negatively affected, when compared to the non-modified peptide.

The amino acid residues that do not substantially contribute to interactions with the T-cell receptor can be modified by replacement with other amino acids whose incorporation does not substantially affect T-cell reactivity and does not eliminate binding to the relevant MHC. Thus, apart from the proviso given, the peptide of the invention may be any peptide (by which term the inventors include oligopeptide or polypeptide), which includes the amino acid sequences or a portion or variant thereof as given.

Table 6: Variants and motif of the peptides according to SEQ ID NO: 2, 5, 8, 62, and 53

Position	1	2	3	4	5	6	7	8	9	10	11
SEQ ID NO. 2	A	L	H	R	P	D	V	Y	L		
Variants									V		
									I		
									A		
		M									
		M							V		
		M							I		
		M							A		
		A									
		A							V		
		A							I		
		A							A		
		V									
		V							V		
		V							I		
		V							A		
		T									
		T							V		
		T							I		
		T							A		
		Q									
		Q							V		
		Q							I		
		Q							A		
Position	1	2	3	4	5	6	7	8	9	10	11
SEQ ID NO 5	A	L	I	F	K	I	A	S	A		
Variants									L		
									I		
									V		
		M									
		M							L		
		M							I		

		M							V		
		A									
		A							L		
		A							I		
		A							V		
		V									
		V							L		
		V							I		
		V							V		
		T									
		T							L		
		T							I		
		T							V		
		Q									
		Q							L		
		Q							I		
		Q							V		
Position	1	2	3	4	5	6	7	8	9	10	11
SEQ ID NO. 8	A	L	A	A	S	A	L	P	A	L	V
Variants											I
											L
											A
		M									
		M									I
		M									L
		M									A
		A									
		A									I
		A									L
		A									A
		V									
		V									I
		V									L
		V									A
		T									
		T									I
		T									L
		T									A
		Q									

		Q								I
		Q								L
		Q								A

Position	1	2	3	4	5	6	7	8	9
SEQ ID 62	G	I	I	D	G	S	P	R	L
Variants		L							V
		L							I
		L							
		L							A

		M							V
		M							I
		M							
		M							A
		A							V
		A							I
		A							
		A							A
		V							V
		V							I
		V							
		V							A
		T							V
		T							I
		T							
		T							A
		Q							V
		Q							I
		Q							
		Q							A
Position	1	2	3	4	5	6	7	8	9
SEQ ID 153	S	L	L	A	E	L	H	V	L
Variants									V
									I
									A
		M							V
		M							I
		M							
		M							A
		A							V
		A							I
		A							
		A							A
		V							V
		V							I
		V							
		V							A
		T							V
		T							I

		T							
		T							A
		Q							V
		Q							I
		Q							
		Q							A

Longer (elongated) peptides may also be suitable. It is possible that MHC class I epitopes, although usually between 8 and 11 amino acids long, are generated by peptide processing from longer peptides or proteins that include the actual epitope. It is preferred that the residues that flank the actual epitope are residues that do not substantially affect proteolytic cleavage necessary to expose the actual epitope during processing.

The peptides of the invention can be elongated by up to four amino acids, that is 1, 2, 3 or 4 amino acids can be added to either end in any combination between 4:0 and 0:4. Combinations of the elongations according to the invention can be found in Table 7.

Table 7: Combinations of the elongations of peptides of the invention

C-terminus	N-terminus
4	0
3	0 or 1
2	0 or 1 or 2
1	0 or 1 or 2 or 3
0	0 or 1 or 2 or 3 or 4
N-terminus	C-terminus
4	0
3	0 or 1
2	0 or 1 or 2
1	0 or 1 or 2 or 3
0	0 or 1 or 2 or 3 or 4

The amino acids for the elongation/extension can be the peptides of the original sequence of the protein or any other amino acid(s). The elongation can be used to enhance the stability or solubility of the peptides.

Thus, the epitopes of the present invention may be identical to naturally occurring tumor-associated or tumor-specific epitopes or may include epitopes that differ by no more than four residues from the reference peptide, as long as they have substantially identical antigenic activity.

In an alternative embodiment, the peptide is elongated on either or both sides by more than 4 amino acids, preferably to a total length of up to 30 amino acids. This may lead to MHC class II binding peptides. Binding to MHC class II can be tested by methods known in the art.

Accordingly, the present invention provides peptides and variants of MHC class I epitopes, wherein the peptide or variant has an overall length of between 8 and 100, preferably between 8 and 30, and most preferred between 8 and 14, namely 8, 9, 10, 11, 12, 13, 14 amino acids, in case of the elongated class II binding peptides the length can also be 15, 16, 17, 18, 19, 20, 21 or 22 amino acids.

Of course, the peptide or variant according to the present invention will have the ability to bind to a molecule of the human major histocompatibility complex (MHC) class I or II. Binding of a peptide or a variant to a MHC complex may be tested by methods known in the art.

Preferably, when the T cells specific for a peptide according to the present invention are tested against the substituted peptides, the peptide concentration at which the substituted peptides achieve half the maximal increase in lysis relative to background is no more than about 1 mM, preferably no more than about 1 µM, more preferably no more than about 1 nM, and still more preferably no more than about 100 pM, and most preferably no more than about 10 pM. It is also preferred that the substituted peptide be recognized by T cells from more than one individual, at least two, and more preferably three individuals.

In a particularly preferred embodiment of the invention the peptide consists or consists essentially of an amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 385.

“Consisting essentially of” shall mean that a peptide according to the present invention, in addition to the sequence according to any of SEQ ID NO: 1 to SEQ ID NO 385 or a variant thereof contains additional N- and/or C-terminally located stretches of amino acids that are not necessarily forming part of the peptide that functions as an epitope for MHC molecules epitope.

Nevertheless, these stretches can be important to provide an efficient introduction of the peptide according to the present invention into the cells. In one embodiment of the present invention, the peptide is part of a fusion protein which comprises, for example, the 80 N-terminal amino acids of the HLA-DR antigen-associated invariant chain (p33, in the following “Ii”) as derived from the NCBI, GenBank Accession number X00497. In other fusions, the peptides of the present invention can be fused to an antibody as described herein, or a functional part thereof, in particular into a sequence of an antibody, so as to be specifically targeted by said antibody, or, for example, to or into an antibody that is specific for dendritic cells as described herein.

In addition, the peptide or variant may be modified further to improve stability and/or binding to MHC molecules in order to elicit a stronger immune response. Methods for such an optimization of a peptide sequence are well known in the art and include, for example, the introduction of reverse peptide bonds or non-peptide bonds.

In a reverse peptide bond amino acid residues are not joined by peptide (-CO-NH-) linkages but the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere et al (1997) (Meziere et al., 1997), incorporated herein by reference. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Meziere et al. (Meziere et al., 1997) show that for MHC binding and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

A non-peptide bond is, for example, $-CH_2-NH$, $-CH_2S-$, $-CH_2CH_2-$, $-CH=CH-$, $-COCH_2-$, $-CH(OH)CH_2-$, and $-CH_2SO-$. US 4,897,445 provides a method for the solid phase synthesis of non-peptide bonds ($-CH_2-NH$) in polypeptide chains which involves polypeptides synthesized by standard procedures and the non-peptide bond synthesized by reacting an amino aldehyde and an amino acid in the presence of $NaCNBH_3$.

Peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, to enhance the stability, bioavailability, and/or affinity of the peptides. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups may be added to the peptides' amino termini. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the peptides' amino termini. Additionally, the hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to the peptides' carboxy termini.

Further, the peptides of the invention may be synthesized to alter their steric configuration. For example, the D-isomer of one or more of the amino acid residues of the peptide may be used, rather than the usual L-isomer. Still further, at least one of the amino acid residues of the peptides of the invention may be substituted by one of the well-known non-naturally occurring amino acid residues. Alterations such as these may serve to increase the stability, bioavailability and/or binding action of the peptides of the invention.

Similarly, a peptide or variant of the invention may be modified chemically by reacting specific amino acids either before or after synthesis of the peptide. Examples for such modifications are well known in the art and are summarized e.g. in R. Lundblad, Chemical Reagents for Protein Modification, 3rd ed. CRC Press, 2004 (Lundblad, 2004), which is incorporated herein by reference. Chemical modification of amino acids includes but is not limited to, modification by acylation, amidination, pyridoxylation of lysine, reductive alkylation, trinitrobenzylation of amino groups with 2,4,6-trinitrobenzene sulphonic acid (TNBS), amide modification of carboxyl groups and sulphydryl modification by performic acid oxidation of cysteine to cysteic acid, formation of mercurial derivatives, formation of mixed disulphides with other thiol compounds, reaction with maleimide, carboxymethylation with iodoacetic acid or iodoacetamide and carbamoylation with cyanate at alkaline pH, although without limitation thereto. In this regard, the skilled person is referred to Chapter 15 of Current Protocols In Protein Science, Eds. Coligan et al. (John Wiley and Sons NY 1995-2000) (Coligan et al., 1995) for more extensive methodology relating to chemical modification of proteins.

Briefly, modification of e.g. arginyl residues in proteins is often based on the reaction of vicinal dicarbonyl compounds such as phenylglyoxal, 2,3-butanedione, and 1,2-cyclohexanedione to form an adduct. Another example is the reaction of methylglyoxal with arginine residues. Cysteine can be modified without concomitant modification of other nucleophilic sites such as lysine and histidine. As a result, a large number of reagents are available for the modification of cysteine. The websites of companies such as Sigma-Aldrich (http://www.sigma-aldrich.com) provide information on specific reagents.

Selective reduction of disulfide bonds in proteins is also common. Disulfide bonds can be formed and oxidized during the heat treatment of biopharmaceuticals. Woodward’s Reagent K may be used to modify specific glutamic acid residues. N-(3-(dimethylamino)propyl)-N’-ethylcarbodiimide can be used to form intra-molecular crosslinks between a lysine residue and a glutamic acid residue. For example, diethylpyrocarbonate is a reagent for the modification of histidyl residues in proteins. Histidine can also be modified using 4-hydroxy-2-nonenal. The reaction of lysine residues and other α-amino groups is, for example, useful in binding of peptides to surfaces or the cross-linking of proteins/peptides. Lysine is the site of attachment of poly(ethylene)glycol and the major site of modification in the glycosylation of proteins.

Methionine residues in proteins can be modified with e.g. iodoacetamide, bromoethylamine, and chloramine T.

Tetranitromethane and N-acetylimidazole can be used for the modification of tyrosyl residues. Cross-linking via the formation of dityrosine can be accomplished with hydrogen peroxide/copper ions.

Recent studies on the modification of tryptophan have used N-bromosuccinimide, 2-hydroxy-5-nitrobenzyl bromide or 3-bromo-3-methyl-2-(2-nitrophenylmercapto)-3H-indole (BPNS-skatole).

Successful modification of therapeutic proteins and peptides with PEG is often associated with an extension of circulatory half-life while cross-linking of proteins with glutaraldehyde, polyethylene glycol diacrylate and formaldehyde is used for the preparation of hydrogels. Chemical modification of allergens for immunotherapy is often achieved by carbamylation with potassium cyanate.

A peptide or variant, wherein the peptide is modified or includes non-peptide bonds is a preferred embodiment of the invention.

Another embodiment of the present invention relates to a non-naturally occurring peptide wherein said peptide consists or consists essentially of an amino acid sequence according to SEQ ID No: 1 to SEQ ID No: 385 and has been synthetically produced (e.g. synthesized) as a pharmaceutically acceptable salt. Methods to synthetically produce peptides are well known in the art. The salts of the peptides according to the present invention differ substantially from the peptides in their state(s) *in vivo*, as the peptides as generated *in vivo* are no salts. The non-natural salt form of the peptide mediates the solubility of the peptide, in particular in the context of pharmaceutical compositions comprising the peptides, e.g. the peptide vaccines as disclosed herein. A sufficient and at least substantial solubility of the peptide(s) is required in order to efficiently provide the peptides to the subject to be treated. Preferably, the salts are pharmaceutically acceptable salts of the peptides. These salts according to the invention include alkaline and earth alkaline salts such as salts of the Hofmeister series comprising as anions PO_4^{3-}, SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3^-, ClO_4^-, I^-, SCN^- and as cations NH_4^+, Rb^+, K^+, Na^+, Cs^+, Li^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+}. Particularly salts are selected from $(NH_4)_3PO_4$, $(NH_4)_2HPO_4$, $(NH_4)H_2PO_4$, $(NH_4)_2SO_4$, NH_4CH_3COO, NH_4Cl, NH_4Br, NH_4NO_3, NH_4ClO_4, NH_4I, NH_4SCN, Rb_3PO_4, Rb_2HPO_4, RbH_2PO_4, Rb_2SO_4, Rb_4CH_3COO, Rb_4Cl, Rb_4Br, Rb_4NO_3, Rb_4ClO_4, Rb_4I, Rb_4SCN, K_3PO_4, K_2HPO_4, KH_2PO_4, K_2SO_4, KCH_3COO, KCl, KBr, KNO_3, $KClO_4$, KI, KSCN, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, Na_2SO_4, $NaCH_3COO$, NaCl, NaBr, $NaNO_3$, $NaClO_4$, NaI, NaSCN, $ZnCl_2$ Cs_3PO_4, Cs_2HPO_4, CsH_2PO_4, Cs_2SO_4, $CsCH_3COO$, CsCl, CsBr, $CsNO_3$, $CsClO_4$, CsI, CsSCN, Li_3PO_4, Li_2HPO_4, LiH_2PO_4, Li_2SO_4, $LiCH_3COO$, LiCl, LiBr, $LiNO_3$, $LiClO_4$, LiI, LiSCN, Cu_2SO_4, $Mg_3(PO_4)_2$, Mg_2HPO_4, $Mg(H_2PO_4)_2$, Mg_2SO_4, $Mg(CH_3COO)_2$, $MgCl_2$, $MgBr_2$, $Mg(NO_3)_2$, $Mg(ClO_4)_2$, MgI_2, $Mg(SCN)_2$, $MnCl_2$, $Ca_3(PO_4)$,, Ca_2HPO_4, $Ca(H_2PO_4)_2$, $CaSO_4$, $Ca(CH_3COO)_2$, $CaCl_2$, $CaBr_2$, $Ca(NO_3)_2$, $Ca(ClO_4)_2$, CaI_2, $Ca(SCN)_2$, $Ba_3(PO_4)_2$, Ba_2HPO_4, $Ba(H_2PO_4)_2$, $BaSO_4$, $Ba(CH_3COO)_2$, $BaCl_2$, $BaBr_2$, $Ba(NO_3)_2$, $Ba(ClO_4)_2$, BaI_2, and $Ba(SCN)_2$. Particularly preferred are NH acetate, $MgCl_2$, KH_2PO_4, Na_2SO_4, KCl, NaCl, and $CaCl_2$, such as, for example, the chloride or acetate (trifluoroacetate) salts.

Generally, peptides and variants (at least those containing peptide linkages between amino acid residues) may be synthesized by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lukas et al. (Lukas et al., 1981) and by references as cited therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is done using 20% piperidine in N, N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalizing agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N, N-dicyclohexyl-carbodiimide/1hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50 % scavenger mix. Scavengers commonly used include ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesized. Also a combination of solid phase and solution phase methodologies for the synthesis of peptides is possible (see, for example, (Bruckdorfer et al., 2004), and the references as cited therein).

Trifluoroacetic acid is removed by evaporation in vacuo, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilization of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from e.g. Calbiochem-Novabiochem (Nottingham, UK).

Purification may be performed by any one, or a combination of, techniques such as re-crystallization, size exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography and (usually) reverse-phase high performance liquid chromatography using e.g. acetonitrile/water gradient separation.

Analysis of peptides may be carried out using thin layer chromatography, electrophoresis, in particular capillary electrophoresis, solid phase extraction (CSPE), reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis, as well as MALDI and ESI-Q-TOF mass spectrometric analysis.

In order to select over-presented peptides, a presentation profile is calculated showing the median sample presentation as well as replicate variation. The profile juxtaposes samples of the tumor entity of interest to a baseline of normal tissue samples. Each of these profiles can then be consolidated into an over-presentation score by calculating the p-value of a Linear Mixed-Effects Model (Pinheiro et al., 2015) adjusting for multiple testing by False Discovery Rate (Benjamini and Hochberg, 1995) (cf. Example 1, Figure 1).

For the identification and relative quantitation of HLA ligands by mass spectrometry, HLA molecules from shock-frozen tissue samples were purified and HLA-associated peptides were isolated. The isolated peptides were separated and sequences were identified by online nano-electrospray-ionization (nanoESI) liquid chromatography-mass spectrometry (LC-MS) experiments. The resulting peptide sequences were verified by comparison of the fragmentation pattern of natural tumor-associated peptides (TUMAPs) recorded from CLL samples (N = 17 A*02-positive samples) with the fragmentation patterns of corresponding synthetic reference peptides of identical sequences. Since the peptides were directly identified as ligands of HLA molecules of primary tumors, these results provide direct evidence for the natural processing and presentation of the identified peptides on primary cancer tissue obtained from 16 CLL patients.

The discovery pipeline XPRESIDENT® v2.1 (see, for example, US 2013-0096016, which is hereby incorporated by reference in its entirety) allows the identification and selection of relevant over-presented peptide vaccine candidates based on direct relative quantitation of HLA-restricted peptide levels on cancer tissues in comparison to several different non-cancerous tissues and organs. This was achieved by the development of label-free differential quantitation using the acquired LC-MS data processed by a proprietary data analysis pipeline, combining algorithms for sequence identification, spectral clustering, ion counting, retention time alignment, charge state deconvolution and normalization.

Presentation levels including error estimates for each peptide and sample were established. Peptides exclusively presented on tumor tissue and peptides over-presented in tumor versus non-cancerous tissues and organs have been identified.

HLA-peptide complexes from CLL tissue samples were purified and HLA-associated peptides were isolated and analyzed by LC-MS (see examples). All TUMAPs contained in the present application were identified with this approach on primary CLL samples confirming their presentation on primary CLL.

TUMAPs identified on multiple CLL and normal tissues were quantified using ion-counting of label-free LC-MS data. The method assumes that LC-MS signal areas of a peptide correlate with its abundance in the sample. All quantitative signals of a peptide in various LC-MS experiments were normalized based on central tendency, averaged per sample and merged into a bar plot, called presentation profile. The presentation profile consolidates different analysis methods like protein database search, spectral clustering, charge state deconvolution (decharging) and retention time alignment and normalization.

Furthermore, the discovery platform Technique XPRESIDENT® v2.x (see for example PCT/EP2011/056056 and PCT/EP2015/079873) allows the direct absolute quantitation of MHC-, preferably HLA-restricted, peptide levels on cancer or other infected tissues. Briefly, the total cell count was calculated from the total DNA content of the analyzed tissue sample. The total peptide amount for a TUMAP in a tissue sample was measured by nanoLC-MS/MS as the ratio of the natural TUMAP and a known amount of an isotope-labelled version of the TUMAP, the so-called internal standard. The efficiency of TUMAP isolation was determined by spiking peptide:MHC complexes of all selected TUMAPs into the tissue lysate at the earliest possible point of the TUMAP isolation procedure and their detection by nanoLC-MS/MS following completion of the peptide isolation procedure. The total cell count and the amount of total peptide were calculated from triplicate measurements per tissue sample. The peptide-specific isolation efficiencies were calculated as an average from 10 spike experiments each measured as a triplicate (see Example 6 and Table 12).

Besides over-presentation of the peptide, mRNA expression of the underlying gene was tested. mRNA data were obtained via RNASeq analyses of normal tissues and cancer tissues (cf. Example 2, Figure 3). An additional source of normal tissue data was a database of publicly available RNA expression data from around 3000 normal tissue samples (Lonsdale, 2013). Peptides which are derived from proteins whose coding mRNA is highly expressed in cancer tissue, but very low or absent in vital normal tissues, were preferably included in the present invention.

The present invention provides peptides that are useful in treating cancers/tumors, preferably CLL that over- or exclusively present the peptides of the invention. These peptides were shown by mass spectrometry to be naturally presented by HLA molecules on primary human CLL samples.

Many of the source gene/proteins (also designated “full-length proteins” or “underlying proteins”) from which the peptides are derived were shown to be highly over-expressed in cancer compared with normal tissues – “normal tissues” in relation to this invention shall mean either healthy adipose tissue, adrenal gland, blood cells, blood vessel, bone marrow, brain, breast, esophagus, eye, gallbladder, heart, kidney, large intestine, liver, lung, lymph node, nerve, pancreas, parathyroid gland, peritoneum, pituitary, pleura, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, thyroid gland, trachea, ureter, and urinary bladder cells or other normal tissue cells, demonstrating a high degree of tumor association of the source genes (see Example 2). Moreover, the peptides themselves are strongly over-presented on tumor tissue – “tumor tissue” in relation to this invention shall mean a sample from a patient suffering from CLL, but not on normal tissues (see Example 1).

HLA-bound peptides can be recognized by the immune system, specifically T lymphocytes. T cells can destroy the cells presenting the recognized HLA/peptide complex, e.g. CLL cells presenting the derived peptides.

The peptides of the present invention have been shown to be capable of stimulating T cell responses and/or are over-presented and thus can be used for the production of antibodies and/or TCRs, such as soluble TCRs, according to the present invention (see Example 3, Example 4). Furthermore, the peptides when complexed with the respective MHC can be used for the production of antibodies and/or TCRs, in particular sTCRs, according to the present invention, as well. Respective methods are well known to the person of skill, and can be found in the respective literature as well. Thus, the peptides of the present invention are useful for generating an immune response in a patient by which tumor cells can be destroyed. An immune response in a patient can be induced by direct administration of the described peptides or suitable precursor substances (e.g. elongated peptides, proteins, or nucleic acids encoding these peptides) to the patient, ideally in combination with an agent enhancing the immunogenicity (i.e. an adjuvant). The immune response originating from such a therapeutic vaccination can be expected to be highly specific against tumor cells because the target peptides of the present invention are not presented on normal tissues in comparable copy numbers, preventing the risk of undesired autoimmune reactions against normal cells in the patient.

The present description further relates to T-cell receptors (TCRs) comprising an alpha chain and a beta chain (“alpha/beta TCRs”). Also provided are peptides according to the invention capable of binding to TCRs and antibodies when presented by an MHC molecule. The present description also relates to nucleic acids, vectors and host cells for expressing TCRs and peptides of the present description; and methods of using the same.

The term “T-cell receptor” (abbreviated TCR) refers to a heterodimeric molecule comprising an alpha polypeptide chain (alpha chain) and a beta polypeptide chain (beta chain), wherein the heterodimeric receptor is capable of binding to a peptide antigen presented by an HLA molecule. The term also includes so-called gamma/delta TCRs.

In one embodiment the description provides a method of producing a TCR as described herein, the method comprising culturing a host cell capable of expressing the TCR under conditions suitable to promote expression of the TCR.

The description in another aspect relates to methods according to the description, wherein the antigen is loaded onto class I or II MHC molecules expressed on the surface of a suitable antigen-presenting cell or artificial antigen-presenting cell by contacting a sufficient amount of the antigen with an antigen-presenting cell or the antigen is loaded onto class I or II MHC tetramers by tetramerizing the antigen/class I or II MHC complex monomers.

The alpha and beta chains of alpha/beta TCR's, and the gamma and delta chains of gamma/delta TCRs, are generally regarded as each having two "domains", namely variable and constant domains. The variable domain consists of a concatenation of variable region (V), and joining region (J). The variable domain may also include a leader region (L). Beta and delta chains may also include a diversity region (D). The alpha and beta constant domains may also include C-terminal transmembrane (TM) domains that anchor the alpha and beta chains to the cell membrane.

With respect to gamma/delta TCRs, the term "TCR gamma variable domain" as used herein refers to the concatenation of the TCR gamma V (TRGV) region without leader region (L), and the TCR gamma J (TRGJ) region, and the term TCR gamma constant domain refers to the extracellular TRGC region, or to a C-terminal truncated TRGC sequence. Likewise the term "TCR delta variable domain" refers to the concatenation of the TCR delta V (TRDV) region without leader region (L) and the TCR delta D/J (TRDD/TRDJ) region, and the term “TCR delta constant domain” refers to the extracellular TRDC region, or to a C-terminal truncated TRDC sequence.

TCRs of the present description preferably bind to an peptide-HLA molecule complex with a binding affinity (KD) of about 100 µM or less, about 50 µM or less, about 25 µM or less, or about 10 µM or less. More preferred are high affinity TCRs having binding affinities of about 1 µM or less, about 100 nM or less, about 50 nM or less, about 25 nM or less. Non-limiting examples of preferred binding affinity ranges for TCRs of the present invention include about 1 nM to about 10 nM; about 10 nM to about 20 nM; about 20 nM to about 30 nM; about 30 nM to about 40 nM; about 40 nM to about 50 nM; about 50 nM to about 60 nM; about 60 nM to about 70 nM; about 70 nM to about 80 nM; about 80 nM to about 90 nM; and about 90 nM to about 100 nM.

As used herein in connect with TCRs of the present description, “specific binding” and grammatical variants thereof are used to mean a TCR having a binding affinity (KD) for a peptide-HLA molecule complex of 100 µM or less.

Alpha/beta heterodimeric TCRs of the present description may have an introduced disulfide bond between their constant domains. Preferred TCRs of this type include those which have a TRAC constant domain sequence and a TRBC1 or TRBC2 constant domain sequence except that Thr 48 of TRAC and Ser 57 of TRBC1 or TRBC2 are replaced by cysteine residues, the said cysteines forming a disulfide bond between the TRAC constant domain sequence and the TRBC1 or TRBC2 constant domain sequence of the TCR.

With or without the introduced inter-chain bond mentioned above, alpha/beta heterodimeric TCRs of the present description may have a TRAC constant domain sequence and a TRBC1 or TRBC2 constant domain sequence, and the TRAC constant domain sequence and the TRBC1 or TRBC2 constant domain sequence of the TCR may be linked by the native disulfide bond between Cys4 of exon 2 of TRAC and Cys2 of exon 2 of TRBC1 or TRBC2.

TCRs of the present description may comprise a detectable label selected from the group consisting of a radionuclide, a fluorophore and biotin. TCRs of the present description may be conjugated to a therapeutically active agent, such as a radionuclide, a chemotherapeutic agent, or a toxin.

In an embodiment, a TCR of the present description having at least one mutation in the alpha chain and/or having at least one mutation in the beta chain has modified glycosylation compared to the unmutated TCR.

In an embodiment, a TCR comprising at least one mutation in the TCR alpha chain and/or TCR beta chain has a binding affinity for, and/or a binding half-life for, a peptide-HLA molecule complex, which is at least double that of a TCR comprising the unmutated TCR alpha chain and/or unmutated TCR beta chain. Affinity-enhancement of tumor-specific TCRs, and its exploitation, relies on the existence of a window for optimal TCR affinities. The existence of such a window is based on observations that TCRs specific for HLA-A2-restricted pathogens have KD values that are generally about 10-fold lower when compared to TCRs specific for HLA-A2-restricted tumor-associated self-antigens. It is now known, although tumor antigens have the potential to be immunogenic, because tumors arise from the individual’s own cells only mutated proteins or proteins with altered translational processing will be seen as foreign by the immune system. Antigens that are upregulated or overexpressed (so called self-antigens) will not necessarily induce a functional immune response against the tumor: T-cells expressing TCRs that are highly reactive to these antigens will have been negatively selected within the thymus in a process known as central tolerance, meaning that only T-cells with low-affinity TCRs for self-antigens remain. Therefore, affinity of TCRs or variants of the present description to pepides can be enhanced by methods well known in the art.

The present description further relates to a method of identifying and isolating a TCR according to the present description, said method comprising incubating PBMCs from HLA-A*02-negative healthy donors with A2/peptide monomers, incubating the PBMCs with tetramer-phycoerythrin (PE) and isolating the high avidity T-cells by fluo-rescence activated cell sorting (FACS)–Calibur analysis.

The present description further relates to a method of identifying and isolating a TCR according to the present description, said method comprising obtaining a transgenic mouse with the entire human TCRαβ gene loci (1.1 and 0.7 Mb), whose T-cells express a diverse human TCR repertoire that compensates for mouse TCR deficiency, immunizing the mouse with a peptide, incubating PBMCs obtained from the transgenic mice with tetramer-phycoerythrin (PE), and isolating the high avidity T-cells by fluorescence activated cell sorting (FACS)–Calibur analysis.

In one aspect, to obtain T-cells expressing TCRs of the present description, nucleic acids encoding TCR-alpha and/or TCR-beta chains of the present description are cloned into expression vectors, such as gamma retrovirus or lentivirus. The recombinant viruses are generated and then tested for functionality, such as antigen specificity and functional avidity. An aliquot of the final product is then used to transduce the target T-cell population (generally purified from patient PBMCs), which is expanded before infusion into the patient.

In another aspect, to obtain T-cells expressing TCRs of the present description, TCR RNAs are synthesized by techniques known in the art, e.g., in vitro transcription systems. The in vitro-synthesized TCR RNAs are then introduced into primary CD8+ T-cells obtained from healthy donors by electroporation to re-express tumor specific TCR-alpha and/or TCR-beta chains.

To increase the expression, nucleic acids encoding TCRs of the present description may be operably linked to strong promoters, such as retroviral long terminal repeats (LTRs), cytomegalovirus (CMV), murine stem cell virus (MSCV) U3, phosphoglycerate kinase (PGK), β-actin, ubiquitin, and a simian virus 40 (SV40)/CD43 composite promoter, elongation factor (EF)-1a and the spleen focus-forming virus (SFFV) promoter. In a preferred embodiment, the promoter is heterologous to the nucleic acid being expressed.

In addition to strong promoters, TCR expression cassettes of the present description may contain additional elements that can enhance transgene expression, including a central polypurine tract (cPPT), which promotes the nuclear translocation of lentiviral constructs (Follenzi et al., 2000), and the woodchuck hepatitis virus posttranscriptional regulatory element (wPRE), which increases the level of transgene expression by increasing RNA stability (Zufferey et al., 1999).

The alpha and beta chains of a TCR of the present invention may be encoded by nucleic acids located in separate vectors, or may be encoded by polynucleotides located in the same vector.

Achieving high-level TCR surface expression requires that both the TCR-alpha and TCR-beta chains of the introduced TCR be transcribed at high levels. To do so, the TCR-alpha and TCR-beta chains of the present description may be cloned into bi-cistronic constructs in a single vector, which has been shown to be capable of over-coming this obstacle. The use of a viral intraribosomal entry site (IRES) between the TCR-alpha and TCR-beta chains results in the coordinated expression of both chains, because the TCR-alpha and TCR-beta chains are generated from a single transcript that is broken into two proteins during translation, ensuring that an equal molar ratio of TCR-alpha and TCR-beta chains are produced. (Schmitt et al. 2009).

Nucleic acids encoding TCRs of the present description may be codon optimized to increase expression from a host cell. Redundancy in the genetic code allows some amino acids to be encoded by more than one codon, but certain codons are less “optimal” than others because of the relative availability of matching tRNAs as well as other factors (Gustafsson et al., 2004). Modifying the TCR-alpha and TCR-beta gene sequences such that each amino acid is encoded by the optimal codon for mammalian gene expression, as well as eliminating mRNA instability motifs or cryptic splice sites, has been shown to significantly enhance TCR-alpha and TCR-beta gene expression (Scholten et al., 2006).

Furthermore, mispairing between the introduced and endogenous TCR chains may result in the acquisition of specificities that pose a significant risk for autoimmunity. For example, the formation of mixed TCR dimers may reduce the number of CD3 molecules available to form properly paired TCR complexes, and therefore can significantly decrease the functional avidity of the cells expressing the introduced TCR (Kuball et al., 2007).

To reduce mispairing, the C-terminus domain of the introduced TCR chains of the present description may be modified in order to promote interchain affinity, while decreasing the ability of the introduced chains to pair with the endogenous TCR. These strategies may include replacing the human TCR-alpha and TCR-beta C-terminus domains with their murine counterparts (murinized C-terminus domain); generating a second interchain disulfide bond in the C-terminus domain by introducing a second cysteine residue into both the TCR-alpha and TCR-beta chains of the introduced TCR (cysteine modification); swapping interacting residues in the TCR-alpha and TCR-beta chain C-terminus domains (“knob-in-hole”); and fusing the variable domains of the TCR-alpha and TCR-beta chains directly to CD3ζ (CD3ζ fusion). (Schmitt et al. 2009).

In an embodiment, a host cell is engineered to express a TCR of the present description. In preferred embodiments, the host cell is a human T-cell or T-cell progenitor. In some embodiments the T-cell or T-cell progenitor is obtained from a cancer patient. In other embodiments the T-cell or T-cell progenitor is obtained from a healthy donor. Host cells of the present description can be allogeneic or autologous with respect to a patient to be treated. In one embodiment, the host is a gamma/delta T-cell transformed to express an alpha/beta TCR.

A “pharmaceutical composition” is a composition suitable for administration to a human being in a medical setting. Preferably, a pharmaceutical composition is sterile and produced according to GMP guidelines.

The pharmaceutical compositions comprise the peptides either in the free form or in the form of a pharmaceutically acceptable salt (see also above). As used herein, "a pharmaceutically acceptable salt" refers to a derivative of the disclosed peptides wherein the peptide is modified by making acid or base salts of the agent. For example, acid salts are prepared from the free base (typically wherein the neutral form of the drug has a neutral –NH2 group) involving reaction with a suitable acid. Suitable acids for preparing acid salts include both organic acids, e.g., acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methane sulfonic acid, ethane sulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid phosphoric acid and the like. Conversely, preparation of basic salts of acid moieties which may be present on a peptide are prepared using a pharmaceutically acceptable base such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine or the like.

In an especially preferred embodiment, the pharmaceutical compositions comprise the peptides as salts of acetic acid (acetates), trifluoro acetates or hydrochloric acid (chlorides).

Preferably, the medicament of the present invention is an immunotherapeutic such as a vaccine. It may be administered directly into the patient, into the affected organ or systemically i.d., i.m., s.c., i.p. and i.v., or applied *ex vivo* to cells derived from the patient or a human cell line which are subsequently administered to the patient, or used *in vitro* to select a subpopulation of immune cells derived from the patient, which are then re-administered to the patient. If the nucleic acid is administered to cells *in vitro*, it may be useful for the cells to be transfected so as to co-express immune-stimulating cytokines, such as interleukin-2. The peptide may be substantially pure, or combined with an immune-stimulating adjuvant (see below) or used in combination with immune-stimulatory cytokines, or be administered with a suitable delivery system, for example liposomes. The peptide may also be conjugated to a suitable carrier such as keyhole limpet haemocyanin (KLH) or mannan (see WO 95/18145 and (Longenecker et al., 1993)). The peptide may also be tagged, may be a fusion protein, or may be a hybrid molecule. The peptides whose sequence is given in the present invention are expected to stimulate CD4 or CD8 T cells. However, stimulation of CD8 T cells is more efficient in the presence of help provided by CD4 T-helper cells. Thus, for MHC Class I epitopes that stimulate CD8 T cells the fusion partner or sections of a hybrid molecule suitably provide epitopes which stimulate CD4-positive T cells. CD4- and CD8-stimulating epitopes are well known in the art and include those identified in the present invention.

In one aspect, the vaccine comprises at least one peptide having the amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 385, and at least one additional peptide, preferably two to 50, more preferably two to 25, even more preferably two to 20 and most preferably two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen or eighteen peptides. The peptide(s) may be derived from one or more specific TAAs and may bind to MHC class I molecules.

A further aspect of the invention provides a nucleic acid (for example a polynucleotide) encoding a peptide or peptide variant of the invention. The polynucleotide may be, for example, DNA, cDNA, PNA, RNA or combinations thereof, either single- and/or double-stranded, or native or stabilized forms of polynucleotides, such as, for example, polynucleotides with a phosphorothioate backbone and it may or may not contain introns so long as it codes for the peptide. Of course, only peptides that contain naturally occurring amino acid residues joined by naturally occurring peptide bonds are encodable by a polynucleotide. A still further aspect of the invention provides an expression vector capable of expressing a polypeptide according to the invention.

A variety of methods have been developed to link polynucleotides, especially DNA, to vectors for example via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc. New Haven, CN, USA.

A desirable method of modifying the DNA encoding the polypeptide of the invention employs the polymerase chain reaction as disclosed by Saiki RK, et al. (Saiki et al., 1988). This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art. If viral vectors are used, pox- or adenovirus vectors are preferred.

The DNA (or in the case of retroviral vectors, RNA) may then be expressed in a suitable host to produce a polypeptide comprising the peptide or variant of the invention. Thus, the DNA encoding the peptide or variant of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed, for example, in US 4,440,859, 4,530,901, 4,582,800, 4,677,063, 4,678,751, 4,704,362, 4,710,463, 4,757,006, 4,766,075, and 4,810,648.

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance.

Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus spec.*), plant cells, animal cells and insect cells. Preferably, the system can be mammalian cells such as CHO cells available from the ATCC Cell Biology Collection.

A typical mammalian cell vector plasmid for constitutive expression comprises the CMV or SV40 promoter with a suitable poly A tail and a resistance marker, such as neomycin. One example is pSVL available from Pharmacia, Piscataway, NJ, USA. An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, TRP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps). CMV promoter-based vectors (for example from Sigma-Aldrich) provide transient or stable expression, cytoplasmic expression or secretion, and N-terminal or C-terminal tagging in various combinations of FLAG, 3xFLAG, c-myc or MAT. These fusion proteins allow for detection, purification and analysis of recombinant protein. Dual-tagged fusions provide flexibility in detection.

The strong human cytomegalovirus (CMV) promoter regulatory region drives constitutive protein expression levels as high as 1 mg/L in COS cells. For less potent cell lines, protein levels are typically ~0.1 mg/L. The presence of the SV40 replication origin will result in high levels of DNA replication in SV40 replication permissive COS cells. CMV vectors, for example, can contain the pMB1 (derivative of pBR322) origin for replication in bacterial cells, the b-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the f1 origin. Vectors containing the pre-pro-trypsin leader (PPT) sequence can direct the secretion of FLAG fusion proteins into the culture medium for purification using ANTI-FLAG antibodies, resins, and plates. Other vectors and expression systems are well known in the art for use with a variety of host cells.

In another embodiment two or more peptides or peptide variants of the invention are encoded and thus expressed in a successive order (similar to “beads on a string” constructs). In doing so, the peptides or peptide variants may be linked or fused together by stretches of linker amino acids, such as for example LLLLLL, or may be linked without any additional peptide(s) between them. These constructs can also be used for cancer therapy, and may induce immune responses both involving MHC I and MHC II.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either prokaryotic or eukaryotic. Bacterial cells may be preferred prokaryotic host cells in some circumstances and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic and colon cell lines. Yeast host cells include YPH499, YPH500 and YPH501, which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 and 293 cells which are human embryonic kidney cells. Preferred insect cells are Sf9 cells which can be transfected with baculovirus expression vectors. An overview regarding the choice of suitable host cells for expression can be found in, for example, the textbook of Paulina Balbás and Argelia Lorence “Methods in Molecular Biology Recombinant Gene Expression, Reviews and Protocols,” Part One, Second Edition, ISBN 978-1-58829-262-9, and other literature known to the person of skill.

Transformation of appropriate cell hosts with a DNA construct of the present invention is accomplished by well-known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen et al. (Cohen et al., 1972) and (Green and Sambrook, 2012) . Transformation of yeast cells is described in Sherman et al. (Sherman et al., 1986) . The method of Beggs (Beggs, 1978) is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA. Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

Successfully transformed cells, i.e. cells that contain a DNA construct of the present invention, can be identified by well-known techniques such as PCR. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

It will be appreciated that certain host cells of the invention are useful in the preparation of the peptides of the invention, for example bacterial, yeast and insect cells. However, other host cells may be useful in certain therapeutic methods. For example, antigen-presenting cells, such as dendritic cells, may usefully be used to express the peptides of the invention such that they may be loaded into appropriate MHC molecules. Thus, the current invention provides a host cell comprising a nucleic acid or an expression vector according to the invention.

In a preferred embodiment, the host cell is an antigen presenting cell, in particular a dendritic cell or antigen presenting cell. APCs loaded with a recombinant fusion protein containing prostatic acid phosphatase (PAP) were approved by the U.S. Food and Drug Administration (FDA) on April 29, 2010, to treat asymptomatic or minimally symptomatic metastatic HRPC (Sipuleucel-T) (Rini et al., 2006; Small et al., 2006).

A further aspect of the invention provides a method of producing a peptide or its variant, the method comprising culturing a host cell and isolating the peptide from the host cell or its culture medium.

In another embodiment, the peptide, the nucleic acid or the expression vector of the invention are used in medicine. For example, the peptide or its variant may be prepared for intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, intramuscular (i.m.) injection. Preferred methods of peptide injection include s.c., i.d., i.p., i.m., and i.v. Preferred methods of DNA injection include i.d., i.m., s.c., i.p. and i.v. Doses of e.g. between 50 µg and 1.5 mg, preferably 125 µg to 500 µg, of peptide or DNA may be given and will depend on the respective peptide or DNA. Dosages of this range were successfully used in previous trials (Walter et al., 2012).

The polynucleotide used for active vaccination may be substantially pure, or contained in a suitable vector or delivery system. The nucleic acid may be DNA, cDNA, PNA, RNA or a combination thereof. Methods for designing and introducing such a nucleic acid are well known in the art. An overview is provided by e.g. Teufel et al. (Teufel et al., 2005). Polynucleotide vaccines are easy to prepare, but the mode of action of these vectors in inducing an immune response is not fully understood. Suitable vectors and delivery systems include viral DNA and/or RNA, such as systems based on adenovirus, vaccinia virus, retroviruses, herpes virus, adeno-associated virus or hybrids containing elements of more than one virus. Non-viral delivery systems include cationic lipids and cationic polymers and are well known in the art of DNA delivery. Physical delivery, such as via a “gene-gun” may also be used. The peptide or peptides encoded by the nucleic acid may be a fusion protein, for example with an epitope that stimulates T cells for the respective opposite CDR as noted above.

The medicament of the invention may also include one or more adjuvants. Adjuvants are substances that non-specifically enhance or potentiate the immune response (e.g., immune responses mediated by CD8-positive T cells and helper-T (TH) cells to an antigen, and would thus be considered useful in the medicament of the present invention. Suitable adjuvants include, but are not limited to, 1018 ISS, aluminum salts, AMPLIVAX®, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, flagellin or TLR5 ligands derived from flagellin, FLT3 ligand, GM-CSF, IC30, IC31, Imiquimod (ALDARA®), resiquimod, ImuFact IMP321, Interleukins as IL-2, IL-13, IL-21, Interferon-alpha or -beta, or pegylated derivatives thereof, IS Patch, ISS, ISCOMATRIX, ISCOMs, JuvImmune®, LipoVac, MALP2, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, water-in-oil and oil-in-water emulsions, OK-432, OM-174, OM-197-MP-EC, ONTAK, OspA, PepTel® vector system, poly(lactid co-glycolid) [PLG]-based and dextran microparticles, talactoferrin SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox, Quil, or Superfos. Adjuvants such as Freund's or GM-CSF are preferred. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described previously (Allison and Krummel, 1995). Also cytokines may be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-), accelerating the maturation of dendritic cells into efficient antigen-presenting cells for T-lymphocytes (e.g., GM-CSF, IL-1 and IL-4) (U.S. Pat. No. 5,849,589, specifically incorporated herein by reference in its entirety) and acting as immunoadjuvants (e.g., IL-12, IL-15, IL-23, IL-7, IFN-alpha. IFN-beta) (Gabrilovich et al., 1996).

CpG immunostimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a vaccine setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non-adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen-specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses, dendritic cell vaccines, autologous cellular vaccines and polysaccharide conjugates in both prophylactic and therapeutic vaccines. More importantly it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of TH1 cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T cell help. The TH1 bias induced by TLR9 stimulation is maintained even in the presence of vaccine adjuvants such as alum or incomplete Freund’s adjuvant (IFA) that normally promote a TH2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nanoparticles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enable the antigen doses to be reduced by approximately two orders of magnitude, with comparable antibody responses to the full-dose vaccine without CpG in some experiments (Krieg, 2006). US 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen to induce an antigen-specific immune response. A CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen (Berlin, Germany) which is a preferred component of the pharmaceutical composition of the present invention. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 may also be used.

Other examples for useful adjuvants include, but are not limited to chemically modified CpGs (e.g. CpR, Idera), dsRNA analogues such as Poly(I:C) and derivates thereof (e.g. AmpliGen®, Hiltonol®, poly-(ICLC), poly(IC-R), poly(I:C12U), non-CpG bacterial DNA or RNA as well as immunoactive small molecules and antibodies such as cyclophosphamide, sunitinib, Bevacizumab®, celebrex, NCX-4016, sildenafil, tadalafil, vardenafil, sorafenib, temozolomide, temsirolimus, XL-999, CP-547632, pazopanib, VEGF Trap, ZD2171, AZD2171, anti-CTLA4, other antibodies targeting key structures of the immune system (e.g. anti-CD40, anti-TGFbeta, anti-TNFalpha receptor) and SC58175, which may act therapeutically and/or as an adjuvant. The amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan without undue experimentation.

Preferred adjuvants are anti-CD40, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, interferon-alpha, CpG oligonucleotides and derivates, poly-(I:C) and derivates, RNA, sildenafil, and particulate formulations with PLG or virosomes.

In a preferred embodiment, the pharmaceutical composition according to the invention the adjuvant is selected from the group consisting of colony-stimulating factors, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim), cyclophosphamide, imiquimod, resiquimod, and interferon-alpha.

In a preferred embodiment, the pharmaceutical composition according to the invention the adjuvant is selected from the group consisting of colony-stimulating factors, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim), cyclophosphamide, imiquimod and resiquimod. In a preferred embodiment of the pharmaceutical composition according to the invention, the adjuvant is cyclophosphamide, imiquimod or resiquimod. Even more preferred adjuvants are Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, poly-ICLC (Hiltonol®) and anti-CD40 mAB, or combinations thereof.

This composition is used for parenteral administration, such as subcutaneous, intradermal, intramuscular or oral administration. For this, the peptides and optionally other molecules are dissolved or suspended in a pharmaceutically acceptable, preferably aqueous carrier. In addition, the composition can contain excipients, such as buffers, binding agents, blasting agents, diluents, flavors, lubricants, etc. The peptides can also be administered together with immune stimulating substances, such as cytokines. An extensive listing of excipients that can be used in such a composition, can be, for example, taken from A. Kibbe, Handbook of Pharmaceutical Excipients (Kibbe, 2000). The composition can be used for a prevention, prophylaxis and/or therapy of adenomatous or cancerous diseases. Exemplary formulations can be found in, for example, EP2112253.

It is important to realize that the immune response triggered by the vaccine according to the invention attacks the cancer in different cell-stages and different stages of development. Furthermore, different cancer associated signaling pathways are attacked. This is an advantage over vaccines that address only one or few targets, which may cause the tumor to easily adapt to the attack (tumor escape). Furthermore, not all individual tumors express the same pattern of antigens. Therefore, a combination of several tumor-associated peptides ensures that every single tumor bears at least some of the targets. The composition is designed in such a way that each tumor is expected to express several of the antigens and cover several independent pathways necessary for tumor growth and maintenance. Thus, the vaccine can easily be used “off-the–shelf” for a larger patient population. This means that a pre-selection of patients to be treated with the vaccine can be restricted to HLA typing, does not require any additional biomarker assessments for antigen expression, but it is still ensured that several targets are simultaneously attacked by the induced immune response, which is important for efficacy (Banchereau et al., 2001; Walter et al., 2012).

As used herein, the term "scaffold" refers to a molecule that specifically binds to an (e.g. antigenic) determinant. In one embodiment, a scaffold is able to direct the entity to which it is attached (e.g. a (second) antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant (e.g. the complex of a peptide with MHC, according to the application at hand). In another embodiment, a scaffold is able to activate signaling through its target antigen, for example a T cell receptor complex antigen. Scaffolds include but are not limited to antibodies and fragments thereof, antigen binding domains of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region, binding proteins comprising at least one ankyrin repeat motif and single domain antigen binding (SDAB) molecules, aptamers, (soluble) TCRs and (modified) cells such as allogenic or autologous T cells. To assess whether a molecule is a scaffold binding to a target, binding assays can be performed.

“Specific” binding means that the scaffold binds the peptide-MHC-complex of interest better than other naturally occurring peptide-MHC-complexes, to an extent that a scaffold armed with an active molecule that is able to kill a cell bearing the specific target is not able to kill another cell without the specific target but presenting other peptide-MHC complex(es). Binding to other peptide-MHC complexes is irrelevant if the peptide of the cross-reactive peptide-MHC is not naturally occurring, i.e. not derived from the human HLA-peptidome. Tests to assess target cell killing are well known in the art. They should be performed using target cells (primary cells or cell lines) with unaltered peptide-MHC presentation, or cells loaded with peptides such that naturally occurring peptide-MHC levels are reached.

Each scaffold can comprise a labelling which provides that the bound scaffold can be detected by determining the presence or absence of a signal provided by the label. For example, the scaffold can be labelled with a fluorescent dye or any other applicable cellular marker molecule. Such marker molecules are well known in the art. For example, a fluorescence-labelling, for example provided by a fluorescence dye, can provide a visualization of the bound aptamer by fluorescence or laser scanning microscopy or flow cytometry. Each scaffold can be conjugated with a second active molecule such as for example IL-21, anti-CD3, anti-CD28. For further information on polypeptide scaffolds see for example the background section of WO 2014/071978A1 and the references cited therein.

The present invention further relates to aptamers. Aptamers (see for example WO 2014/191359 and the literature as cited therein) are short single-stranded nucleic acid molecules, which can fold into defined three-dimensional structures and recognize specific target structures. They have appeared to be suitable alternatives for developing targeted therapies. Aptamers have been shown to selectively bind to a variety of complex targets with high affinity and specificity.

Aptamers recognizing cell surface located molecules have been identified within the past decade and provide means for developing diagnostic and therapeutic approaches. Since aptamers have been shown to possess almost no toxicity and immunogenicity they are promising candidates for biomedical applications. Indeed, aptamers, for example prostate-specific membrane-antigen recognizing aptamers, have been successfully employed for targeted therapies and shown to be functional in xenograft *in vivo* models. Furthermore, aptamers recognizing specific tumor cell lines have been identified.

DNA aptamers can be selected to reveal broad-spectrum recognition properties for various cancer cells, and particularly those derived from solid tumors, while non-tumorigenic and primary healthy cells are not recognized. If the identified aptamers recognize not only a specific tumor sub-type but rather interact with a series of tumors, this renders the aptamers applicable as so-called broad-spectrum diagnostics and therapeutics.

Further, investigation of cell-binding behavior with flow cytometry showed that the aptamers revealed very good apparent affinities that are within the nanomolar range.

Aptamers are useful for diagnostic and therapeutic purposes. Further, it could be shown that some of the aptamers are taken up by tumor cells and thus can function as molecular vehicles for the targeted delivery of anti-cancer agents such as siRNA into tumor cells.

Aptamers can be selected against complex targets such as cells and tissues and complexes of the peptides comprising, preferably consisting of, a sequence according to any of SEQ ID NO 1 to SEQ ID NO 385, according to the invention at hand with the MHC molecule, using the cell-SELEX (Systematic Evolution of Ligands by Exponential enrichment) technique.

The peptides of the present invention can be used to generate and develop specific antibodies against MHC/peptide complexes. These can be used for therapy, targeting toxins or radioactive substances to the diseased tissue. Another use of these antibodies can be targeting radionuclides to the diseased tissue for imaging purposes such as PET. This use can help to detect small metastases or to determine the size and precise localization of diseased tissues.

Therefore, it is a further aspect of the invention to provide a method for producing a recombinant antibody specifically binding to a human major histocompatibility complex (MHC) class I or II being complexed with a HLA-restricted antigen, the method comprising: immunizing a genetically engineered non-human mammal comprising cells expressing said human major histocompatibility complex (MHC) class I or II with a soluble form of a MHC class I or II molecule being complexed with said HLA-restricted antigen; isolating mRNA molecules from antibody producing cells of said non-human mammal; producing a phage display library displaying protein molecules encoded by said mRNA molecules; and isolating at least one phage from said phage display library, said at least one phage displaying said antibody specifically binding to said human major histocompatibility complex (MHC) class I or II being complexed with said HLA-restricted antigen.

It is a further aspect of the invention to provide an antibody that specifically binds to a human major histocompatibility complex (MHC) class I or II being complexed with a

HLA-restricted antigen, wherein the antibody preferably is a polyclonal antibody, monoclonal antibody, bi-specific antibody and/or a chimeric antibody.

Respective methods for producing such antibodies and single chain class I major histocompatibility complexes, as well as other tools for the production of these antibodies are disclosed in WO 03/068201, WO 2004/084798, WO 01/72768, WO 03/070752, and in publications (Cohen et al., 2003a; Cohen et al., 2003b; Denkberg et al., 2003), which for the purposes of the present invention are all explicitly incorporated by reference in their entireties.

Preferably, the antibody is binding with a binding affinity of below 20 nanomolar, preferably of below 10 nanomolar, to the complex, which is also regarded as “specific” in the context of the present invention.

The present invention relates to a peptide comprising a sequence that is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 385, or a variant thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 385 or a variant thereof that induces T cells cross-reacting with said peptide, wherein said peptide is not the underlying full-length polypeptide.

The present invention further relates to a peptide comprising a sequence that is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 385 or a variant thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 385, wherein said peptide or variant has an overall length of between 8 and 100, preferably between 8 and 30, and most preferred between 8 and 14 amino acids.

The present invention further relates to the peptides according to the invention that have the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I or -II.

The present invention further relates to the peptides according to the invention wherein the peptide consists or consists essentially of an amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 385.

The present invention further relates to the peptides according to the invention, wherein the peptide is (chemically) modified and/or includes non-peptide bonds.

The present invention further relates to the peptides according to the invention, wherein the peptide is part of a fusion protein, in particular comprising N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii), or wherein the peptide is fused to (or into) an antibody, such as, for example, an antibody that is specific for dendritic cells.

The present invention further relates to a nucleic acid, encoding the peptides according to the invention, provided that the peptide is not the complete (full) human protein.

The present invention further relates to the nucleic acid according to the invention that is DNA, cDNA, PNA, RNA or combinations thereof.

The present invention further relates to an expression vector capable of expressing a nucleic acid according to the present invention.

The present invention further relates to a peptide according to the present invention, a nucleic acid according to the present invention or an expression vector according to the present invention for use in medicine, in particular in the treatment of CLL.

The present invention further relates to a host cell comprising a nucleic acid according to the invention or an expression vector according to the invention.

The present invention further relates to the host cell according to the present invention that is an antigen presenting cell, and preferably a dendritic cell.

The present invention further relates to a method of producing a peptide according to the present invention, said method comprising culturing the host cell according to the present invention, and isolating the peptide from said host cell or its culture medium.

The present invention further relates to the method according to the present invention, where-in the antigen is loaded onto class I or II MHC molecules expressed on the surface of a suitable antigen-presenting cell by contacting a sufficient amount of the antigen with an antigen-presenting cell.

The present invention further relates to the method according to the invention, wherein the antigen-presenting cell comprises an expression vector capable of expressing or expressing said peptide containing SEQ ID NO: 1 to SEQ ID NO: 385 or said variant amino acid sequence.

The present invention further relates to activated T cells, produced by the method according to the present invention, wherein said T cells selectively recognizes a cell which aberrantly expresses a polypeptide comprising an amino acid sequence according to the present invention.

The present invention further relates to a method of killing target cells in a patient which target cells aberrantly express a polypeptide comprising any amino acid sequence according to the present invention, the method comprising administering to the patient an effective number of T cells as according to the present invention.

The present invention further relates to the use of any peptide described, a nucleic acid according to the present invention, an expression vector according to the present invention, a cell according to the present invention, or an activated cytotoxic T lymphocyte according to the present invention as a medicament or in the manufacture of a medicament. The present invention further relates to a use according to the present invention, wherein the medicament is active against cancer.

The present invention further relates to a use according to the invention, wherein the medicament is a vaccine. The present invention further relates to a use according to the invention, wherein the medicament is active against cancer.

The present invention further relates to a use according to the invention, wherein said cancer cells are CLL cells or other solid or hematological tumor cells such as acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer.

The present invention further relates to particular marker proteins and biomarkers based on the peptides according to the present invention, herein called “targets” that can be used in the diagnosis and/or prognosis of CLL. The present invention also relates to the use of these novel targets for cancer treatment.

The term “antibody” or ”antibodies“ is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact or “full” immunoglobulin molecules, also included in the term ”antibodies“ are fragments (e.g. CDRs, Fv, Fab and Fc fragments) or polymers of those immunoglobulin molecules and humanized versions of immunoglobulin molecules, as long as they exhibit any of the desired properties (e.g., specific binding of a CLL marker (poly)peptide, delivery of a toxin to a CLL cell expressing a cancer marker gene at an increased level, and/or inhibiting the activity of a CLL marker polypeptide) according to the invention.

Whenever possible, the antibodies of the invention may be purchased from commercial sources. The antibodies of the invention may also be generated using well-known methods. The skilled artisan will understand that either full length CLL marker polypeptides or fragments thereof may be used to generate the antibodies of the invention. A polypeptide to be used for generating an antibody of the invention may be partially or fully purified from a natural source, or may be produced using recombinant DNA techniques.

For example, a cDNA encoding a peptide according to the present invention, such as a peptide according to SEQ ID NO: 1 to SEQ ID NO: 385 polypeptide, or a variant or fragment thereof, can be expressed in prokaryotic cells (e.g., bacteria) or eukaryotic cells (e.g., yeast, insect, or mammalian cells), after which the recombinant protein can be purified and used to generate a monoclonal or polyclonal antibody preparation that specifically bind the CLL marker polypeptide used to generate the antibody according to the invention.

One of skill in the art will realize that the generation of two or more different sets of monoclonal or polyclonal antibodies maximizes the likelihood of obtaining an antibody with the specificity and affinity required for its intended use (e.g., ELISA, immunohistochemistry, *in vivo* imaging, immunotoxin therapy). The antibodies are tested for their desired activity by known methods, in accordance with the purpose for which the antibodies are to be used (e.g., ELISA, immunohistochemistry, immunotherapy, etc.; for further guidance on the generation and testing of antibodies, see, e.g., Greenfield, 2014 (Greenfield, 2014)). For example, the antibodies may be tested in ELISA assays or, Western blots, immunohistochemical staining of formalin-fixed cancers or frozen tissue sections. After their initial *in vitro* characterization, antibodies intended for therapeutic or *in vivo* diagnostic use are tested according to known clinical testing methods.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, i.e.; the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired antagonistic activity (US 4,816,567, which is hereby incorporated in its entirety).

Monoclonal antibodies of the invention may be prepared using hybridoma methods. In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in US 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies).

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 and US 4,342,566. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a F(ab')2 fragment and a pFc' fragment.

The antibody fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the antibody fragment must possess a bioactive property, such as binding activity, regulation of binding at the binding domain, etc. Functional or active regions of the antibody may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antibody fragment.

The antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab' or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. Human antibodies can also be produced in phage display libraries.

Antibodies of the invention are preferably administered to a subject in a pharmaceutically acceptable carrier. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of antibody being administered.

The antibodies can be administered to the subject, patient, or cell by injection (e.g., intravenous, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. The antibodies may also be administered by intratumoral or peritumoral routes, to exert local as well as systemic therapeutic effects. Local or intravenous injection is preferred.

Effective dosages and schedules for administering the antibodies may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of antibodies that must be administered will vary depending on, for example, the subject that will receive the antibody, the route of administration, the particular type of antibody used and other drugs being administered. A typical daily dosage of the antibody used alone might range from about 1 (µg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. Following administration of an antibody, preferably for treating CLL, the efficacy of the therapeutic antibody can be assessed in various ways well known to the skilled practitioner. For instance, the size, number, and/or distribution of cancer in a subject receiving treatment may be monitored using standard tumor imaging techniques. A therapeutically-administered antibody that arrests tumor growth, results in tumor shrinkage, and/or prevents the development of new tumors, compared to the disease course that would occurs in the absence of antibody administration, is an efficacious antibody for treatment of cancer.

It is a further aspect of the invention to provide a method for producing a soluble T-cell receptor (sTCR) recognizing a specific peptide-MHC complex. Such soluble T-cell receptors can be generated from specific T-cell clones, and their affinity can be increased by mutagenesis targeting the complementarity-determining regions. For the purpose of T-cell receptor selection, phage display can be used (US 2010/0113300, (Liddy et al., 2012)). For the purpose of stabilization of T-cell receptors during phage display and in case of practical use as drug, alpha and beta chain can be linked e.g. by non-native disulfide bonds, other covalent bonds (single-chain T-cell receptor), or by dimerization domains (Boulter et al., 2003; Card et al., 2004; Willcox et al., 1999). The

T-cell receptor can be linked to toxins, drugs, cytokines (see, for example, US 2013/0115191), domains recruiting effector cells such as an anti-CD3 domain, etc., in order to execute particular functions on target cells. Moreover, it could be expressed in T cells used for adoptive transfer. Further information can be found in WO 2004/033685A1 and WO 2004/074322A1. A combination of sTCRs is described in WO 2012/056407A1. Further methods for the production are disclosed in WO 2013/057586A1.

In addition, the peptides and/or the TCRs or antibodies or other binding molecules of the present invention can be used to verify a pathologist's diagnosis of a cancer based on a biopsied sample.

The antibodies or TCRs may also be used for *in vivo* diagnostic assays. Generally, the antibody is labeled with a radionucleotide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{3}H, ^{32}P or ^{35}S) so that the tumor can be localized using immunoscintiography. In one embodiment, antibodies or fragments thereof bind to the extracellular domains of two or more targets of a protein selected from the group consisting of the above-mentioned proteins, and the affinity value (Kd) is less than 1 x 10µM.

Antibodies for diagnostic use may be labeled with probes suitable for detection by various imaging methods. Methods for detection of probes include, but are not limited to, fluorescence, light, confocal and electron microscopy; magnetic resonance imaging and spectroscopy; fluoroscopy, computed tomography and positron emission tomography. Suitable probes include, but are not limited to, fluorescein, rhodamine, eosin and other fluorophores, radioisotopes, gold, gadolinium and other lanthanides, paramagnetic iron, fluorine-18 and other positron-emitting radionuclides. Additionally, probes may be bi- or multi-functional and be detectable by more than one of the methods listed. These antibodies may be directly or indirectly labeled with said probes. Attachment of probes to the antibodies includes covalent attachment of the probe, incorporation of the probe into the antibody, and the covalent attachment of a chelating compound for binding of probe, amongst others well recognized in the art. For immunohistochemistry, the disease tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin. The fixed or embedded section contains the sample are contacted with a labeled primary antibody and secondary antibody, wherein the antibody is used to detect the expression of the proteins in situ.

Another aspect of the present invention includes an *in vitro* method for producing activated T cells, the method comprising contacting *in vitro* T cells with antigen loaded human MHC molecules expressed on the surface of a suitable antigen-presenting cell for a period of time sufficient to activate the T cell in an antigen specific manner, wherein the antigen is a peptide according to the invention. Preferably a sufficient amount of the antigen is used with an antigen-presenting cell.

Preferably the mammalian cell lacks or has a reduced level or function of the TAP peptide transporter. Suitable cells that lack the TAP peptide transporter include T2, RMA-S and Drosophila cells. TAP is the transporter associated with antigen processing.

The human peptide loading deficient cell line T2 is available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA under Catalogue No CRL 1992; the Drosophila cell line Schneider line 2 is available from the ATCC under Catalogue No CRL 19863; the mouse RMA-S cell line is described in Ljunggren et al. (Ljunggren and Karre, 1985).

Preferably, before transfection the host cell expresses substantially no MHC class I molecules. It is also preferred that the stimulator cell expresses a molecule important for providing a co-stimulatory signal for T-cells such as any of B7.1, B7.2, ICAM-1 and LFA 3. The nucleic acid sequences of numerous MHC class I molecules and of the co-stimulator molecules are publicly available from the GenBank and EMBL databases.

In case of a MHC class I epitope being used as an antigen, the T cells are CD8-positive T cells.

If an antigen-presenting cell is transfected to express such an epitope, preferably the cell comprises an expression vector capable of expressing a peptide containing SEQ ID NO: 1 to SEQ ID NO: 385, or a variant amino acid sequence thereof.

A number of other methods may be used for generating T cells in vitro. For example, autologous tumor-infiltrating lymphocytes can be used in the generation of CTL. Plebanski et al. (Plebanski et al., 1995) made use of autologous peripheral blood lymphocytes (PLBs) in the preparation of T cells. Furthermore, the production of autologous T cells by pulsing dendritic cells with peptide or polypeptide, or via infection with recombinant virus is possible. Also, B cells can be used in the production of autologous T cells. In addition, macrophages pulsed with peptide or polypeptide, or infected with recombinant virus, may be used in the preparation of autologous T cells. S. Walter et al. (Walter et al., 2003) describe the in vitro priming of T cells by using artificial antigen presenting cells (aAPCs), which is also a suitable way for generating T cells against the peptide of choice. In the present invention, aAPCs were generated by the coupling of preformed MHC:peptide complexes to the surface of polystyrene particles (microbeads) by biotin:streptavidin biochemistry. This system permits the exact control of the MHC density on aAPCs, which allows to selectively elicit high- or low-avidity antigen-specific T cell responses with high efficiency from blood samples. Apart from MHC:peptide complexes, aAPCs should carry other proteins with co-stimulatory activity like anti-CD28 antibodies coupled to their surface. Furthermore, such aAPC-based systems often require the addition of appropriate soluble factors, e. g. cytokines, like interleukin-12.

Allogeneic cells may also be used in the preparation of T cells and a method is described in detail in WO 97/26328, incorporated herein by reference. For example, in addition to *Drosophila* cells and T2 cells, other cells may be used to present antigens such as CHO cells, baculovirus-infected insect cells, bacteria, yeast, vaccinia-infected target cells. In addition plant viruses may be used (see, for example, Porta et al. (Porta et al., 1994) which describes the development of cowpea mosaic virus as a high-yielding system for the presentation of foreign peptides.

The activated T cells that are directed against the peptides of the invention are useful in therapy. Thus, a further aspect of the invention provides activated T cells obtainable by the foregoing methods of the invention.

Activated T cells, which are produced by the above method, will selectively recognize a cell that aberrantly expresses a polypeptide that comprises an amino acid sequence of SEQ ID NO: 1 to SEQ ID NO 385.

Preferably, the T cell recognizes the cell by interacting through its TCR with the HLA/peptide-complex (for example, binding). The T cells are useful in a method of killing target cells in a patient whose target cells aberrantly express a polypeptide comprising an amino acid sequence of the invention wherein the patient is administered an effective number of the activated T cells. The T cells that are administered to the patient may be derived from the patient and activated as described above (i.e. they are autologous T cells). Alternatively, the T cells are not from the patient but are from another individual. Of course, it is preferred if the individual is a healthy individual. By “healthy individual” the inventors mean that the individual is generally in good health, preferably has a competent immune system and, more preferably, is not suffering from any disease that can be readily tested for, and detected.

In vivo, the target cells for the CD8-positive T cells according to the present invention can be cells of the tumor (which sometimes express MHC class II) and/or stromal cells surrounding the tumor (tumor cells) (which sometimes also express MHC class II; (Dengjel et al., 2006)).

The T cells of the present invention may be used as active ingredients of a therapeutic composition. Thus, the invention also provides a method of killing target cells in a patient whose target cells aberrantly express a polypeptide comprising an amino acid sequence of the invention, the method comprising administering to the patient an effective number of T cells as defined above.

By ”aberrantly expressed“ the inventors also mean that the polypeptide is over-expressed compared to levels of expression in normal tissues or that the gene is silent in the tissue from which the tumor is derived but in the tumor it is expressed. By ”over-expressed“ the inventors mean that the polypeptide is present at a level at least 1.2-fold of that present in normal tissue; preferably at least 2-fold, and more preferably at least 5-fold or 10-fold the level present in normal tissue.

T cells may be obtained by methods known in the art, e.g. those described above.

Protocols for this so-called adoptive transfer of T cells are well known in the art. Reviews can be found in: Gattioni et al. and Morgan et al. (Gattinoni et al., 2006; Morgan et al., 2006).

Another aspect of the present invention includes the use of the peptides complexed with MHC to generate a T-cell receptor whose nucleic acid is cloned and is introduced into a host cell, preferably a T cell. This engineered T cell can then be transferred to a patient for therapy of cancer.

Any molecule of the invention, i.e. the peptide, nucleic acid, antibody, expression vector, cell, activated T cell, T-cell receptor or the nucleic acid encoding it, is useful for the treatment of disorders, characterized by cells escaping an immune response. Therefore, any molecule of the present invention may be used as medicament or in the manufacture of a medicament. The molecule may be used by itself or combined with other molecule(s) of the invention or (a) known molecule(s).

The present invention is further directed at a kit comprising:
(a) a container containing a pharmaceutical composition as described above, in solution or in lyophilized form;

(b) optionally a second container containing a diluent or reconstituting solution for the lyophilized formulation; and
(c) optionally, instructions for (i) use of the solution or (ii) reconstitution and/or use of the lyophilized formulation.

The kit may further comprise one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, or (v) a syringe. The container is preferably a bottle, a vial, a syringe or test tube; and it may be a multi-use container. The pharmaceutical composition is preferably lyophilized.

Kits of the present invention preferably comprise a lyophilized formulation of the present invention in a suitable container and instructions for its reconstitution and/or use. Suitable containers include, for example, bottles, vials (e.g. dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. Preferably the kit and/or container contain/s instructions on or associated with the container that indicates directions for reconstitution and/or use. For example, the label may indicate that the lyophilized formulation is to be reconstituted to peptide concentrations as described above. The label may further indicate that the formulation is useful or intended for subcutaneous administration.

The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6 administrations) of the reconstituted formulation. The kit may further comprise a second container comprising a suitable diluent (e.g., sodium bicarbonate solution).

Upon mixing of the diluent and the lyophilized formulation, the final peptide concentration in the reconstituted formulation is preferably at least 0.15 mg/mL/peptide (=75 µg) and preferably not more than 3 mg/mL/peptide (=1500 µg). The kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

Kits of the present invention may have a single container that contains the formulation of the pharmaceutical compositions according to the present invention with or without other components (e.g., other compounds or pharmaceutical compositions of these other compounds) or may have distinct container for each component.

Preferably, kits of the invention include a formulation of the invention packaged for use in combination with the co-administration of a second compound (such as adjuvants (e.g. GM-CSF), a chemotherapeutic agent, a natural product, a hormone or antagonist, an anti-angiogenesis agent or inhibitor, an apoptosis-inducing agent or a chelator) or a pharmaceutical composition thereof. The components of the kit may be pre-complexed or each component may be in a separate distinct container prior to administration to a patient. The components of the kit may be provided in one or more liquid solutions, preferably, an aqueous solution, more preferably, a sterile aqueous solution. The components of the kit may also be provided as solids, which may be converted into liquids by addition of suitable solvents, which are preferably provided in another distinct container.

The container of a therapeutic kit may be a vial, test tube, flask, bottle, syringe, or any other means of enclosing a solid or liquid. Usually, when there is more than one component, the kit will contain a second vial or other container, which allows for separate dosing. The kit may also contain another container for a pharmaceutically acceptable liquid. Preferably, a therapeutic kit will contain an apparatus (e.g., one or more needles, syringes, eye droppers, pipette, etc.), which enables administration of the agents of the invention that are components of the present kit.

The present formulation is one that is suitable for administration of the peptides by any acceptable route such as oral (enteral), nasal, ophthal, subcutaneous, intradermal, intramuscular, intravenous or transdermal. Preferably, the administration is s.c., and most preferably i.d. administration may be by infusion pump.

Since the peptides of the invention were isolated from CLL, the medicament of the invention is preferably used to treat CLL.

The present invention further relates to a method for producing a personalized pharmaceutical for an individual patient comprising manufacturing a pharmaceutical composition comprising at least one peptide selected from a warehouse of pre-screened TUMAPs, wherein the at least one peptide used in the pharmaceutical composition is selected for suitability in the individual patient. In one embodiment, the pharmaceutical composition is a vaccine. The method could also be adapted to produce T cell clones for down-stream applications, such as TCR isolations, or soluble antibodies, and other treatment options.

A “personalized pharmaceutical” shall mean specifically tailored therapies for one individual patient that will only be used for therapy in such individual patient, including actively personalized cancer vaccines and adoptive cellular therapies using autologous patient tissue.

As used herein, the term “warehouse” shall refer to a group or set of peptides that have been pre-screened for immunogenicity and/or over-presentation in a particular tumor type. The term “warehouse” is not intended to imply that the particular peptides included in the vaccine have been pre-manufactured and stored in a physical facility, although that possibility is contemplated. It is expressly contemplated that the peptides may be manufactured *de novo* for each individualized vaccine produced, or may be pre-manufactured and stored. The warehouse (e.g. in the form of a database) is composed of tumor-associated peptides which were highly overexpressed in the tumor tissue of CLL patients with various HLA-A HLA-B and HLA-C alleles. It may contain MHC class I and MHC class II peptides or elongated MHC class I peptides. In addition to the tumor associated peptides collected from several CLL tissues, the warehouse may contain

HLA-A*02 and HLA-A*24 marker peptides. These peptides allow comparison of the magnitude of T-cell immunity induced by TUMAPS in a quantitative manner and hence allow important conclusion to be drawn on the capacity of the vaccine to elicit anti-tumor responses. Secondly, they function as important positive control peptides derived from a "non-self" antigen in the case that any vaccine-induced T-cell responses to TUMAPs derived from "self" antigens in a patient are not observed. And thirdly, it may allow conclusions to be drawn, regarding the status of immunocompetence of the patient.

TUMAPs for the warehouse are identified by using an integrated functional genomics approach combining gene expression analysis, mass spectrometry, and T-cell immunology (XPresident ®). The approach assures that only TUMAPs truly present on a high percentage of tumors but not or only minimally expressed on normal tissue, are chosen for further analysis. For initial peptide selection, CLL samples from patients and blood from healthy donors were analyzed in a stepwise approach:

1. HLA ligands from the malignant material were identified by mass spectrometry
2. Genome-wide messenger ribonucleic acid (mRNA) expression analysis was used to identify genes over-expressed in the malignant tissue (CLL) compared with a range of normal organs and tissues
3. Identified HLA ligands were compared to gene expression data. Peptides over-presented or selectively presented on tumor tissue, preferably encoded by selectively expressed or over-expressed genes as detected in step 2 were considered suitable TUMAP candidates for a multi-peptide vaccine.
4. Literature research was performed in order to identify additional evidence supporting the relevance of the identified peptides as TUMAPs
5. The relevance of over-expression at the mRNA level was confirmed by redetection of selected TUMAPs from step 3 on tumor tissue and lack of (or infrequent) detection on healthy tissues.
6. In order to assess, whether an induction of *in vivo* T-cell responses by the selected peptides may be feasible, in vitro immunogenicity assays were performed using human T cells from healthy donors as well as from CLL patients.

In an aspect, the peptides are pre-screened for immunogenicity before being included in the warehouse. By way of example, and not limitation, the immunogenicity of the peptides included in the warehouse is determined by a method comprising *in vitro* T-cell priming through repeated stimulations of CD8+ T cells from healthy donors with artificial antigen presenting cells loaded with peptide/MHC complexes and anti-CD28 antibody.

This method is preferred for rare cancers and patients with a rare expression profile. In contrast to multi-peptide cocktails with a fixed composition as currently developed, the warehouse allows a significantly higher matching of the actual expression of antigens in the tumor with the vaccine. Selected single or combinations of several “off-the-shelf” peptides will be used for each patient in a multitarget approach. In theory, an approach based on selection of e.g. 5 different antigenic peptides from a library of 50 would already lead to approximately 17 million possible drug product (DP) compositions.

In one aspect, the peptides are selected for inclusion in the vaccine based on their suitability for the individual patient based on the method according to the present invention as described herein, or as below.

The HLA phenotype, transcriptomic and peptidomic data is gathered from the patient’s tumor material, and blood samples to identify the most suitable peptides for each patient containing “warehouse” and patient-unique (i.e. mutated) TUMAPs. Those peptides will be chosen, which are selectively or over-expressed in the patients’ tumor and, where possible, show strong *in vitro* immunogenicity if tested with the patients’ individual PBMCs.

Preferably, the peptides included in the vaccine are identified by a method comprising: (a) identifying tumor-associated peptides (TUMAPs) presented by a tumor sample from the individual patient; (b) comparing the peptides identified in (a) with a warehouse (database) of peptides as described above; and (c) selecting at least one peptide from the warehouse (database) that correlates with a tumor-associated peptide identified in the patient. For example, the TUMAPs presented by the tumor sample are identified by:

(a1) comparing expression data from the tumor sample to expression data from a sample of normal tissue corresponding to the tissue type of the tumor sample to identify proteins that are over-expressed or aberrantly expressed in the tumor sample; and (a2) correlating the expression data with sequences of MHC ligands bound to MHC class I and/or class II molecules in the tumor sample to identify MHC ligands derived from proteins over-expressed or aberrantly expressed by the tumor. Preferably, the sequences of MHC ligands are identified by eluting bound peptides from MHC molecules isolated from the tumor sample, and sequencing the eluted ligands. Preferably, the tumor sample and the normal tissue are obtained from the same patient.

In addition to, or as an alternative to, selecting peptides using a warehousing (database) model, TUMAPs may be identified in the patient *de novo*, and then included in the vaccine. As one example, candidate TUMAPs may be identified in the patient by (a1) comparing expression data from the tumor sample to expression data from a sample of normal tissue corresponding to the tissue type of the tumor sample to identify proteins that are over-expressed or aberrantly expressed in the tumor sample; and (a2) correlating the expression data with sequences of MHC ligands bound to MHC class I and/or class II molecules in the tumor sample to identify MHC ligands derived from proteins over-expressed or aberrantly expressed by the tumor. As another example, proteins may be identified containing mutations that are unique to the tumor sample relative to normal corresponding tissue from the individual patient, and TUMAPs can be identified that specifically target the mutation. For example, the genome of the tumor and of corresponding normal tissue can be sequenced by whole genome sequencing: For discovery of non-synonymous mutations in the protein-coding regions of genes, genomic DNA and RNA are extracted from tumor tissues and normal non-mutated genomic germline DNA is extracted from peripheral blood mononuclear cells (PBMCs). The applied NGS approach is confined to the re-sequencing of protein coding regions (exome re-sequencing). For this purpose, exonic DNA from human samples is captured using vendor-supplied target enrichment kits, followed by sequencing with e.g. a HiSeq2000 (Illumina). Additionally, tumor mRNA is sequenced for direct quantification of gene expression and validation that mutated genes are expressed in the patients' tumors. The resultant millions of sequence reads are processed through software algorithms. The output list contains mutations and gene expression. Tumor-specific somatic mutations are determined by comparison with the PBMC-derived germline variations and prioritized. The *de novo* identified peptides can then be tested for immunogenicity as described above for the warehouse, and candidate TUMAPs possessing suitable immunogenicity are selected for inclusion in the vaccine.

In one exemplary embodiment, the peptides included in the vaccine are identified by: (a) identifying tumor-associated peptides (TUMAPs) presented by a tumor sample from the individual patient by the method as described above; (b) comparing the peptides identified in a) with a warehouse of peptides that have been prescreened for immunogenicity and overpresentation in tumors as compared to corresponding normal tissue; (c) selecting at least one peptide from the warehouse that correlates with a tumor-associated peptide identified in the patient; and (d) optionally, selecting at least one peptide identified de novo in (a) confirming its immunogenicity.

In one exemplary embodiment, the peptides included in the vaccine are identified by: (a) identifying tumor-associated peptides (TUMAPs) presented by a tumor sample from the individual patient; and (b) selecting at least one peptide identified de novo in (a) and confirming its immunogenicity.

Once the peptides for a personalized peptide based vaccine are selected, the vaccine is produced. The vaccine preferably is a liquid formulation consisting of the individual peptides dissolved in between 20-40% DMSO, preferably about 30-35% DMSO, such as about 33% DMSO.

Each peptide to be included into a product is dissolved in DMSO. The concentration of the single peptide solutions has to be chosen depending on the number of peptides to be included into the product. The single peptide-DMSO solutions are mixed in equal parts to achieve a solution containing all peptides to be included in the product with a concentration of ~2.5 mg/ml per peptide. The mixed solution is then diluted 1:3 with water for injection to achieve a concentration of 0.826 mg/ml per peptide in 33% DMSO. The diluted solution is filtered through a 0.22 µm sterile filter. The final bulk solution is obtained.

Final bulk solution is filled into vials and stored at -20°C until use. One vial contains 700 µL solution, containing 0.578 mg of each peptide. Of this, 500 µL (approx. 400 µg per peptide) will be applied for intradermal injection.

In addition to being useful for treating cancer, the peptides of the present invention are also useful as diagnostics. Since the peptides were generated from CLL cells and since it was determined that these peptides are not or at lower levels present in normal tissues, these peptides can be used to diagnose the presence of a cancer.

The presence of claimed peptides on tissue biopsies in blood samples can assist a pathologist in diagnosis of cancer. Detection of certain peptides by means of antibodies, mass spectrometry or other methods known in the art can tell the pathologist that the tissue sample is malignant or inflamed or generally diseased, or can be used as a biomarker for CLL. Presence of groups of peptides can enable classification or sub-classification of diseased tissues.

The detection of peptides on diseased tissue specimen can enable the decision about the benefit of therapies involving the immune system, especially if T-lymphocytes are known or expected to be involved in the mechanism of action. Loss of MHC expression is a well described mechanism by which infected of malignant cells escape immuno-surveillance. Thus, presence of peptides shows that this mechanism is not exploited by the analyzed cells.

The peptides of the present invention might be used to analyze lymphocyte responses against those peptides such as T cell responses or antibody responses against the peptide or the peptide complexed to MHC molecules. These lymphocyte responses can be used as prognostic markers for decision on further therapy steps. These responses can also be used as surrogate response markers in immunotherapy approaches aiming to induce lymphocyte responses by different means, e.g. vaccination of protein, nucleic acids, autologous materials, adoptive transfer of lymphocytes. In gene therapy settings, lymphocyte responses against peptides can be considered in the assessment of side effects. Monitoring of lymphocyte responses might also be a valuable tool for follow-up examinations of transplantation therapies, e.g. for the detection of graft versus host and host versus graft diseases.

The present invention will now be described in the following examples which describe preferred embodiments thereof, and with reference to the accompanying figures, nevertheless, without being limited thereto. For the purposes of the present invention, all references as cited herein are incorporated by reference in their entireties.

FIGURES

Figure 1A to V show the overpresentation of various peptides in normal tissues (white bars) and CLL (black bars). Figure 1A) Gene: IGHM, Peptide: ALHRPDVYL (SEQ ID NO.: 2). Tissues from left to right: 2 adipose tissues, 3 adrenal glands, 4 blood cells, 10 blood vessels, 6 bone marrows, 7 brains, 6 breasts, 2 cartilages, 2 eyes, 3 gallbladders, 6 hearts, 14 kidneys, 19 large intestines, 20 livers, 45 lungs, 6 lymph nodes, 7 nerves, 3 ovaries, 10 pancreases, 3 parathyroid glands, 1 peritoneum, 5 pituitary glands, 6 placentas, 3 pleuras, 3 prostates, 7 salivary glands, 5 skeletal muscles, 6 skins, 4 small intestines, 9 spleens, 5 stomachs, 6 testes, 2 thymi, 3 thyroid glands, 9 tracheas, 3 ureters, 6 urinary bladders, 2 uteri, 6 esophagi, 17 chronic lymphocytic leukemia samples. The peptide has additionally been detected on 2/21 non-Hodgkin lymphoma samples. Figure 1B) Gene: IGHM, Peptide: VIAELPPKV (SEQ ID NO.: 3). Tissues from left to right: 2 adipose tissues, 3 adrenal glands, 4 blood cells, 10 blood vessels, 6 bone marrows, 7 brains, 6 breasts, 2 cartilages, 2 eyes, 3 gallbladders, 6 hearts, 14 kidneys, 19 large intestines, 20 livers, 45 lungs, 6 lymph nodes, 7 nerves, 3 ovaries, 10 pancreases, 3 parathyroid glands, 1 peritoneum, 5 pituitary glands, 6 placentas, 3 pleuras, 3 prostates, 7 salivary glands, 5 skeletal muscles, 6 skins, 4 small intestines, 9 spleens, 5 stomachs, 6 testes, 2 thymi, 3 thyroid glands, 9 tracheas, 3 ureters, 6 urinary bladders, 2 uteri, 6 esophagi, 17 chronic lymphocytic leukemia samples. The peptide has additionally been detected on 5/21 non-Hodgkin lymphoma samples and 1/90 lung cancers. Figure 1C), Gene: IGHM, Peptide: VIAELPPKVSV (SEQ ID No.: 4), Tissues from left to right: 2 adipose tissues, 3 adrenal glands, 4 blood cells, 10 blood vessels, 6 bone marrows, 7 brains, 6 breasts, 2 cartilages, 2 eyes, 3 gallbladders, 6 hearts, 14 kidneys, 19 large intestines, 20 livers, 45 lungs, 6 lymph nodes, 7 nerves, 3 ovaries, 10 pancreases, 3 parathyroid glands, 1 peritoneum, 5 pituitary glands, 6 placentas, 3 pleuras, 3 prostates, 7 salivary glands, 5 skeletal muscles, 6 skins, 4 small intestines, 9 spleens, 5 stomachs, 6 testes, 2 thymi, 3 thyroid glands, 9 tracheas, 3 ureters, 6 urinary bladders, 2 uteri, 6 esophagi, 17 chronic lymphocytic leukemia samples. Figure 1D), Gene: NUP210, Peptide: RLYEITIEV (SEQ ID No.: 121). Samples from left to right: 1 PBMC culture, 1 benign prostate, 8 normal tissues (3 lungs, 4 spleens, 1 trachea), 49 cancer tissues (2 brain cancers, 2 breast cancers, 1 colon cancer, 13 leukocytic leukemia cancers, 13 lung cancers, 8 lymph node cancers, 1 myeloid cells cancer, 4 ovarian cancers, 2 skin cancers, 1 urinary bladder cancer, 2 uterus cancers). Discrepancies regarding the list of tumor types between figure 1D and table 4 may be due to the more stringent selection criteria applied in table 4 (for details please refer to table 4). The normal tissue panel and the cancer cell lines and xenografts tested were the same as in Figure 1A-C. Figure 1E), Gene: COBRA1, Peptide: ALLRLLPGL (SEQ ID No.: 14). Samples from left to right: 1 cell line, 11 cancer tissues (1 breast cancer, 1 colon cancer, 1 head and neck cancer, 2 leukocytic leukemia cancers, 1 liver cancer, 1 lung cancer, 2 lymph node cancers, 1 myeloid cell cancer, 1 skin cancer). Figure 1F), Gene: KIAA0226L, Peptide: GIIDGSPRL (SEQ ID No.: 62). Samples from left to right: 2 normal tissues (1 lymph node, 1 spleen), 18 cancer tissues (2 colon cancers, 9 leukocytic leukemia cancers, 6 lymph node cancers, 1 rectum cancer). Figure 1G), Gene: DDX3X, Peptide: GLDQQFAGLDL (SEQ ID No.: 68). Samples from left to right: 1 cell line, 2 primary cultures, 21 cancer tissues (1 bile duct cancer, 1 brain cancer, 1 breast cancer, 1 head and neck cancer, 2 kidney cancers, 3 leukocytic leukemia cancers, 2 liver cancers, 2 lung cancers, 2 lymph node cancers, 3 ovarian cancers, 1 rectum cancer, 1 skin cancer, 1 uterus cancer). Figure 1H), Gene: LIG1, Peptide: KTLDVDATYEI (SEQ ID No.: 112). Samples from left to right: 2 cell lines, 1 primary culture, 13 cancer tissues (1 esophageal cancer, 2 leukocytic leukemia cancers, 1 liver cancer, 3 lung cancers, 2 lymph node cancers, 2 ovarian cancers, 1 skin cancer, 1 uterus cancer). Figure 1I), Gene: SMCHD1, Peptide: SIIEGPIIKL (SEQ ID No.: 146). Samples from left to right: 14 cancer tissues (1 head and neck cancer, 3 leukocytic leukemia cancers, 6 lung cancers, 1 lymph node cancer, 1 ovarian cancer, 2 urinary bladder cancers). Figure 1J), Gene: MMS22L, Peptide: SILETVATL (SEQ ID No.: 147). Samples from left to right: 1 primary culture, 11 cancer tissues (1 colon cancer, 4 leukocytic leukemia cancers, 1 lung cancer, 3 lymph node cancers, 1 myeloid cell cancer, 1 skin cancer). Figure 1K), Gene: FCRL3, Peptide: SLLAELHVL (SEQ ID No.: 153). Samples from left to right: 1 cell line, 9 normal tissues (1 lymph node, 1 rectum, 7 spleens), 27 cancer tissues (1 breast cancer, 1 kidney cancer, 10 leukocytic leukemia cancers, 14 lymph node cancers, 1 skin cancer). Figure 1L), Gene: FAM126A, Peptide: SLQEEKLIYV (SEQ ID No.: 159). Samples from left to right: 2 cell lines, 23 cancer tissues (5 brain cancers, 1 breast cancer, 1 esophageal cancer, 3 head and neck cancers, 2 leukocytic leukemia cancers, 5 lung cancers, 3 lymph node cancers, 1 skin cancer, 2 urinary bladder cancers). Figure 1M), Gene: HNRNPC, Peptide: SVHKGFAFV (SEQ ID No.: 164). Samples from left to right: 10 cell lines, 1 primary culture, 1 normal tissue (1 adrenal gland), 36 cancer tissues (12 brain cancers, 1 colon cancer, 2 head and neck cancers, 2 kidney cancers, 3 leukocytic leukemia cancers, 1 liver cancer, 5 lung cancers, 1 myeloid cell cancer, 1 ovarian cancer, 1 rectum cancer, 1 skin cancer, 2 stomach cancers, 2 urinary bladder cancers, 2 uterus cancers). Figure 1N), Gene: RASGRF1, Peptide: TLDTSKLYV (SEQ ID No.: 167). Samples from left to right: 13 cancer tissues (1 brain cancer, 8 leukocytic leukemia cancers, 4 lymph node cancers). Figure 1O), Gene: RASGRF1, Peptide: YLLDQSFVM (SEQ ID No.: 168). Samples from left to right: 16 cancer tissues (10 leukocytic leukemia cancers, 6 lymph node cancers). Figure 1P), Gene: RNF213, Peptide: YIQEYLTLL (SEQ ID No.: 192). Samples from left to right: 2 cell lines, 18 cancer tissues (1 colon cancer, 1 head and neck cancer, 1 kidney cancer, 4 leukocytic leukemia cancers, 5 lung cancers, 3 lymph node cancers, 1 myeloid cell cancer, 1 stomach cancer, 1 urinary bladder cancer). Figure 1Q), Gene: DCK, Peptide: YLQEVPILTL (SEQ ID No.: 197). Samples from left to right: 11 cancer tissues (5 leukocytic leukemia cancers, 4 lymph node cancers, 1 skin cancer, 1 uterus cancer). Figure 1R), Gene: EIF3H, Peptide: YMFEEVPIVI (SEQ ID No.: 200). Samples from left to right: 4 cell lines, 2 primary cultures, 34 cancer tissues (1 bile duct cancer, 2 breast cancers, 1 colon cancer, 1 esophageal cancer, 1 gallbladder cancer, 5 head and neck cancers, 2 leukocytic leukemia cancers, 2 liver cancers, 9 lung cancers, 4 lymph node cancers, 2 ovarian cancers, 1 rectum cancer, 2 skin cancers, 1 urinary bladder cancer). Figure 1S), Gene: UBLCP1, Peptide: LIDVKPLGV (SEQ ID No.: 211). Samples from left to right: 3 cell lines, 23 cancer tissues (5 brain cancers, 1 breast cancer, 1 esophageal cancer, 1 head and neck cancer, 1 kidney cancer, 3 leukocytic leukemia cancers, 2 liver cancers, 2 lung cancers, 4 lymph node cancers, 1 ovarian cancer, 1 prostate cancer, 1 skin cancer). Figure 1T), Gene: TMCO6, Peptide: QLLPVSNVVSV (SEQ ID No.: 256). Samples from left to right: 2 primary cultures, 13 cancer tissues (2 head and neck cancers, 3 leukocytic leukemia cancers, 1 liver cancer, 2 lung cancers, 4 lymph node cancers, 1 ovarian cancer). Figure 1U), Gene: MYO1G, Peptide: LLYNSTDPTL (SEQ ID No.: 312). Samples from left to right: 1 cell line, 2 primary cultures, 14 cancer tissues (1 colon cancer, 4 leukocytic leukemia cancers, 2 lung cancers, 5 lymph node cancers, 1 ovarian cancer, 1 urinary bladder cancer). Figure 1V), Gene: PIM1, Peptide: VLLPQETAEIHL (SEQ ID No.: 328). Samples from left to right: 3 primary cultures, 27 cancer tissues (1 breast cancer, 1 colon cancer, 5 leukocytic leukemia cancers, 5 lung cancers, 7 lymph node cancers, 2 ovarian cancers, 2 skin cancers, 1 stomach cancer, 3 urinary bladder cancers).

Figures 2A to D show exemplary expression profiles of source genes of the present invention that are highly over-expressed or exclusively expressed in CLL in a panel of normal tissues (white bars) and 17 CLL samples (black bars). Tissues from left to right: 6 arteries, 1 blood cells, 1 brain, 1 heart, 2 livers, 2 lungs, 2 veins, 1 adipose tissue, 1 adrenal gland, 6 bone marrows, 1 cartilage, 1 colon, 1 esophagus, 2 eyes, 2 gallbladders, 1 kidney, 6 lymph nodes, 5 pancreases, 2 pituitary glands, 1 rectum, 1 salivary gland, 1 skeletal muscle, 1 skin, 1 small intestine, 1 spleen, 1 stomach, 1 thyroid gland, 7 tracheas, 1 urinary bladder, 1 breast, 5 ovaries, 3 placentas, 1 prostate, 1 testis, 1 thymus, 1 uterus, 10 chronic lymphocytic leukemia samples. Figure 2A) Gene symbol: FCER2; Figure 2B) Gene symbol: KIAA0226L; Figure 2C) Gene symbol: PAX5; Figure 2D) Gene symbol: CLEC17A.

Figure 3 shows exemplary immunogenicity data: flow cytometry results after peptide-specific multimer staining. CD8+ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*02 in complex with SeqID No 1 peptide (C, left panel), SeqID No 5 peptide (D left panel), SeqID No 32 peptide (E, left panel) and SeqID No 220 peptide (F, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*02/SeqID No 1 (C), A*02/SeqID No 5 (D), A*02/SeqID No 32 (E) or A*02/SeqID No 220 (F). Right panels (C, D, E and F) show control staining of cells stimulated with irrelevant A*02/peptide complexes. Viable singlet cells were gated for CD8+ lymphocytes. Boolean gates helped excluding false-positive events detected with multimers specific for different peptides. Frequencies of specific multimer+ cells among CD8+ lymphocytes are indicated.

EXAMPLES

EXAMPLE 1

Identification and quantitation of tumor associated peptides presented on the cell surface

Tissue samples

Patients' tumor tissues were obtained from: ProteoGenex Inc. (Culver City, CA, USA); University Hospital Bonn (Bonn, Germany); University Hospital Tübingen (Tübingen, Germany).

Normal tissues were obtained from Asterand (Detroit, MI, USA & Royston, Herts, UK); Bio-Options Inc. (Brea, CA, USA); BioServe (Beltsville, MD, USA); Capital BioScience Inc. (Rockville, MD, USA); Geneticist Inc. (Glendale, CA, USA); Kyoto Prefectural University of Medicine (KPUM) (Kyoto, Japan); ProteoGenex Inc. (Culver City, CA, USA); Tissue Solutions Ltd (Glasgow, UK); University Hospital Geneva (Geneva, Switzerland); University Hospital Heidelberg (Heidelberg, Germany); University Hospital Munich (Munich, Germany); University Hospital Tübingen (Tübingen, Germany).

Written informed consents of all patients had been given before surgery or autopsy. Tissues were shock-frozen immediately after excision and stored until isolation of TUMAPs at -70°C or below.

Isolation of HLA peptides from tissue samples

HLA peptide pools from shock-frozen tissue samples were obtained by immune precipitation from solid tissues according to a slightly modified protocol (Falk et al., 1991; Seeger et al., 1999) using the HLA-A*02-specific antibody BB7.2, the HLA-A, -B, -C-specific antibody W6/32, CNBr-activated sepharose, acid treatment, and ultrafiltration.

Mass spectrometry analyses

The HLA peptide pools as obtained were separated according to their hydrophobicity by reversed-phase chromatography (nanoAcquity UPLC system, Waters) and the eluting peptides were analyzed in LTQ- velos and fusion hybrid mass spectrometers (ThermoElectron) equipped with an ESI source. Peptide pools were loaded directly onto the analytical fused-silica micro-capillary column (75 µm i.d. x 250 mm) packed with 1.7 µm C18 reversed-phase material (Waters) applying a flow rate of 400 nL per minute. Subsequently, the peptides were separated using a two-step 180 minute-binary gradient from 10% to 33% B at a flow rate of 300 nL per minute. The gradient was composed of Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A gold coated glass capillary (PicoTip, New Objective) was used for introduction into the nanoESI source. The LTQ-Orbitrap mass spectrometers were operated in the data-dependent mode using a TOP5 strategy. In brief, a scan cycle was initiated with a full scan of high mass accuracy in the orbitrap (R = 30 000), which was followed by MS/MS scans also in the orbitrap (R = 7500) on the 5 most abundant precursor ions with dynamic exclusion of previously selected ions. Tandem mass spectra were interpreted by SEQUEST and additional manual control. The identified peptide sequence was assured by comparison of the generated natural peptide fragmentation pattern with the fragmentation pattern of a synthetic sequence-identical reference peptide.

Label-free relative LC-MS quantitation was performed by ion counting i.e. by extraction and analysis of LC-MS features (Mueller et al., 2007). The method assumes that the peptide’s LC-MS signal area correlates with its abundance in the sample. Extracted features were further processed by charge state deconvolution and retention time alignment (Mueller et al., 2008; Sturm et al., 2008). Finally, all LC-MS features were cross-referenced with the sequence identification results to combine quantitative data of different samples and tissues to peptide presentation profiles. The quantitative data were normalized in a two-tier fashion according to central tendency to account for variation within technical and biological replicates. Thus each identified peptide can be associated with quantitative data allowing relative quantification between samples and tissues. In addition, all quantitative data acquired for peptide candidates was inspected manually to assure data consistency and to verify the accuracy of the automated analysis. For each peptide, a presentation profile was calculated showing the mean sample presentation as well as replicate variations. The profiles juxtapose CLL samples to a baseline of normal tissue samples. Presentation profiles of exemplary over-presented peptides are shown in Figure 1. Presentation scores for exemplary peptides are shown in Table 8.

Table 8: Presentation scores. The table lists peptides that are very highly over-presented on tumors compared to a panel of normal tissues (+++), highly over-presented on tumors compared to a panel of normal tissues (++) or over-presented on tumors compared to a panel of normal tissues (+).The panel of normal tissues considered relevant for comparison with tumors consisted of: adipose tissue, adrenal gland, blood cells, blood vessel, bone marrow, brain, breast, esophagus, eye, gallbladder, heart, kidney, large intestine, liver, lung, lymph node, nerve, pancreas, parathyroid gland, peritoneum, pituitary, pleura, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thymus, thyroid gland, trachea, ureter, urinary bladder.

SEQ ID No.	Sequence	Peptide Presentation
1	AIPPSFASIFL	+++
2	ALHRPDVYL	+++
3	VIAELPPKV	+++
4	VIAELPPKVSV	+++
5	ALIFKIASA	+++
6	ALDTLEDDMTI	+++
7	ALLERTGYTL	+++
8	ALAASALPALV	+++
9	ALCDTLITV	+++
10	FVYGESVEL	+++
11	ALFTFJPLTV	+++
12	ALGEDEITL	+++
13	VVDGMPPGV	++
14	ALLRLLPGL	+
15	ALPEVSVEA	+
16	ALPGGAAVAAV	++
17	ALTKTNLQL	+++
18	LLGEFSIKM	+++
19	QVMEKLAAV	+
20	ALVDPGPDFVV	+
21	ALWAGLLTL	+++
22	ALWDPVIEL	+++
23	ALYLTEVFL	+++
24	AMAGDVVYA	+++
25	ATYSGLESQSV	+
27	AVLGLVWLL	++
28	AVLHLLLSV	+++
29	AVLQAVTAV	+++
30	ELLEGSEIYL	+
31	ELMHGVAGV	++
32	FIDKFTPPV	+++
33	FIINSSNIFL	+++
35	FILPSSLYL	+++
36	FILTHVDQL	+++
37	FIMEGGAMVL	+++
39	FLDALLTDV	+++
40	FLDEDDMSL	+
41	FLDPSLDPLL	+++
43	FLLGPEALSFA	+++

45	FLPELPADLEA	++
47	FLSDQPEPYL	++
48	FLSPQQPPLL	+++
49	FLTDLFAQL	+
50	FLFEPVVKAFL	+++
51	FLVEAPHDWDL	++
52	FLVETGFLHV	+++
53	FLWQHVELVL	+++
54	FLYPFPLALF	+++
56	FVFEAPYTL	+++
57	GLSEISLRL	+++
58	YIQQGIFSV	++
59	FVFGDENGTVSL	+++
60	FVLDHEDGLNL	++
61	FVYFIVREV	+++
62	GIIDGSPRL	+
63	SLAHVAGCEL	+++
64	KLLESVASA	++
65	GLDDMKANL	+++
66	SLAGGLDDMKA	+++
68	GLDQQFAGLDL	+++
69	GLHQREIFL	+++
70	FVPDTPVGV	++
71	GLKHDIARV	+++
72	GLLDAGKMYV	+++
73	GLLEVISALQL	+++
74	GLLRASFLL	+++
76	GLLRIIPYL	++
77	GLLRLTWFL	++
78	GLPSFLTEV	+++
79	GLQAKIQEA	+++
80	VLIEDELEEL	+++
81	WLVGQEFEL	+++
82	GLQSGVDIGV	+
83	GQGEVLVYV	+
86	HLYPGAVTI	+++
88	ILDEIGADVQA	+++
89	ILDFGTFQL	+++
90	VIADLGLIRV	+++
92	ILEPLNPLL	+++
93	ILFNTQINI	+++

94	ILFPLRFTL	+++
95	ILGYMAHEHKV	+++
96	ILIDKTSFV	+
97	LLFATQITL	+
98	SLIKYFLFV	+++
99	ILIFHSVAL	+++
100	ILNNEVFAI	+
101	ILVVIEPLL	+++
102	IQDRAVPSL	+++
103	KLGGTPAPA	++
104	KLILLDTPLFL	+++
105	KLMNDIADI	+++
106	FMASHLDYL	+++
107	ILYNLYDLL	++
108	VIYTLIHYI	+
109	KLWEGLTELV	++
112	KTLDVDATYEI	+++
113	KVPAEEVLVAV	+
114	LIPEGPPQV	+++
115	LLFDKLYLL	+++
116	LLIGATMQV	+++
117	LLILENILL	++
118	RLLILENILL	+++
119	VLPAEFFEV	+++
121	RLYEITIEV	++
122	LLIPVVPGV	++
123	LLLAEAELLTL	+++
124	LLLEETEKQAV	++
125	LLLEIGEVGKLFV	++
126	LLPEGGITAI	+++
127	LLPTAPTTV	+++
128	LLSEEEYHL	+++
129	LLVGTLDVV	+
130	LLVLIPVYL	+++
131	LQALEVLKI	+++
132	LVYEAIIMV	+++
133	YLLSGDISEA	+++
134	MLLEHGITLV	+++
135	MTAGFSTIAGSV	+++
137	NLIKTVIKL	+
138	NLLDIDAPVTV	++

139	NLTDVVEKL	+++
140	QIAELPATSV	+++
141	QILSEIVEA	++
142	QLDEPAPQV	+++
144	QLPPFPREL	+++
145	SALDTITTV	+++
146	SIIEGPIIKL	+++
147	SILETVATL	+++
148	SIVASLITV	+++
149	SLDNGGYYI	+++
153	SLLAELHVL	++
154	SLLAELHVLTV	++
155	SLMLEVPAL	+++
157	SLNIRDFTM	+++
160	SLSFLVPSL	+++
162	SMKDDLENV	+++
163	SQLDISEPYKV	+
164	SVHKGFAFV	+++
165	TLDDDLDTV	+++
166	TLDPNQVSL	+++
167	TLDTSKLYV	+++
168	YLLDQSFVM	+++
169	TLLLGLTEV	+++
170	TLTFRVETV	+++
171	TLVPPAALISI	+++
172	TLYDMLASI	+++
174	VLAELPIIVV	+++
175	FTVPRVVAV	++
176	VLAEQNIIPSA	+++
177	VLDDRELLL	+++
178	VLFFNVQEV	+++
179	VLLGLEMTL	+++
181	VLLSIPFVSV	+
182	VLLSVPGPPV	+++
185	VMDDQRDLI	+
186	VMDPTKILI	+++
187	VMDTHLVNI	+++
189	VMLEMTPEL	+++
190	VVMGTVPRL	+++
191	YIFDGSDGGV	+++
192	YIQEYLTLL	+++

193	YLDLSNNRL	+++
194	YLDNVLAEL	+
195	YLGGFALSV	+++
197	YLQEVPILTL	+++
198	YLTFLPAEV	+++
199	YLVELSSLL	+++
200	YMFEEVPIVI	+++
201	YQLELHGIEL	+++
202	YVDDVFLRV	+++
204	GLLQINDKIAL	++
205	GLSQANFTL	+
206	HMQDVRVLL	+++
208	ILLKTEGINL	++
209	ILQAELPSL	++
210	KLLVQDFFL	+++
211	LIDVKPLGV	+++
212	NIIEAINELLV	++
213	RLLYQLVFL	++
214	RLQELTEKL	+
215	VMQDIVYKL	+
217	ALDEPPYLTV	+
218	ALGEEWKGYVV	+
219	ALLNLLESA	++
221	ALVSTIIMV	+
225	ILQERELLPV	+
226	AMNISVPQV	+
227	FLAEASVMTQL	++
228	FLGGLSPGV	++
229	FLLNLQNCHL	++
231	FLYIRQLAI	+
232	FMHQIIDQV	+
234	GLDDAEYAL	+
235	GLDDLLLFL	+
236	GLLESGRHYL	+++
237	GLQENLDVVV	+
238	GLVETELQL	+
240	ILARDILEI	++
241	ILGDILLKV	+
242	ILLGIQELL	++
243	ILPTLEKELFL	+
244	ILQALAVHL	++

245	KIMDYSLLLGV	+
248	KTVEPPISQV	+
249	LLPTGVFQV	+
251	LLYDNVPGA	+
254	QLIPKLIFL	+++
255	YLFEEAISM	+
258	RLDYITAEI	++
259	RLLDEQFAVL	+
260	SLDDVEGMSV	++
261	SLVEAQGWLV	++
263	SQWEDIHVV	++
264	TILDYINVV	+++
266	TLLDQLDTQL	+
267	TLLDWQDSL	+
268	TLLQVFHLL	++
270	TVLPVPPLSV	+++
271	VIRNIVEAA	++
273	VLGEYSYLL	+
275	VLLFIEHSV	+
276	VLNDGAPNV	+
277	VMILKLPFL	+
278	YLDDLLPKL	++
280	YTLDSLYWSV	+
281	NLLDDRGMTAL	+++
282	LLRDGIELV	+++
283	ILQPMDIHV	+++
284	LLSAAEPVPA	+++
286	FLLEDLSQKL	+++
287	FLWEEKFNSL	+++
289	ILEEQPMDMLL	+++
290	LANPHELSL	+++
291	ILLNEDDLVTI	+++
292	AAALIIHHV	+
294	ALLDQLHTLL	+
297	FLVEPQEDTRL	++
298	IILPVEVEV	+++
299	ILEENIPVL	++
300	ILLNPAYDVYL	++
302	ILQDLTFVHL	+
304	LAIVPVNTL	+++
305	LLFPQIEGIKI	+

307	LLLTKPTEA	++
308	SLYDVSRMYV	+++
309	ILYGTQFVL	+
310	LLSTLHLLV	+
311	LLVDVEPKV	+
312	LLYNSTDPTL	+++
314	LMKDCEAEV	++
316	MLLEHGITL	+++
317	NLLAHIWAL	+
318	NLQVTQPTV	+++
320	TIAPVTVAV	+
322	SLASIHVPL	+++
323	SLDLFNCEV	+++
327	VLIKWFPEV	+++
328	VLLPQETAEIHL	++
329	VLMDGSVKL	+
330	VLMWEIYSL	++
331	VLWELAHLPTL	+++
332	VMIQHVENL	+++
334	YLLEEKIASL	+++
336	YMAVTTQEV	+++
337	YMYEKESEL	++
338	FLDMTNWNL	+
339	GLWGTVVNI	+
340	KLLEEICNL	++
341	LLAELPASVHA	++
342	SLITPLQAV	+++
343	TLLEALDCI	++
344	VLAFENPQV	++
345	YLIEPDVELQRI	+
346	VLVQVSPSL	+++
347	YLGPVSPSL	+
349	ALATHILSL	++
350	ALEDRVWEL	+
351	ALSEKLARL	+
352	ALVFELHYV	+
353	ATPMPTPSV	++
354	FIMDDPAGNSYL	+
355	FIWPMLIHI	+++
356	FLHDHQAEL	+
357	FLIQEIKTL	++

358	FLTDYLNDL	+
359	FMQDPMEVFV	++
360	HLIDTNKIQL	+
362	ILTELGGFEV	+
363	ITTEVVNELYV	+
364	KMDWIFHTI	+
365	LISPLLLPV	++
367	NLWSLVAKV	++
368	QLQPTDALLCV	+
371	SLADDSVLERL	+
372	SLFGPLPGPGPALV	+
374	VLSVITEEL	++
375	VLWFKPVEL	++
377	VVDGTCVAV	+
378	YILGKFFAL	+
379	YLAELVTPIL	+
380	YLDRKLLTL	+
382	YLLPLLQRL	+++
383	YLLREWVNL	+++
384	YMIGSEVGNYL	+
385	YTIPLAIKL	+++

EXAMPLE 2

Expression profiling of genes encoding the peptides of the invention

Over-presentation or specific presentation of a peptide on tumor cells compared to normal cells is sufficient for its usefulness in immunotherapy, and some peptides are tumor-specific despite their source protein occurring also in normal tissues. Still, mRNA expression profiling adds an additional level of safety in selection of peptide targets for immunotherapies. Especially for therapeutic options with high safety risks, such as affinity-matured TCRs, the ideal target peptide will be derived from a protein that is unique to the tumor and not found on normal tissues.

RNA sources and preparation

Surgically removed tissue specimens were provided as indicated above (see Example 1) after written informed consent had been obtained from each patient. Tumor tissue specimens were snap-frozen immediately after surgery and later homogenized with mortar and pestle under liquid nitrogen. Total RNA was prepared from these samples using TRI Reagent (Ambion, Darmstadt, Germany) followed by a cleanup with RNeasy (QIAGEN, Hilden, Germany); both methods were performed according to the manufacturer's protocol.

Total RNA from healthy human tissues for RNASeq experiments was obtained from: Asterand (Detroit, MI, USA & Royston, Herts, UK); BioCat GmbH (Heidelberg, Germany); BioServe (Beltsville, MD, USA); Geneticist Inc. (Glendale, CA, USA); Istituto Nazionale Tumori "Pascale" (Naples, Italy); ProteoGenex Inc. (Culver City, CA, USA); University Hospital Heidelberg (Heidelberg, Germany).

Total RNA from tumor tissues for RNASeq experiments was obtained from: Tissue Solutions Ltd (Glasgow, UK); University Hospital Bonn (Bonn, Germany)

Quality and quantity of all RNA samples were assessed on an Agilent 2100 Bioanalyzer (Agilent, Waldbronn, Germany) using the RNA 6000 Pico LabChip Kit (Agilent).

RNAseq experiments

Gene expression analysis of - tumor and normal tissue RNA samples was performed by next generation sequencing (RNAseq) by CeGaT (Tübingen, Germany). Briefly, sequencing libraries are prepared using the Illumina HiSeq v4 reagent kit according to the provider’s protocol (Illumina Inc, San Diego, CA, USA), which includes RNA fragmentation, cDNA conversion and addition of sequencing adaptors. Libraries derived from multiple samples are mixed equimolarly and sequenced on the Illumina HiSeq 2500 sequencer according to the manufacturer’s instructions, generating 50 bp single end reads. Processed reads are mapped to the human genome (GRCh38) using the STAR software. Expression data are provided on transcript level as RPKM (Reads Per Kilobase per Million mapped reads, generated by the software Cufflinks) and on exon level (total reads, generated by the software Bedtools), based on annotations of the ensembl sequence database (Ensembl77). Exon reads are normalized for exon length and alignment size to obtain RPKM values. Exemplary expression profiles of source genes of the present invention that are highly over-expressed or exclusively expressed in CLL are shown in Figure 3. Expression scores for further exemplary genes are shown in Table 9.

Table 9: Expression scores. The table lists peptides from genes that are very highly over-expressed in tumors compared to a panel of normal tissues (+++), highly over-expressed in tumors compared to a panel of normal tissues (++) or over-expressed in tumors compared to a panel of normal tissues (+). The baseline for this score was calculated from measurements of the following relevant normal tissues: adipose tissue, adrenal gland, artery, blood cells, bone marrow, brain, cartilage, colon, esophagus, eye, gallbladder, heart, kidney, liver, lung, lymph node, pancreas, pituitary, rectum, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, thyroid gland, trachea, urinary bladder, and vein. In case expression data for several samples of the same tissue type were available, the arithmetic mean of all respective samples was used for the calculation.

SEQ ID No	Sequence	Gene Expression
1	AIPPSFASIFL	++
2	ALHRPDVYL	++
3	VIAELPPKV	+++
4	VIAELPPKVSV	+++
5	ALIFKIASA	+++
9	ALCDTLITV	+++
10	FVYGESVEL	+++
17	ALTKTNLQL	+++
18	LLGEFSIKM	+++
19	QVMEKLAAV	+++
21	ALWAGLLTL	+++
24	AMAGDVVYA	+++
31	ELMHGVAGV	+++
46	YIIDSAQAV	++
62	GIIDGSPRL	+++
63	SLAHVAGCEL	+++
64	KLLESVASA	+++
65	GLDDMKANL	+++
66	SLAGGLDDMKA	+++

71	GLKHDIARV	+++
74	GLLRASFLL	++
75	GLSIFAQDLRL	++
85	HLMLHTAAL	+
86	HLYPGAVTI	+++
91	ILDLNTYNV	+
96	ILIDKTSFV	+++
97	LLFATQITL	+++
98	SLIKYFLFV	+++
105	KLMNDIADI	++
106	FMASHLDYL	+
107	ILYNLYDLL	++
108	VIYTLIHYI	+
113	KVPAEEVLVAV	++
116	LLIGATMQV	+
117	LLILENILL	++
118	RLLILENILL	++
119	VLPAEFFEV	++
120	AIDAALTSV	++
121	RLYEITIEV	++
127	LLPTAPTTV	+
137	NLIKTVIKL	+
146	SIIEGPIIKL	+++
149	SLDNGGYYI	+++
150	SLFDQPLSII	+++
153	SLLAELHVL	+++
154	SLLAELHVLTV	+++
156	SLNIGDVQL	+++
163	SQLDISEPYKV	+++
177	VLDDRELLL	++
189	VMLEMTPEL	+++
193	YLDLSNNRL	+
195	YLGGFALSV	++
205	GLSQANFTL	+++
206	HMQDVRVLL	+++
213	RLLYQLVFL	+++
220	ALPEILFAKV	++
221	ALVSTIIMV	+
223	ALWVSQPPEI	++
235	GLDDLLLFL	+
251	LLYDNVPGA	+++

254	QLIPKLIFL	+++
255	YLFEEAISM	+++
257	RIINGIIISV	+++
262	SLWNAGTSV	++
275	VLLFIEHSV	++
284	LLSAAEPVPA	+++
285	GVATAGCVNEV	+++
286	FLLEDLSQKL	++
294	ALLDQLHTLL	+
299	ILEENIPVL	++
300	ILLNPAYDVYL	++
301	AASPIITLV	+
307	LLLTKPTEA	++
308	SLYDVSRMYV	+
309	ILYGTQFVL	++
312	LLYNSTDPTL	+
319	QVIPQLQTV	++
320	TIAPVTVAV	++
330	VLMWEIYSL	++
332	VMIQHVENL	+++
337	YMYEKESEL	+++
352	ALVFELHYV	+++
356	FLHDHQAEL	+
360	HLIDTNKIQL	+
378	YILGKFFAL	+
380	YLDRKLLTL	++

EXAMPLE 3

In vitro immunogenicity for MHC class I presented peptides

In order to obtain information regarding the immunogenicity of the TUMAPs of the present invention, the inventors performed investigations using an *in vitro* T-cell priming assay based on repeated stimulations of CD8+ T cells with artificial antigen presenting cells (aAPCs) loaded with peptide/MHC complexes and anti-CD28 antibody. This way the inventors could show immunogenicity for HLA-A*0201 restricted TUMAPs of the invention, demonstrating that these peptides are T-cell epitopes against which CD8+ precursor T cells exist in humans (Table 10).

In vitro priming of CD8+ T cells

In order to perform *in vitro* stimulations by artificial antigen presenting cells loaded with peptide-MHC complex (pMHC) and anti-CD28 antibody, the inventors first isolated CD8+ T cells from fresh HLA-A*02 leukapheresis products via positive selection using CD8 microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) of healthy donors obtained from the University clinics Mannheim, Germany, after informed consent.

PBMCs and isolated CD8+ lymphocytes were incubated in T-cell medium (TCM) until use consisting of RPMI-Glutamax (Invitrogen, Karlsruhe, Germany) supplemented with 10% heat inactivated human AB serum (PAN-Biotech, Aidenbach, Germany), 100 U/ml Penicillin/100 µg/ml Streptomycin (Cambrex, Cologne, Germany), 1 mM sodium pyruvate (CC Pro, Oberdorla, Germany), 20 µg/ml Gentamycin (Cambrex). 2.5 ng/ml IL-7 (PromoCell, Heidelberg, Germany) and 10 U/ml IL-2 (Novartis Pharma, Nürnberg, Germany) were also added to the TCM at this step.

Generation of pMHC/anti-CD28 coated beads, T-cell stimulations and readout was performed in a highly defined *in vitro* system using four different pMHC molecules per stimulation condition and 8 different pMHC molecules per readout condition.

The purified co-stimulatory mouse IgG2a anti human CD28 Ab 9.3 (Jung et al., 1987) was chemically biotinylated using Sulfo-N-hydroxysuccinimidobiotin as recommended by the manufacturer (Perbio, Bonn, Germany). Beads used were 5.6 µm diameter streptavidin coated polystyrene particles (Bangs Laboratories, Illinois, USA).

pMHC used for positive and negative control stimulations were A*0201/MLA-001 (peptide ELAGIGILTV (SEQ ID NO. 523) from modified Melan-A/MART-1) and A*0201/DDX5-001 (YLLPAIVHI from DDX5, SEQ ID NO. 524), respectively.

800.000 beads/200 µl were coated in 96-well plates in the presence of 4 x 12.5 ng different biotin-pMHC, washed and 600 ng biotin anti-CD28 were added subsequently in a volume of 200 µl. Stimulations were initiated in 96-well plates by co-incubating $1x10^6$ CD8+ T cells with $2x10^5$ washed coated beads in 200 µl TCM supplemented with 5 ng/ml IL-12 (PromoCell) for 3 days at 37°C. Half of the medium was then exchanged by fresh TCM supplemented with 80 U/ml IL-2 and incubating was continued for 4 days at 37°C. This stimulation cycle was performed for a total of three times. For the pMHC multimer readout using 8 different pMHC molecules per condition, a two-dimensional combinatorial coding approach was used as previously described (Andersen et al., 2012) with minor modifications encompassing coupling to 5 different fluorochromes. Finally, multimeric analyses were performed by staining the cells with Live/dead near IR dye (Invitrogen, Karlsruhe, Germany), CD8-FITC antibody clone SK1 (BD, Heidelberg, Germany) and fluorescent pMHC multimers. For analysis, a BD LSRII SORP cytometer equipped with appropriate lasers and filters was used. Peptide specific cells were calculated as percentage of total CD8+ cells. Evaluation of multimeric analysis was done using the FlowJo software (Tree Star, Oregon, USA). *In vitro* priming of specific multimer+ CD8+ lymphocytes was detected by comparing to negative control stimulations. Immunogenicity for a given antigen was detected if at least one evaluable *in vitro* stimulated well of one healthy donor was found to contain a specific CD8+ T-cell line after *in vitro* stimulation (i.e. this well contained at least 1% of specific multimer+ among CD8+ T-cells and the percentage of specific multimer+ cells was at least 10x the median of the negative control stimulations).

In vitro immunogenicity for CLL peptides

For tested HLA class I peptides, *in vitro* immunogenicity could be demonstrated by generation of peptide specific T-cell lines. Exemplary flow cytometry results after TUMAP-specific multimer staining for two peptides of the invention are shown in Figure 3 together with corresponding negative controls. Results for two peptides from the invention are summarized in Table 10A, others in Table 10B.

Table 10A: in vitro immunogenicity of HLA class I peptides of the invention

Exemplary results of *in vitro* immunogenicity experiments conducted by the applicant for the peptides of the invention. <20 % = +; 20 % - 49 % = ++; 50 % - 69 %= +++; >= 70 % = ++++

Seq ID	Sequence	wells

Seq ID	Sequence	wells
427	LLYDAVHIV	++
521	YLYGQTTTYL	++

Table 10B: In vitro immunogenicity of additional HLA class I peptides of the invention

Exemplary results of *in vitro* immunogenicity experiments conducted by the applicant for HLA-A*02 restricted peptides of the invention. Results of *in vitro* immunogenicity experiments are indicated. Percentage of positive wells and donors (among evaluable) are summarized as indicated <20 % = +; 20 % - 49 % = ++; 50 % - 69 %= +++; >= 70 %= ++++

SEQ ID NO:	Sequence	Wells positive [%]
1	AIPPSFASIFL	"+++"
2	ALHRPDVYL	"+"
5	ALIFKIASA	"+++"
17	ALTKTNLQL	"+"
26	AVLLVLPLV	"+"
32	FIDKFTPPV	"++++"
49	FLTDLFAQL	"++"
54	FLYPFPLALF	"+"
56	FVFEAPYTL	"++"
57	GLSEISLRL	"+"
69	GLHQREIFL	"+"
81	WLVGQEFEL	"+"
86	HLYPGAVTI	"+"
96	ILIDKTSFV	"+"
100	ILNNEVFAI	"++"
101	ILVVIEPLL	"+"
102	IQDRAVPSL	"+"
112	KTLDVDATYEI	"+"
114	LIPEGPPQV	"+"
154	SLLAELHVLTV	"+"
192	YIQEYLTLL	"+"
197	YLQEVPILTL	"+"
200	YMFEEVPIVI	"+"
201	YQLELHGIEL	"+"
202	YVDDVFLRV	"++"

SEQ ID NO:	Sequence	Wells positive [%]
220	ALPEILFAKV	"++"
240	ILARDILEI	"+"
281	NLLDDRGMTAL	"+"
282	LLRDGIELV	"++"
307	LLLTKPTEA	"+"
320	TIAPVTVAV	"+"
322	SLASIHVPL	"+"
323	SLDLFNCEV	"+"
329	VLMDGSVKL	"+++"
332	VMIQHVENL	"+"
380	YLDRKLLTL	"++++"

EXAMPLE 4

Synthesis of peptides

All peptides were synthesized using standard and well-established solid phase peptide synthesis using the Fmoc-strategy. Identity and purity of each individual peptide have been determined by mass spectrometry and analytical RP-HPLC. The peptides were obtained as white to off-white lyophilizates (trifluoro acetate salt) in purities of >50%. All TUMAPs are preferably administered as trifluoro-acetate salts or acetate salts, other salt-forms are also possible.

EXAMPLE 5

MHC Binding Assays

Candidate peptides for T cell based therapies according to the present invention were further tested for their MHC binding capacity (affinity). The individual peptide-MHC complexes were produced by UV-ligand exchange, where a UV-sensitive peptide is cleaved upon UV-irradiation, and exchanged with the peptide of interest as analyzed. Only peptide candidates that can effectively bind and stabilize the peptide-receptive MHC molecules prevent dissociation of the MHC complexes. To determine the yield of the exchange reaction, an ELISA was performed based on the detection of the light chain (β2m) of stabilized MHC complexes. The assay was performed as generally described in Rodenko et al. (Rodenko et al., 2006).

96 well MAXISorp plates (NUNC) were coated over night with 2ug/ml streptavidin in PBS at room temperature, washed 4x and blocked for1h at 37°C in 2% BSA containing blocking buffer. Refolded HLA-A*02:01/MLA-001 monomers served as standards, covering the range of 15-500 ng/ml. Peptide-MHC monomers of the UV-exchange reaction were diluted 100 fold in blocking buffer. Samples were incubated for 1h at 37°C, washed four times, incubated with 2ug/ml HRP conjugated anti-β2m for 1h at 37°C, washed again and detected with TMB solution that is stopped with NH2SO4. Absorption was measured at 450nm. Candidate peptides that show a high exchange yield (preferably higher than 50%, most preferred higher than 75 %) are generally preferred for a generation and production of antibodies or fragments thereof, and/or T cell receptors or fragments thereof, as they show sufficient avidity to the MHC molecules and prevent dissociation of the MHC complexes.

Table 11: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-A*02:01 was ranged by peptide exchange yield: $\geq$10% = +; $\geq$20% = ++; $\geq$50 = +++; $\geq$ 75% = ++++

SEQID	Sequence	Peptide Exchange
1	AIPPSFASIFL	"+++"
2	ALHRPDVYL	"+++"
3	VIAELPPKV	"++"
4	VIAELPPKVSV	"+++"
5	ALIFKIASA	"+++"
6	ALDTLEDDMTI	"+++"
7	ALLERTGYTL	"+++"
8	ALAASALPALV	"+++"
9	ALCDTLITV	"+++"
10	FVYGESVEL	"+++"
11	ALFTFJPLTV	"+++"
12	ALGEDEITL	"+++"
13	VVDGMPPGV	"+++"
14	ALLRLLPGL	"+++"
15	ALPEVSVEA	"+++"
16	ALPGGAAVAAV	"++++"
17	ALTKTNLQL	"+++"
18	LLGEFSIKM	"+++"
19	QVMEKLAAV	"+++"

SEQID	Sequence	Peptide Exchange
20	ALVDPGPDFVV	"+++"
21	ALWAGLLTL	"+++"
22	ALWDPVIEL	"+++"
23	ALYLTEVFL	"++++"
24	AMAGDVVYA	"+++"
25	ATYSGLESQSV	"++"
26	AVLLVLPLV	"+++"
27	AVLGLVWLL	"+++"
28	AVLHLLLSV	"++"
29	AVLQAVTAV	"+++"
30	ELLEGSEIYL	"+++"
31	ELMHGVAGV	"+++"
32	FIDKFTPPV	"++++"
34	YLPYIFPNI	"+++"
35	FILPSSLYL	"+++"
36	FILTHVDQL	"++++"
37	FIMEGGAMVL	"++++"
38	FIMEGGAMV	"+++"
39	FLDALLTDV	"+++"
40	FLDEDDMSL	"++"
41	FLDPSLDPLL	"+++"
42	FLEEGGVVTV	"+++"
43	FLLGPEALSFA	"++++"
44	FLLSINDFL	"+++"
45	FLPELPADLEA	"+++"
46	YIIDSAQAV	"+++"
47	FLSDQPEPYL	"++"
48	FLSPQQPPLL	"+++"
49	FLTDLFAQL	"++++"
50	FLFEPVVKAFL	"+++"
51	FLVEAPHDWDL	"++++"
52	FLVETGFLHV	"++++"
53	FLWQHVELVL	"+++"
54	FLYPFPLALF	"+++"
55	FMEPTLLML	"+++"
56	FVFEAPYTL	"+++"
57	GLSEISLRL	"++++"
58	YIQQGIFSV	"+++"
59	FVFGDENGTVSL	"+++"
60	FVLDHEDGLNL	"+++"
61	FVYFIVREV	"+++"
62	GIIDGSPRL	"+++"
63	SLAHVAGCEL	"+++"

SEQID	Sequence	Peptide Exchange
64	KLLESVASA	"+++"
65	GLDDMKANL	"++"
66	SLAGGLDDMKA	"+++"
67	GLDDVTVEV	"++"
68	GLDQQFAGLDL	"+"
69	GLHQREIFL	"+++"
70	FVPDTPVGV	"+++"
71	GLKHDIARV	"+++"
72	GLLDAGKMYV	"+++"
73	GLLEVISALQL	"+++"
74	GLLRASFLL	"++"
75	GLSIFAQDLRL	"++"
76	GLLRIIPYL	"+++"
78	GLPSFLTEV	"+++"
79	GLQAKIQEA	"+++"
80	VLIEDELEEL	"++"
81	WLVGQEFEL	"+++"
82	GLQSGVDIGV	"+++"
83	GQGEVLVYV	"+++"
84	GVMDVNTAL	"++"
85	HLMLHTAAL	"++++"
86	HLYPGAVTI	"+++"
87	HQIEAVDGEEL	"++"
88	ILDEIGADVQA	"+++"
89	ILDFGTFQL	"+++"
90	VIADLGLIRV	"+++"
91	ILDLNTYNV	"+++"
92	ILEPLNPLL	"+++"
93	ILFNTQINI	"+++"
94	ILFPLRFTL	"+++"
95	ILGYMAHEHKV	"++"
96	ILIDKTSFV	"+++"
97	LLFATQITL	"++"
98	SLIKYFLFV	"+"
99	ILIFHSVAL	"+++"
100	ILNNEVFAI	"+++"
101	ILVVIEPLL	"++++"
102	IQDRAVPSL	"++"
103	KLGGTPAPA	"++"
104	KLILLDTPLFL	"+++"
105	KLMNDIADI	"++"
106	FMASHLDYL	"+++"
107	ILYNLYDLL	"+++"

SEQID	Sequence	Peptide Exchange
108	VIYTLIHYI	"+++"
109	KLWEGLTELV	"+++"
110	LLFDHLEPMEL	"+++"
111	KLWNVAAPLYL	"+++"
112	KTLDVDATYEI	"+++"
113	KVPAEEVLVAV	"+++"
114	LIPEGPPQV	"+++"
115	LLFDKLYLL	"+++"
116	LLIGATMQV	"+++"
117	LLILENILL	"+++"
118	RLLILENILL	"+++"
119	VLPAEFFEV	"+++"
120	AIDAALTSV	"+++"
121	RLYEITIEV	"++++"
122	LLIPVVPGV	"+++"
123	LLLAEAELLTL	"++"
124	LLLEETEKQAV	"+++"
125	LLLEIGEVGKLFV	"+++"
126	LLPEGGITAI	"+++"
127	LLPTAPTTV	"+++"
128	LLSEEEYHL	"+++"
130	LLVLIPVYL	"+"
131	LQALEVLKI	"+"
132	LVYEAIIMV	"+++"
133	YLLSGDISEA	"+++"
134	MLLEHGITLV	"++++"
135	MTAGFSTIAGSV	"+"
136	NLDKLWTLV	"++"
137	NLIKTVIKL	"+++"
138	NLLDIDAPVTV	"+++"
139	NLTDVVEKL	"+++"
140	QIAELPATSV	"+++"
141	QILSEIVEA	"++"
142	QLDEPAPQV	"++"
143	QLLDTYFTL	"+++"
145	SALDTITTV	"+++"
146	SIIEGPIIKL	"+++"
147	SILETVATL	"+++"
148	SIVASLITV	"+++"
149	SLDNGGYYI	"+++"
150	SLFDQPLSII	"+++"
151	SLFDSAYGA	"+++"
152	SLIRILQTI	"+++"

SEQID	Sequence	Peptide Exchange
153	SLLAELHVL	"+++"
154	SLLAELHVLTV	"++++"
155	SLMLEVPAL	"+++"
156	SLNIGDVQL	"+++"
157	SLNIRDFTM	"+++"
158	SLPEAPLDV	"+++"
159	SLQEEKLIYV	"+++"
160	SLSFLVPSL	"+++"
161	SMDDGMINV	"+++"
162	SMKDDLENV	"++"
163	SQLDISEPYKV	"+++"
164	SVHKGFAFV	"+++"
165	TLDDDLDTV	"++"
166	TLDPNQVSL	"++"
167	TLDTSKLYV	"+++"
168	YLLDQSFVM	"++++"
169	TLLLGLTEV	"+++"
170	TLTFRVETV	"+++"
171	TLVPPAALISI	"+++"
172	TLYDMLASI	"+++"
173	TVIENIHTI	"++"
174	VLAELPIIVV	"+++"
175	FTVPRVVAV	"+++"
176	VLAEQNIIPSA	"+++"
177	VLDDRELLL	"+++"
178	VLFFNVQEV	"+++"
179	VLLGLEMTL	"+++"
180	LLKDGPEIGL	"++"
181	VLLSIPFVSV	"+++"
182	VLLSVPGPPV	"+++"
183	VLMPTVYQQGV	"+++"
184	VLSHNLYTV	"+++"
185	VMDDQRDLI	"++"
186	VMDPTKILI	"++++"
187	VMDTHLVNI	"++++"
188	VMGDIPAAV	"++"
189	VMLEMTPEL	"+++"
190	VVMGTVPRL	"+++"
191	YIFDGSDGGV	"+++"
192	YIQEYLTLL	"+++"
193	YLDLSNNRL	"+++"
194	YLDNVLAEL	"++++"
195	YLGGFALSV	"++++"

SEQID	Sequence	Peptide Exchange
196	YLLLQTYVL	"+"
197	YLQEVPILTL	"++++"
198	YLTFLPAEV	"+++"
199	YLVELSSLL	"++++"
200	YMFEEVPIVI	"++++"
201	YQLELHGIEL	"++++"
202	YVDDVFLRV	"++++"
203	ALLSSQLAL	"+++"
204	GLLQINDKIAL	"+++"
205	GLSQANFTL	"+++"
206	HMQDVRVLL	"+++"
207	IIADLDTTIMFA	"+++"
208	ILLKTEGINL	"+++"
209	ILQAELPSL	"+++"
210	KLLVQDFFL	"+++"
211	LIDVKPLGV	"+++"
214	RLQELTEKL	"+++"
215	VMQDIVYKL	"+++"
216	WLAGDVPAA	"+++"
217	ALDEPPYLTV	"+++"
218	ALGEEWKGYVV	"++"
219	ALLNLLESA	"+++"
220	ALPEILFAKV	"+++"
221	ALVSTIIMV	"+"
222	ALWELSLKI	"+++"
223	ALWVSQPPEI	"+++"
224	AMEALVVEV	"+++"
225	ILQERELLPV	"+++"
226	AMNISVPQV	"+++"
227	FLAEASVMTQL	"+++"
228	FLGGLSPGV	"+++"
229	FLLNLQNCHL	"++++"
230	FLQDSKVIFV	"+++"
231	FLYIRQLAI	"+++"
232	FMHQIIDQV	"++++"
233	GIIDINVRL	"++"
234	GLDDAEYAL	"++"
235	GLDDLLLFL	"+++"
236	GLLESGRHYL	"+++"
237	GLQENLDVVV	"++++"
238	GLVETELQL	"+++"
239	ILAGEMLSV	"+++"
240	ILARDILEI	"+++"

SEQID	Sequence	Peptide Exchange
241	ILGDILLKV	"+++"
242	ILLGIQELL	"+++"
243	ILPTLEKELFL	"+++"
244	ILQALAVHL	"+++"
245	KIMDYSLLLGV	"++++"
246	KLDETGVAL	"++"
247	KLKDRLPSI	"++"
248	KTVEPPISQV	"++"
249	LLPTGVFQV	"+++"
250	LLVQEPDGLMV	"+++"
251	LLYDNVPGA	"+++"
252	NLLDPGSSYLL	"+++"
253	NLWSVDGEVTV	"+++"
254	QLIPKLIFL	"+++"
255	YLFEEAISM	"+++"
256	QLLPVSNVVSV	"+++"
258	RLDYITAEI	"+++"
259	RLLDEQFAVL	"+++"
260	SLDDVEGMSV	"+++"
261	SLVEAQGWLV	"+++"
262	SLWNAGTSV	"+++"
263	SQWEDIHVV	"+++"
264	TILDYINVV	"+++"
265	TLLADDLEIKL	"+++"
266	TLLDQLDTQL	"++"
267	TLLDWQDSL	"+++"
268	TLLQVFHLL	"+++"
269	TLTDEQFLV	"++"
270	TVLPVPPLSV	"+++"
271	VIRNIVEAA	"++"
272	VLDELPPLI	"+++"
273	VLGEYSYLL	"+++"
274	VLLEYHIAYL	"+++"
275	VLLFIEHSV	"+++"
276	VLNDGAPNV	"+++"
277	VMILKLPFL	"++"
278	YLDDLLPKL	"+++"
279	YMAPEVVEA	"+++"
280	YTLDSLYWSV	"+++"
281	NLLDDRGMTAL	"+++"
282	LLRDGIELV	"+++"
283	ILQPMDIHV	"+++"
284	LLSAAEPVPA	"++"

SEQID	Sequence	Peptide Exchange
285	GVATAGCVNEV	"++++"
286	FLLEDLSQKL	"+++"
287	FLWEEKFNSL	"++++"
288	GLAESTGLLAV	"+++"
289	ILEEQPMDMLL	"+++"
291	ILLNEDDLVTI	"+++"
292	AAALIIHHV	"+++"
293	ALDIMIPMV	"+++"
294	ALLDQLHTLL	"++++"
295	ALLQKLQQL	"+++"
296	FIAPTGHSL	"++"
297	FLVEPQEDTRL	"+++"
298	IILPVEVEV	"+++"
299	ILEENIPVL	"+++"
300	ILLNPAYDVYL	"+++"
301	AASPIITLV	"+++"
302	ILQDLTFVHL	"+++"
303	ILSQPTPSL	"+++"
304	LAIVPVNTL	"+"
305	LLFPQIEGIKI	"+++"
306	VVAEELENV	"++"
307	LLLTKPTEA	"+++"
308	SLYDVSRMYV	"++++"
309	ILYGTQFVL	"+++"
310	LLSTLHLLV	"+++"
311	LLVDVEPKV	"+++"
312	LLYNSTDPTL	"+++"
313	LMADLEGLHL	"+++"
314	LMKDCEAEV	"+++"
315	LVYEAPETV	"+++"
316	MLLEHGITL	"+++"
317	NLLAHIWAL	"+++"
318	NLQVTQPTV	"+++"
319	QVIPQLQTV	"+++"
320	TIAPVTVAV	"++++"
321	RLLEFELAQL	"+++"
322	SLASIHVPL	"+++"
323	SLDLFNCEV	"++++"
324	SLYSALQQA	"+++"
325	TLENGVPCV	"+++"
326	VLAFLVHEL	"++++"
327	VLIKWFPEV	"++++"
328	VLLPQETAEIHL	"++++"

SEQID	Sequence	Peptide Exchange
329	VLMDGSVKL	"++++"
330	VLMWEIYSL	"+++"
331	VLWELAHLPTL	"+++"
332	VMIQHVENL	"+++"
333	VTLEFPQLIRV	"+++"
334	YLLEEKIASL	"+++"
335	YLYQEQYFI	"+++"
336	YMAVTTQEV	"++++"
337	YMYEKESEL	"++"
338	FLDMTNWNL	"++++"
339	GLWGTVVNI	"+++"
340	KLLEEICNL	"++++"
341	LLAELPASVHA	"+++"
342	SLITPLQAV	"+++"
344	VLAFENPQV	"+++"
345	YLIEPDVELQRI	"+++"
346	VLVQVSPSL	"+++"
347	YLGPVSPSL	"+++"
348	ALAKPPVVSV	"+++"
349	ALATHILSL	"+++"
350	ALEDRVWEL	"+++"
351	ALSEKLARL	"+++"
352	ALVFELHYV	"+++"
353	ATPMPTPSV	"++"
354	FIMDDPAGNSYL	"+++"
355	FIWPMLIHI	"+++"
356	FLHDHQAEL	"++"
357	FLIQEIKTL	"+++"
358	FLTDYLNDL	"+++"
359	FMQDPMEVFV	"+++"
360	HLIDTNKIQL	"+++"
361	ILQEFESKL	"+++"
362	ILTELGGFEV	"+++"
363	ITTEVVNELYV	"+++"
364	KMDWIFHTI	"+++"
365	LISPLLLPV	"+++"
367	NLWSLVAKV	"+++"
369	RLLDLENSLLGL	"+++"
370	SIFASPESV	"+++"
371	SLADDSVLERL	"+++"
372	SLFGPLPGPGPALV	"+++"
373	TLLADQGEIRV	"++"
374	VLSVITEEL	"+++"

SEQID	Sequence	Peptide Exchange
375	VLWFKPVEL	"+++"
376	VLYNQRVEEI	"+++"
377	VVDGTCVAV	"+++"
378	YILGKFFAL	"+++"
379	YLAELVTPIL	"+++"
380	YLDRKLLTL	"+++"
381	YLLEENKIKL	"+++"
382	YLLPLLQRL	"++++"
383	YLLREWVNL	"++++"
384	YMIGSEVGNYL	"++++"
385	YTIPLAIKL	"+++"

EXAMPLE 6

Absolute quantitation of tumor associated peptides presented on the cell surface.

The generation of binders, such as antibodies and/or TCRs, is a laborious process, which may be conducted only for a number of selected targets. In the case of tumor-associated and –specific peptides, selection criteria include but are not restricted to exclusiveness of presentation and the density of peptide presented on the cell surface. In addition to the isolation and relative quantitation of peptides as described in EXAMPLE 1, the inventors did analyze absolute peptide copies per cell as described in patent x . The quantitation of TUMAP copies per cell in solid tumor samples requires the absolute quantitation of the isolated TUMAP, the efficiency of TUMAP isolation, and the cell count of the tissue sample analyzed. An overview on our experimental approach is given in Figure 4, experimental steps are described below.

Peptide quantitation by nanoLC-MS/MS

For an accurate quantitation of peptides by mass spectrometry, a calibration curve was generated for each peptide using the internal standard method. The internal standard is a double-isotope-labelled variant of each peptide, i.e. two isotope-labelled amino acids were included in TUMAP synthesis. It differs from the tumor-associated peptide only in its mass but shows no difference in other physicochemical properties (Anderson et al., 2012). The internal standard was spiked to each MS sample and all MS signals were normalized to the MS signal of the internal standard to level out potential technical variances between MS experiments. The calibration curves were prepared in at least three different matrices, i.e. HLA peptide eluates from natural samples similar to the routine MS samples, and each preparation was measured in duplicate MS runs. For evaluation, MS signals were normalized to the signal of the internal standard and a calibration curve was calculated by logistic regression. For the quantitation of tumor-associated peptides from tissue samples, the respective samples were also spiked with the internal standard; the MS signals were normalized to the internal standard and quantified using the peptide calibration curve.

Efficiency of peptide/MHC isolation

As for any protein purification process, the isolation of proteins from tissue samples is associated with a certain loss of the protein of interest. To determine the efficiency of TUMAP isolation, peptide/MHC complexes were generated for all TUMAPs selected for absolute quantitation. To be able to discriminate the spiked from the natural peptide/MHC complexes, single-isotope-labelled versions of the TUMAPs were used, i.e. one isotope-labelled amino acid was included in TUMAP synthesis. These complexes were spiked into the freshly prepared tissue lysates, i.e. at the earliest possible point of the TUMAP isolation procedure, and then captured like the natural peptide/MHC complexes in the following affinity purification. Measuring the recovery of the single-labelled TUMAPs therefore allows conclusions regarding the efficiency of isolation of individual natural TUMAPs.

The efficiency of isolation was analyzed in a low number of samples and was comparable among these tissue samples. In contrast, the isolation efficiency differs between individual peptides. This suggests that the isolation efficiency, although determined in only a limited number of tissue samples, may be extrapolated to any other tissue preparation. However, it is necessary to analyze each TUMAP individually as the isolation efficiency may not be extrapolated from one peptide to others.

Determination of the cell count in solid, frozen tissue

In order to determine the cell count of the tissue samples subjected to absolute peptide quantitation, the inventors applied DNA content analysis. This method is applicable to a wide range of samples of different origin and, most importantly, frozen samples (Alcoser et al., 2011; Forsey and Chaudhuri, 2009; Silva et al., 2013). During the peptide isolation protocol, a tissue sample is processed to a homogenous lysate, from which a small lysate aliquot is taken. The aliquot is divided in three parts, from which DNA is isolated (QiaAmp DNA Mini Kit, Qiagen, Hilden, Germany). The total DNA content from each DNA isolation is quantified using a fluorescence-based DNA quantitation assay (Qubit dsDNA HS Assay Kit, Life Technologies, Darmstadt, Germany) in at least two replicates.

In order to calculate the cell number, a DNA standard curve from aliquots of single healthy blood cells, with a range of defined cell numbers, has been generated. The standard curve is used to calculate the total cell content from the total DNA content from each DNA isolation. The mean total cell count of the tissue sample used for peptide isolation is extrapolated considering the known volume of the lysate aliquots and the total lysate volume.

Peptide copies per cell

With data of the aforementioned experiments, the inventors calculated the number of TUMAP copies per cell by dividing the total peptide amount by the total cell count of the sample, followed by division through isolation efficiency. Copy cell number for selected peptides are shown in Table 12.

Table 12: Absolute copy numbers. The table lists the results of absolute peptide quantitation in NSCLC tumor samples. The median number of copies per cell are indicated for each peptide: <100 = +; >=100 = ++; >=1,000 +++; >=10,000 = ++++. The number of samples, in which evaluable, high quality MS data are available, is indicated.

Seq ID	Sequence	Copy Number Category	Number of quantifiable samples
62	GIIDGSPRL	+	17

153	SLLAELHVL	++	17

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CLAIMS

1. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385, and variant sequences thereof which are at least 88% homologous to SEQ ID No. 1 to SEQ ID No. 385, and wherein said variant binds to molecule(s) of the major histocompatibility complex (MHC) and/or induces T cells cross-reacting with said variant peptide; and a pharmaceutical acceptable salt thereof, wherein said peptide is not a full-length polypeptide.

2. The peptide according to claim 1, wherein said peptide has the ability to bind to an MHC class-I or –II molecule, and wherein said peptide, when bound to said MHC, is capable of being recognized by CD4 and/or CD8 T cells.

3. The peptide or variant thereof according to claim 1 or 2, wherein the amino acid sequence thereof comprises a continuous stretch of amino acids according to any one of SEQ ID No. 1 to SEQ ID No. 385.

4. The peptide or variant thereof according to any of claims 1 to 3, wherein said peptide or variant thereof has an overall length of from 8 to 100, preferably from 8 to 30, and more preferred from 8 to 16 amino acids, and most preferred wherein the peptide consists or consists essentially of an amino acid sequence according to any of SEQ ID No. 1 to SEQ ID No. 385.

5. The peptide or variant thereof according to any of Claims 1 to 4, wherein said peptide is modified and/or includes non-peptide bonds.

6. The peptide or variant thereof according to any of Claims 1 to 5, wherein said peptide is part of a fusion protein, in particular comprising N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii).

7. A nucleic acid, encoding a peptide or variant thereof according to any one of claims 1 to 6, optionally linked to a heterologous promoter sequence.

8. An expression vector capable of expressing the nucleic acid according to claim 7.

9. A recombinant host cell comprising the peptide according to claim 1 to 6, the nucleic acid according to claim 7 or the expression vector according to claim 8, wherein said host cell preferably is an antigen presenting cell such as a dendritic cell.

10. The peptide or variant thereof according to any one of claims 1 to 6, the nucleic acid according to claim 7, the expression vector according to claim 8, or the host cell according to claim 9 for use in medicine.

11. A method for producing the peptide or variant thereof according to any one of claims 1 to 6, the method comprising culturing the host cell according to claim 9 that presents the peptide according to claim 1 to 6, or expresses the nucleic acid according to claim 7 or comprises the expression vector according to claim 8, and isolating the peptide or variant thereof from the host cell or its culture medium.

12. An *in vitro* method for producing activated T lymphocytes, the method comprising contacting *in vitro* T cells with antigen loaded human class I or II MHC molecules expressed on the surface of a suitable antigen-presenting cell or an artificial construct mimicking an antigen-presenting cell for a period of time sufficient to activate said T cells in an antigen specific manner, wherein said antigen is a peptide according to any one of claims 1 to 4.

13. An activated T lymphocyte, produced by the method according to claim 12, that selectively recognizes a cell which presents a polypeptide comprising an amino acid sequence given in any one of claims 1 to 4.

14. A method for killing target cells in a patient which target cells present a polypeptide comprising an amino acid sequence given in any one of claims 1 to 4, the method comprising administering to the patient an effective number of activated T cells as defined in claim 13.

15. An antibody, in particular a soluble or membrane-bound antibody, that specifically recognizes the peptide or variant thereof according to any of claims 1 to 5, preferably the peptide or variant thereof according to any of claims 1 to 5 when bound to an MHC molecule.

16. Use of a peptide according to any one of claims 1 to 6, the nucleic acid according to claim 7, the expression vector according to claim 8, the cell according to claim 9, the activated T lymphocyte according to claim 13 or the antibody according to claim 15 for use in diagnosis and/or treatment of cancer, or for use in the manufacture of a medicament against cancer.

17. The use according to claim 16, wherein said cancer is selected from the group of acute myelogenous leukemia, bile duct cancer, brain cancer, breast cancer, colorectal carcinoma, esophageal cancer, gallbladder cancer, gastric cancer, hepatocellular cancer, Merkel cell carcinoma, melanoma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small cell lung cancer, urinary bladder cancer and uterine cancer, CLL and other tumors that show an overexpression of a protein from which a peptide SEQ ID No. 1 to SEQ ID No. 385 is derived from.

18. A kit comprising:

(a) a container comprising a pharmaceutical composition containing the peptide(s) or the variant according to any one of claims 1 to 6, the nucleic acid(s) according to claim 7, the expression vector(s) according to claim 8, the cell(s) according to claim 10, the activated T lymphocyte(s) according to claim 13 or the antibody according to claim 15, in solution or in lyophilized form;

(b) optionally, a second container containing a diluent or reconstituting solution for the lyophilized formulation;

(c) optionally, at least one more peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 522, and

(d) optionally, instructions for (i) use of the solution or (ii) reconstitution and/or use of the lyophilized formulation.

19. The kit according to claim 18, further comprising one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, or (v) a syringe.

20. The kit according to claim 18 or 19, wherein said peptide is selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385.

21. A method for producing a personalized anti-cancer vaccine or a compound-based and/or cellular therapy for an individual patient, said method comprising:
a) identifying tumor-associated peptides (TUMAPs) presented by a tumor sample from said individual patient;
b) comparing the peptides as identified in a) with a warehouse of peptides that have been pre-screened for immunogenicity and/or over-presentation in tumors as compared to normal tissues
c) selecting at least one peptide from the warehouse that matches a TUMAP identified in the patient; and
d) manufacturing and / or formulating the personalized vaccine or compound-based or cellular therapy based on step c).

22. The method according to claim 21, wherein said TUMAPs are identified by:
a1) comparing expression data from the tumor sample to expression data from a sample of normal tissue corresponding to **the** tissue type of the tumor sample to identify proteins that are over-expressed or aberrantly expressed in the tumor sample; and
a2) correlating the expression data with sequences of MHC ligands bound to MHC class I and/or class II molecules in the tumor sample to identify MHC ligands derived from proteins over-expressed or aberrantly expressed by the tumor.

23. The method according to claim 21 or 22, wherein the sequences of MHC ligands are identified by eluting bound peptides from MHC molecules isolated from the tumor sample, and sequencing the eluted ligands.

24. The method according to any of claims 21 to 23, wherein the normal tissue corresponding to the tissue type of the tumor sample is obtained from the same patient.

25. The method according to any of claims 21 to 24, wherein the peptides included in the warehouse are identified based on the following steps:

    aa. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;

    ab. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step aa, and

    ac. Determining an induction of in vivo T-cell responses by the peptides as selected comprising *in vitro* immunogenicity assays using human T cells from healthy donors or said patient; or

    ba. Identifying HLA ligands from said tumor sample using mass spectrometry;

    bb. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;

    bc. Comparing the identified HLA ligands to said gene expression data;

    bd. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step bc;

    be. Re-detecting of selected TUMAPs from step bd on tumor tissue and lack of or infrequent detection on healthy tissues and confirming the relevance of over-expression at the mRNA level; and

    bf. Determining an induction of in vivo T-cell responses by the peptides as selected comprising *in vitro* immunogenicity assays using human T cells from healthy donors or said patient.

26. The method according to any of claims 21 to 25, wherein the immunogenicity of the peptides included in the warehouse is determined by a method comprising in vitro immunogenicity assays, patient immunomonitoring for individual HLA binding, MHC multimer staining, ELISPOT assays and/or intracellular cytokine staining.

27. The method according to any of claims 21 to 26, wherein said warehouse comprises a plurality of peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 522.

28. The method according to any of claims 21 to 27, further comprising identifying at least one mutation that is unique to the tumor sample relative to normal corresponding tissue from the individual patient, and selecting a peptide that correlates with the mutation for inclusion in the vaccine or for the generation of cellular therapies.

29. The method according to claim 28, wherein said at least one mutation is identified by whole genome sequencing.

30. A T-cell receptor, preferably soluble or membrane-bound, that is reactive with an HLA ligand, wherein said ligand has at least 75% identity to an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385.

31. The T-cell receptor according to claim 30, wherein said amino acid sequence is at least 88% identical to SEQ ID No. 1 to SEQ ID No. 385.

32. The T-cell receptor according to claim 30 or 31, wherein said amino acid sequence consists any of SEQ ID No. 1 to SEQ ID No. 385.

33. The T-cell receptor according to any of claims 30 to 32, wherein said T-cell receptor is provided as a soluble molecule and optionally carries a further effector function such as an immune stimulating domain or toxin.

34. A nucleic acid, encoding for a TCR according to any one of claims 30 to 33, optionally linked to a heterologous promoter sequence.

35. An expression vector capable of expressing the nucleic acid according to claim 34.

36. A host cell comprising the nucleic acid according to claim 34 or the nucleic acid encoding an antibody according to claim 15 or the expression vector according to claim 35, wherein said host cell preferably is a T cell or NK cell.

37. A method for producing the T cell receptor according to any claims 30 to 33, said method comprising culturing a host cell according to Claim 36, and isolating said T cell receptor from said host cell and/or its culture medium.

38. A pharmaceutical composition comprising at least one active ingredient selected from the group consisting of

a) a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385;

b) a T-cell receptor reactive with a peptide and/or the peptide-MHC complex according to a);

c) a fusion protein comprising a peptide according to a), and the N-terminal amino acids 1 to 80 of the HLA-DR antigen-associated invariant chain (Ii);

d) a nucleic acid encoding for any of a) to c) or an expression vector comprising said nucleic acid,

e) a host cell comprising the expression vector of d,

f) an activated T-lymphocyte, obtained by a method comprising contacting in vitro T cells with a peptide according to a) expressed on the surface of a suitable antigen presenting cell for a period of time sufficient to activate said T cell in an antigen specific manner, as well as a method to transfer these activated T cells into the autologous or other patients;

g) an antibody, or soluble T-cell receptor, reactive to a peptide and/or the peptide – MHC complex according to a) and/or a cell presenting a peptide according to a), and potentially modified by fusion with for example immune-activating domains or toxins,

h) an aptamer recognizing a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385 and/or a complex of a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 385 with a MHC molecule,

i) a conjugated or labelled peptide or scaffold according to any of a) to h) and a pharmaceutically acceptable carrier, and optionally, pharmaceutically acceptable excipients and/or stabilizers.

39. An aptamer that specifically recognizes the peptide or variant thereof according to any of claims 1 to 5, preferably the peptide or variant thereof according to any of claims 1 to 5 that is bound to an MHC molecule.

Figure 1A

Peptide: ALHRPDVYL (A*02)
Seq ID NO: 2

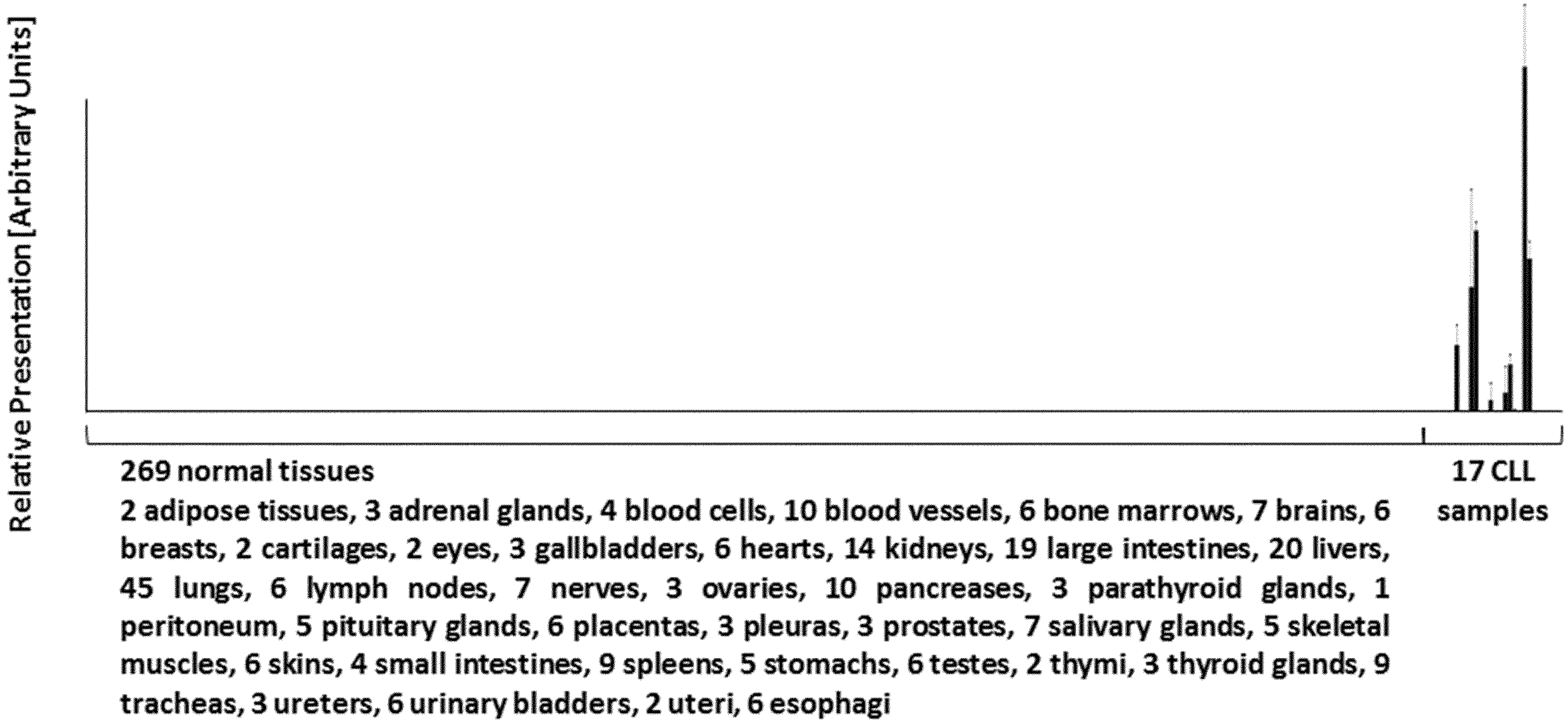


Figure 1B

Peptide: VIAELPPKV (A*02)
SEQ ID NO: 3

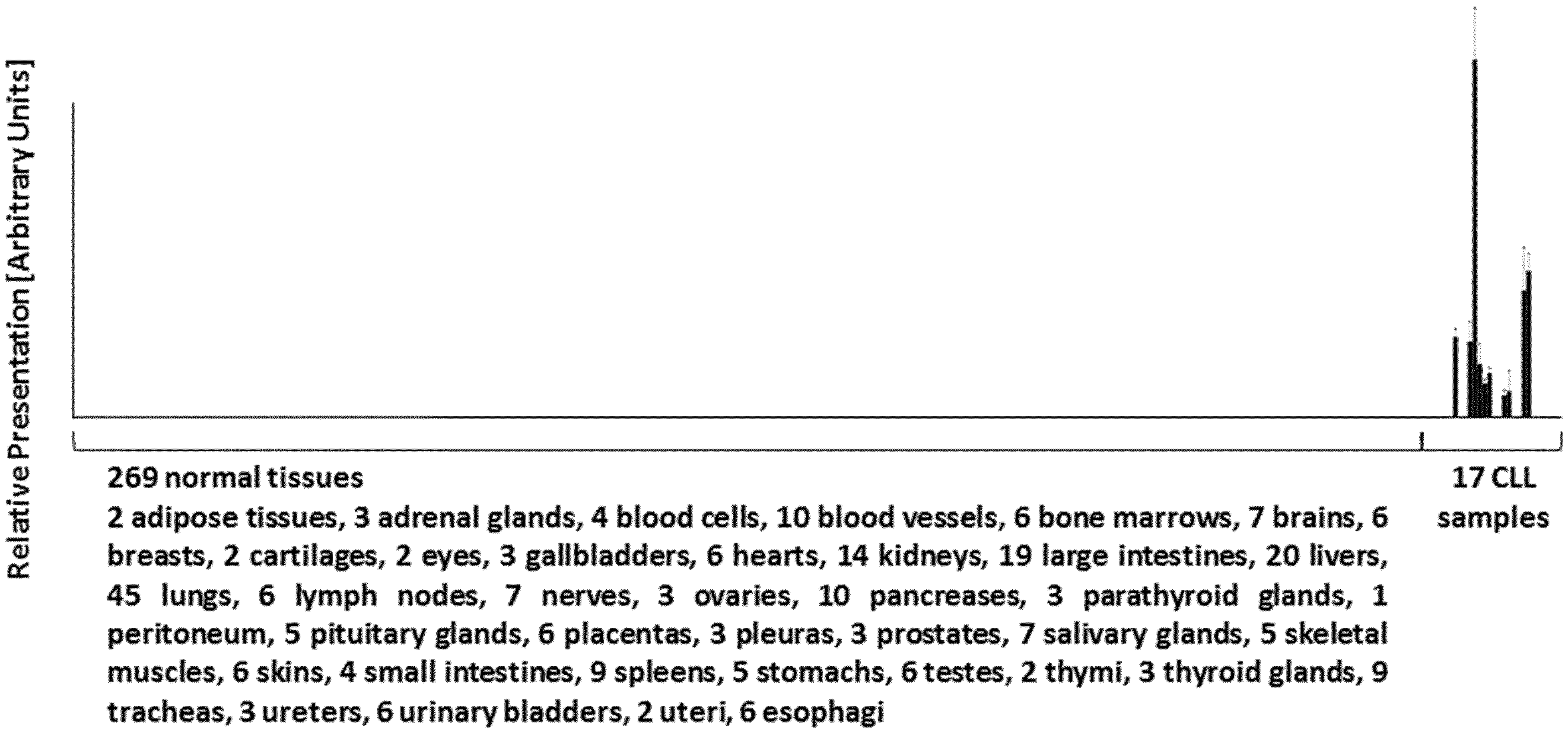


Figure 1C

Peptide: VIAELPPKVSV (A*02)
SEQ ID NO: 4

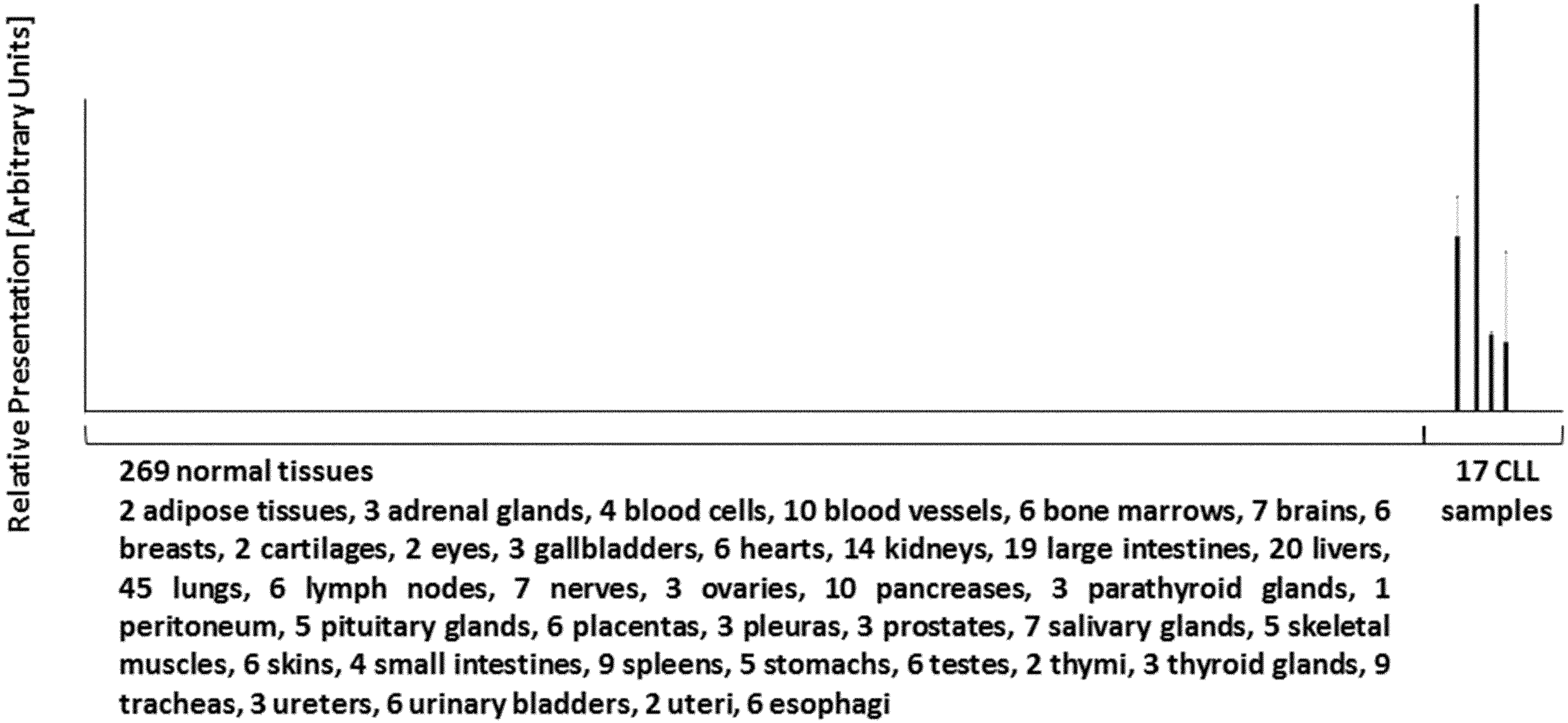
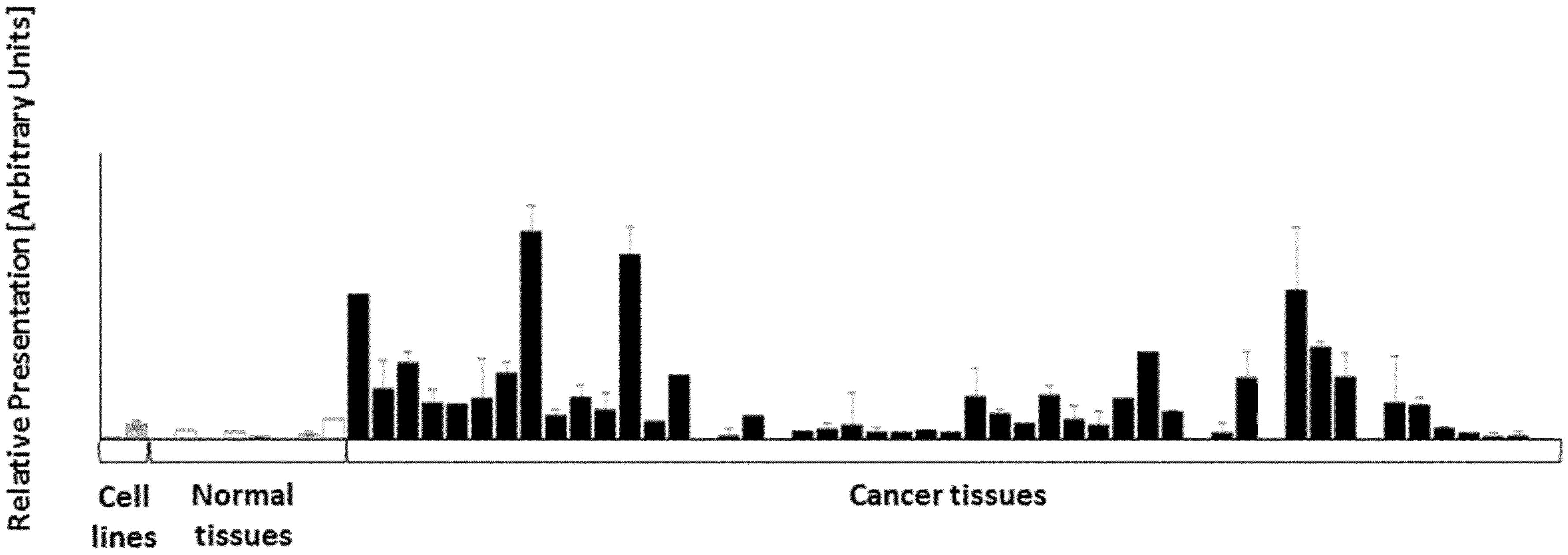


Figure 1D

Peptide: RLYEITIEV (A*02)
SEQ ID NO: 121

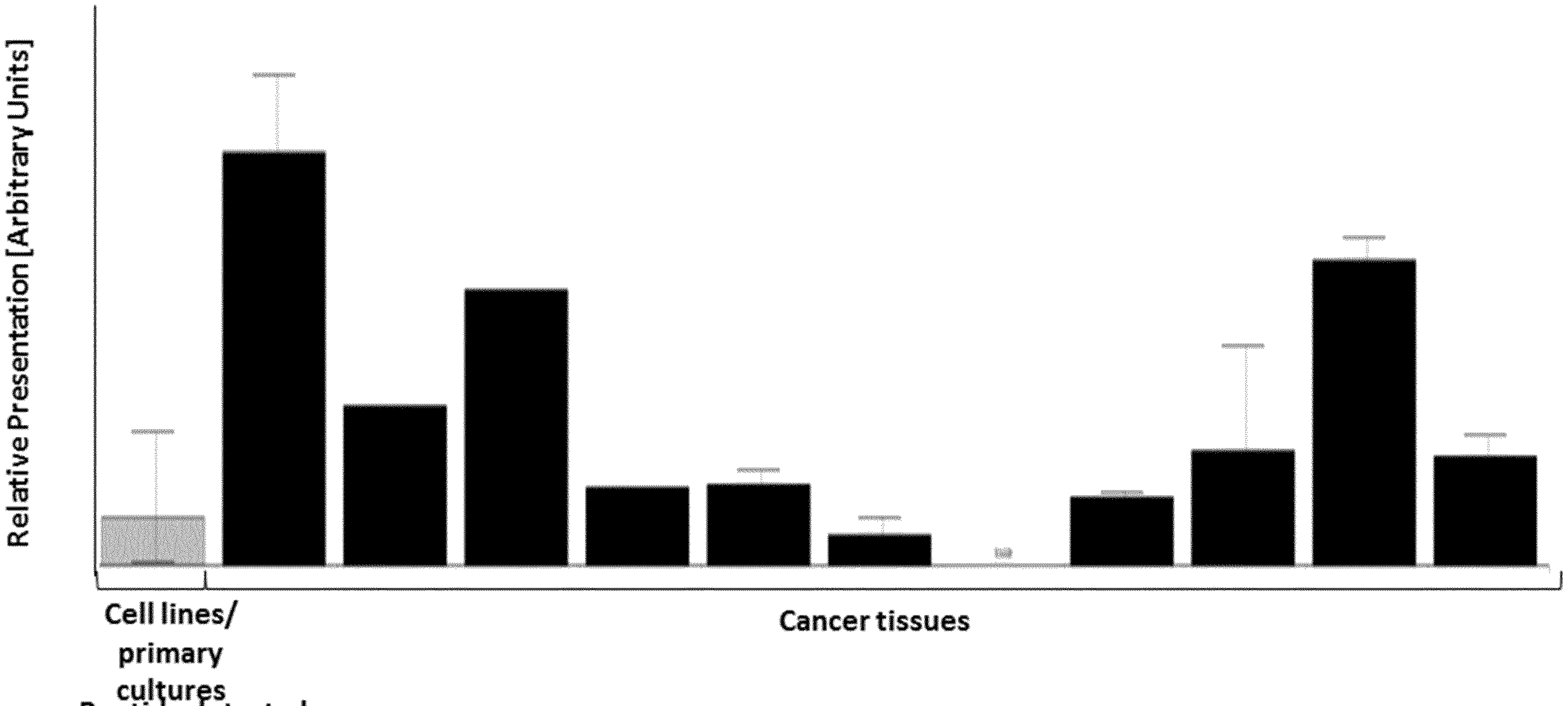


Peptide detected on
1 PBMC culture, 1 benign prostate, 8 normal tissues (3 lungs, 4 spleens, 1 trachea), 49 cancer tissues (2 brain cancers, 2 breast cancers, 1 colon cancer, 13 leukocytic leukemia cancers, 13 lung cancers, 8 lymph node cancers, 1 myeloid cells cancer, 4 ovarian cancers, 2 skin cancers, 1 urinary bladder cancer, 2 uterus cancers) (from left to right)

Figure 1E

Peptide: ALLRLLPGL (A*02)

SEQ ID NO: 14



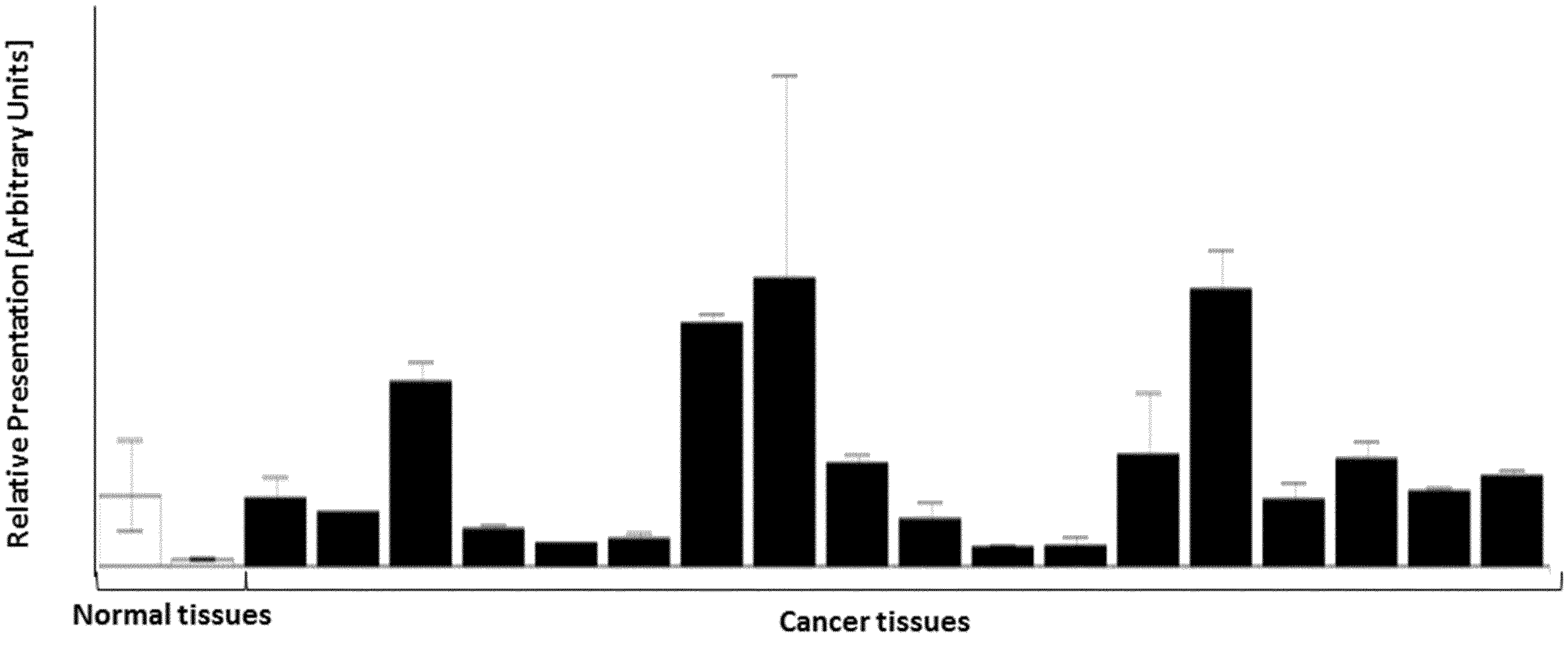
Peptide detected on

1 cell line, 11 cancer tissues (1 breast cancer, 1 colon cancer, 1 head and neck cancer, 2 leukocytic leukemia cancers, 1 liver cancer, 1 lung cancer, 2 lymph node cancers, 1 myeloid cell cancer, 1 skin cancer) (from left to right)

Figure 1F

Peptide: GIIDGSPRL (A*02)

SEQ ID NO: 62

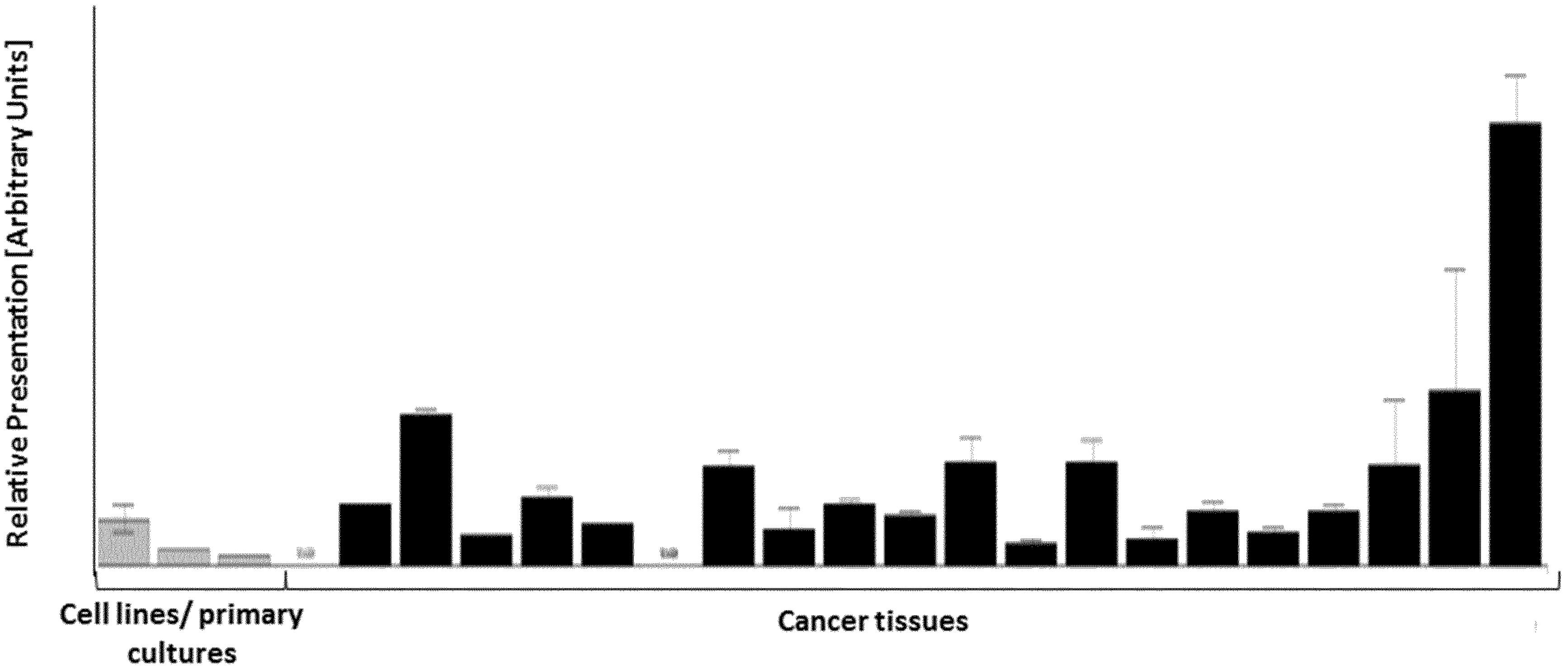


Peptide detected on
2 normal tissues (1 lymph node, 1 spleen), 18 cancer tissues (2 colon cancers, 9 leukocytic leukemia cancers, 6 lymph node cancers, 1 rectum cancer) (from left to right)

Figure 1G

Peptide: GLDQQFAGLDL (A*02)

SEQ ID NO: 68



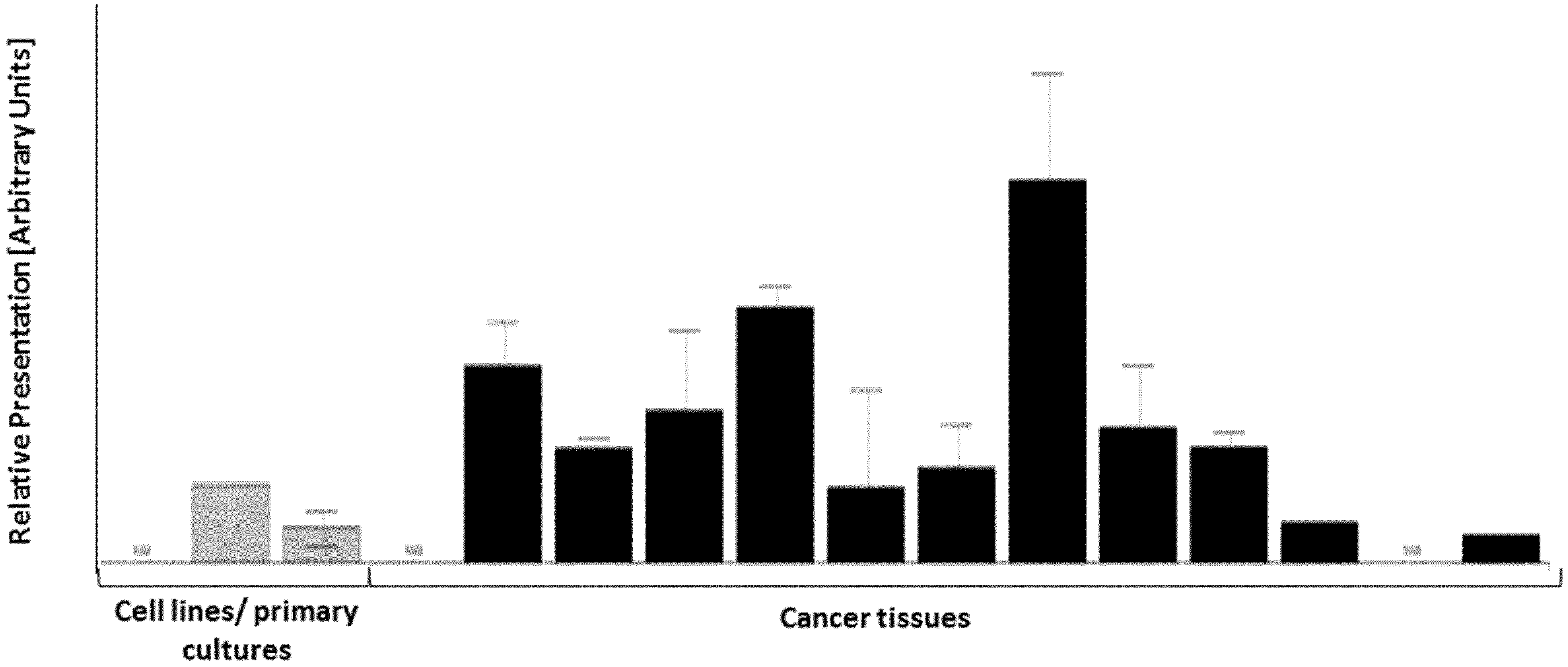
Peptide detected on

1 cell line, 2 primary cultures, 21 cancer tissues (1 bile duct cancer, 1 brain cancer, 1 breast cancer, 1 head and neck cancer, 2 kidney cancers, 3 leukocytic leukemia cancers, 2 liver cancers, 2 lung cancers, 2 lymph node cancers, 3 ovarian cancers, 1 rectum cancer, 1 skin cancer, 1 uterus cancer) (from left to right)

Figure 1H

Peptide: KTLDVDATYEI (A*02)

SEQ ID NO: 112

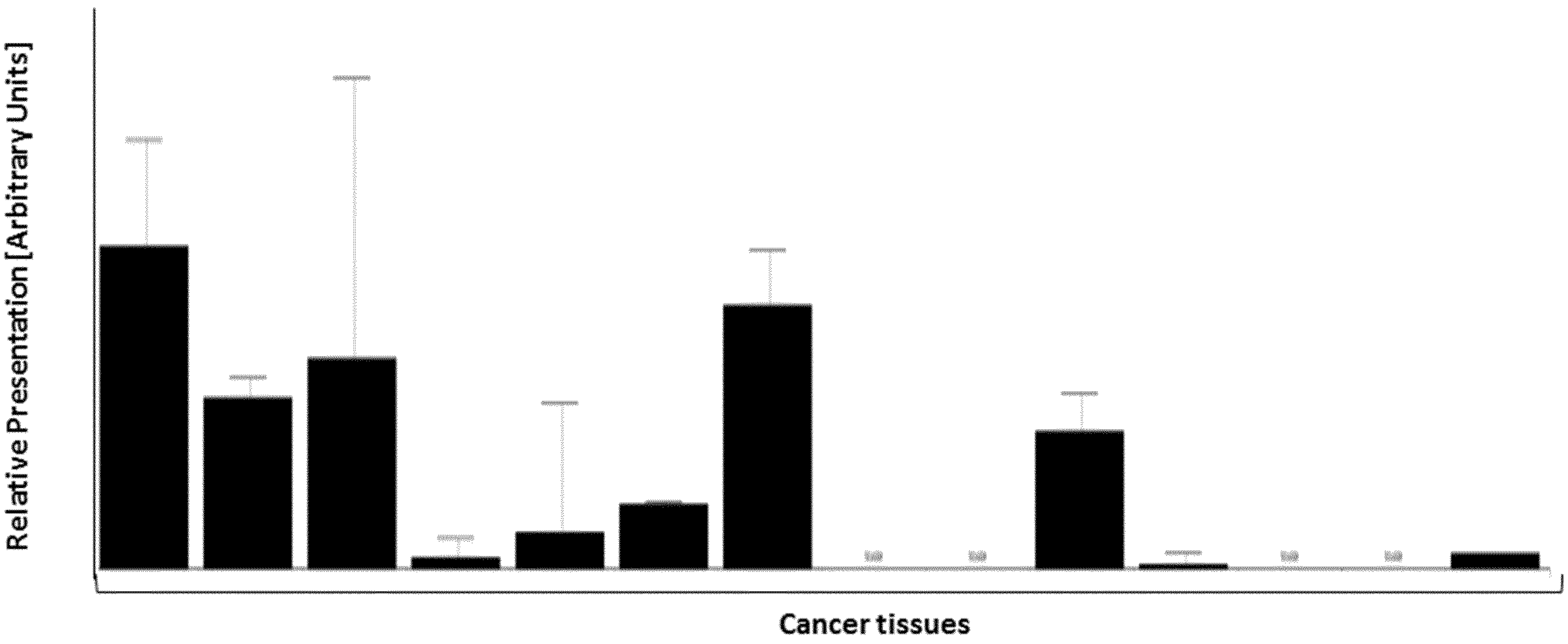


Peptide detected on
2 cell lines, 1 primary culture, 13 cancer tissues (1 esophageal cancer, 2 leukocytic leukemia cancers, 1 liver cancer, 3 lung cancers, 2 lymph node cancers, 2 ovarian cancers, 1 skin cancer, 1 uterus cancer) (from left to right)

Figure 1I

Peptide: SIIEGPIIKL (A*02)

SEQ ID NO: 146

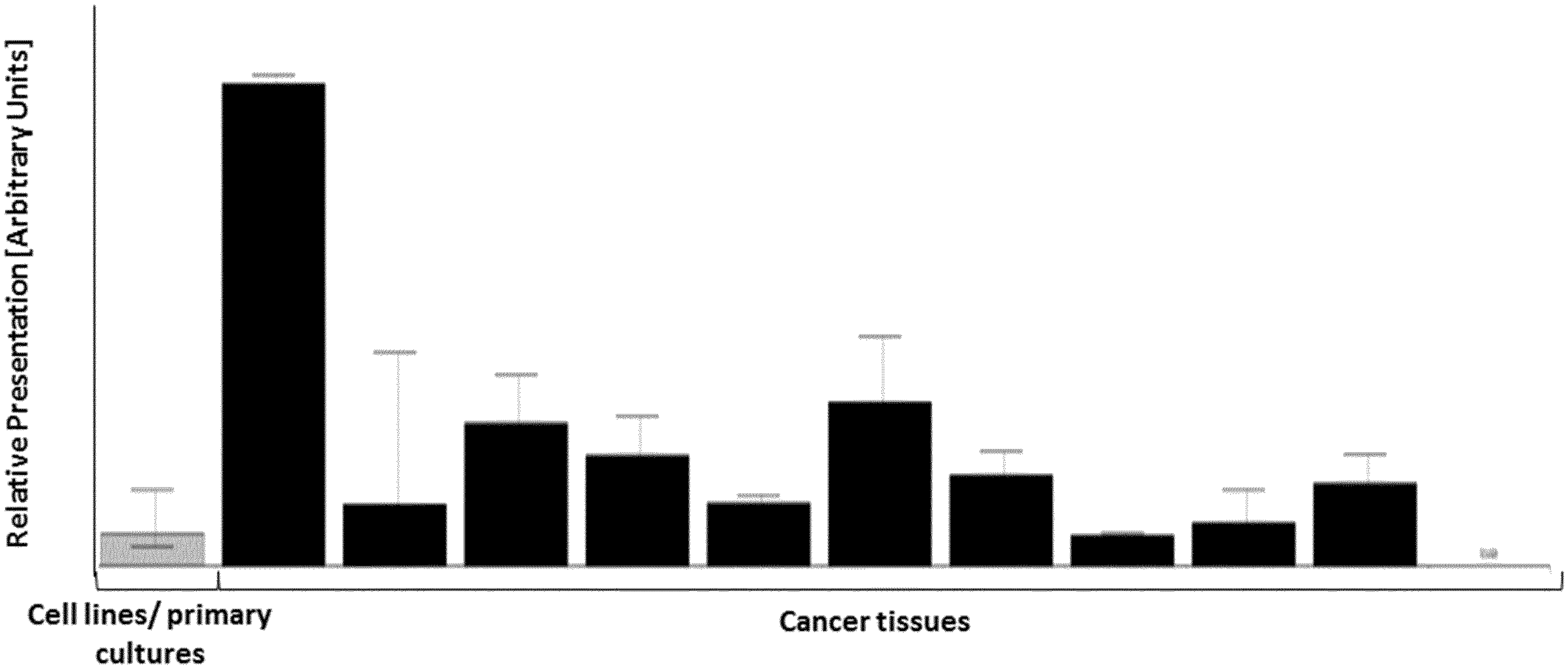


Peptide detected on
14 cancer tissues (1 head and neck cancer, 3 leukocytic leukemia cancers, 6 lung cancers, 1 lymph node cancer, 1 ovarian cancer, 2 urinary bladder cancers) (from left to right)

Figure 1J

Peptide: SILETVATL (A*02)

SEQ ID NO: 147



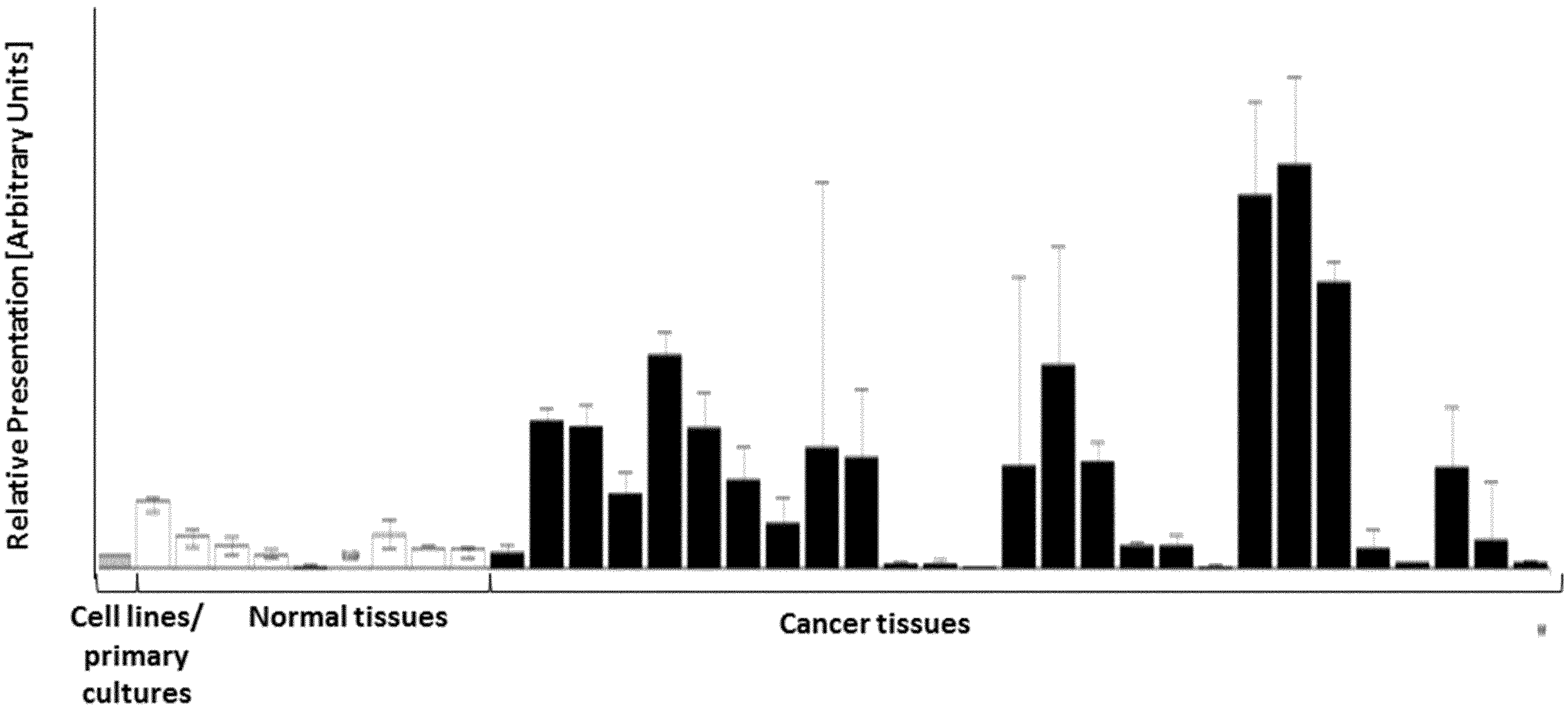
Peptide detected on

1 primary culture, 11 cancer tissues (1 colon cancer, 4 leukocytic leukemia cancers, 1 lung cancer, 3 lymph node cancers, 1 myeloid cell cancer, 1 skin cancer) (from left to right)

Figure 1K

Peptide: SLLAELHVL (A*02)

SEQ ID NO: 153



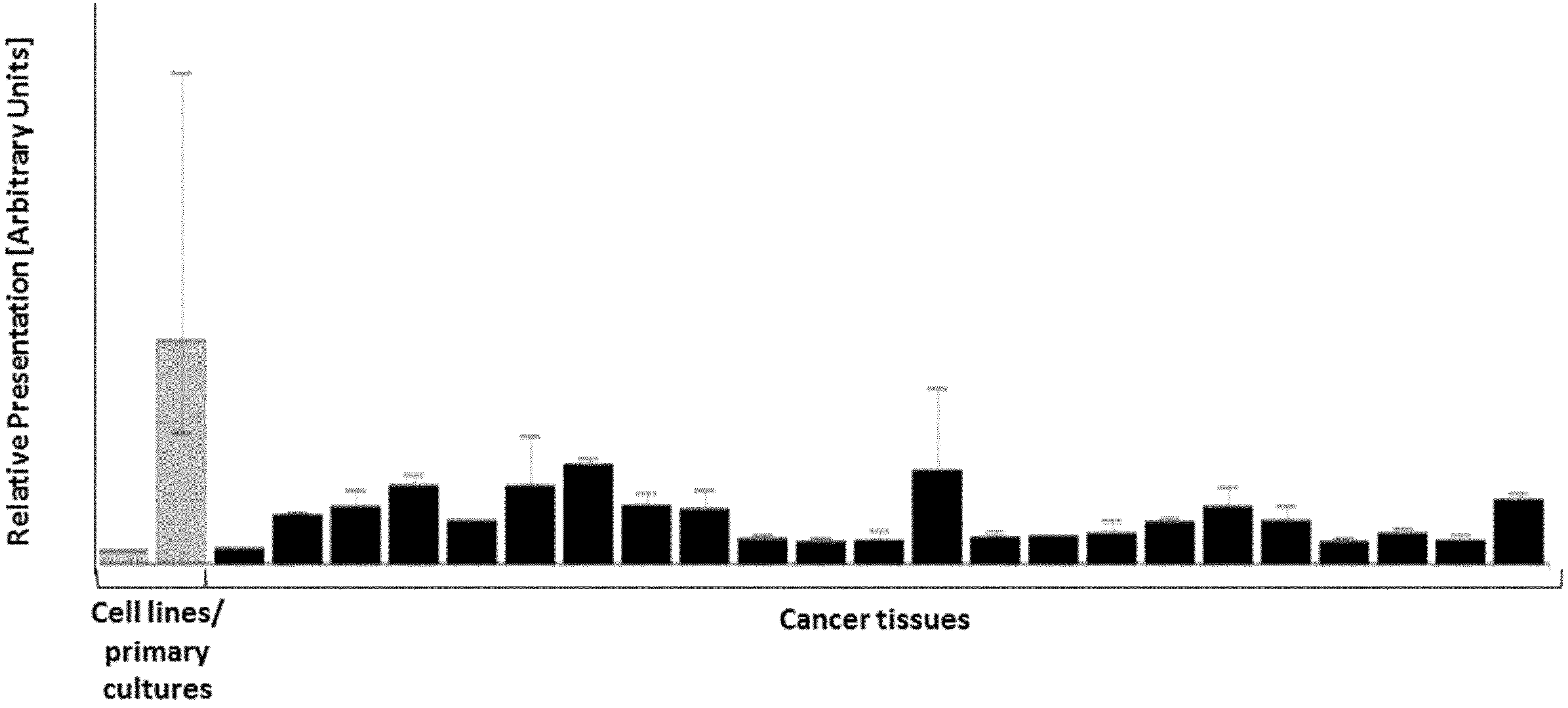
Peptide detected on

1 cell line, 9 normal tissues (1 lymph node, 1 rectum, 7 spleens), 27 cancer tissues (1 breast cancer, 1 kidney cancer, 10 leukocytic leukemia cancers, 14 lymph node cancers, 1 skin cancer) (from left to right)

Figure 1L

Peptide: SLQEEKLIYV (A*02)

SEQ ID NO: 159

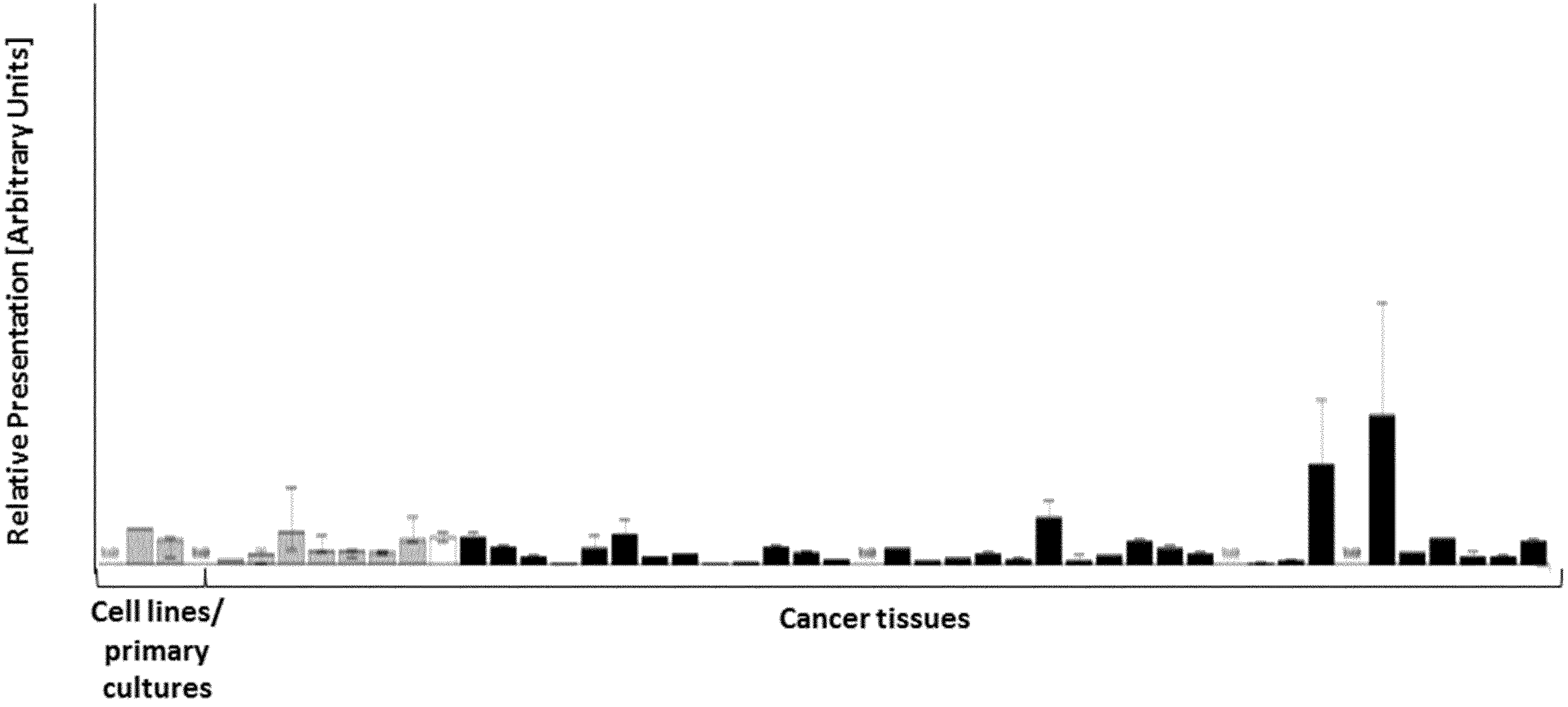


Peptide detected on
2 cell lines, 23 cancer tissues (5 brain cancers, 1 breast cancer, 1 esophageal cancer, 3 head and neck cancers, 2 leukocytic leukemia cancers, 5 lung cancers, 3 lymph node cancers, 1 skin cancer, 2 urinary bladder cancers) (from left to right)

Figure 1M

Peptide: SVHKGFAFV (A*02)

SEQ ID NO: 164



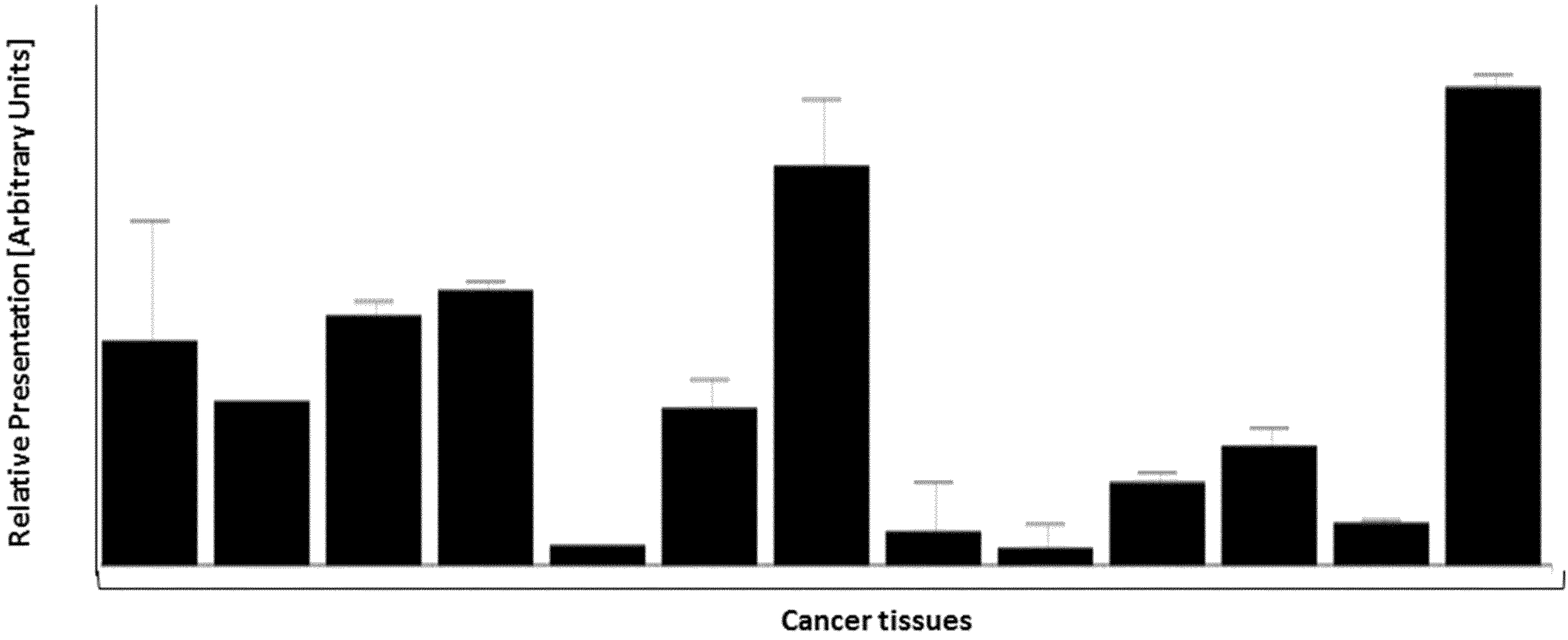
Peptide detected on

10 cell lines, 1 primary culture, 1 normal tissue (1 adrenal gland), 36 cancer tissues (12 brain cancers, 1 colon cancer, 2 head and neck cancers, 2 kidney cancers, 3 leukocytic leukemia cancers, 1 liver cancer, 5 lung cancers, 1 myeloid cell cancer, 1 ovarian cancer, 1 rectum cancer, 1 skin cancer, 2 stomach cancers, 2 urinary bladder cancers, 2 uterus cancers) (from left to right)

Figure 1N

Peptide: TLDTSKLYV (A*02)

SEQ ID NO: 167

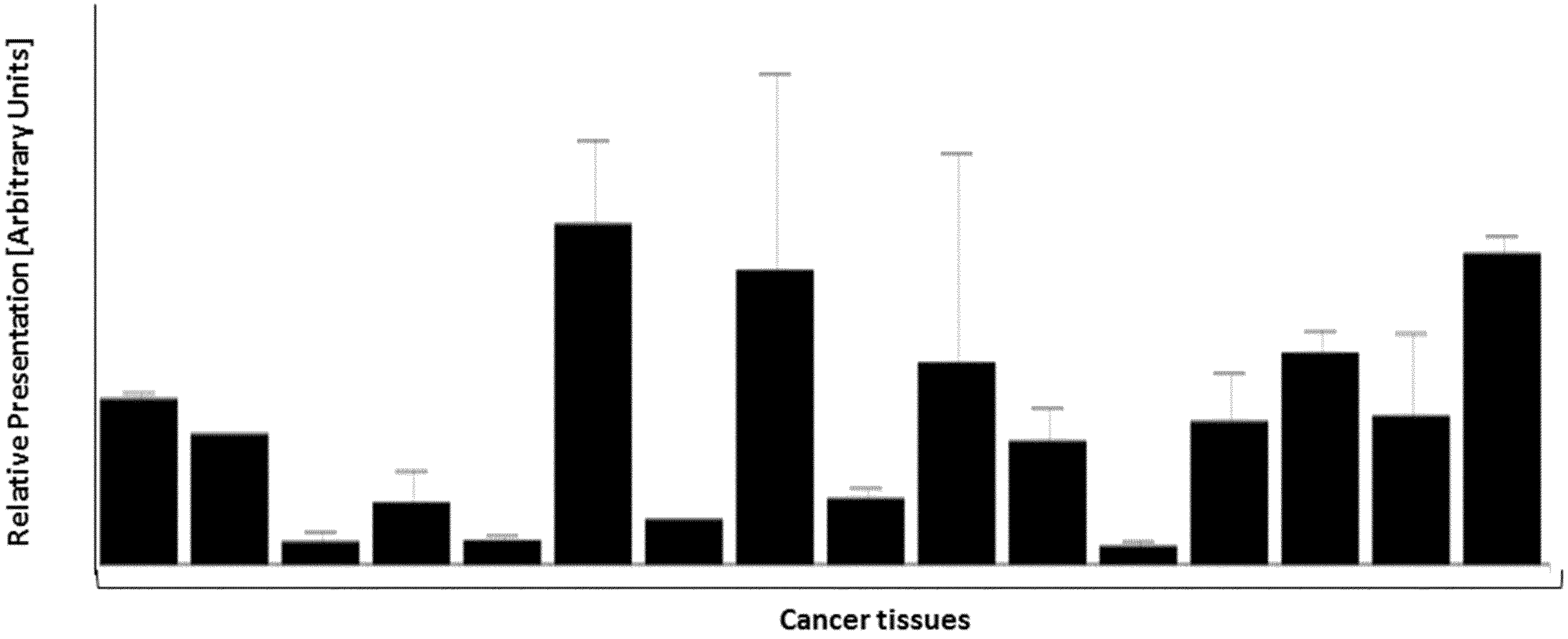


Peptide detected on
13 cancer tissues (1 brain cancer, 8 leukocytic leukemia cancers, 4 lymph node cancers) (from left to right)

Figure 1O

Peptide: YLLDQSFVM (A*02)

SEQ ID NO: 168

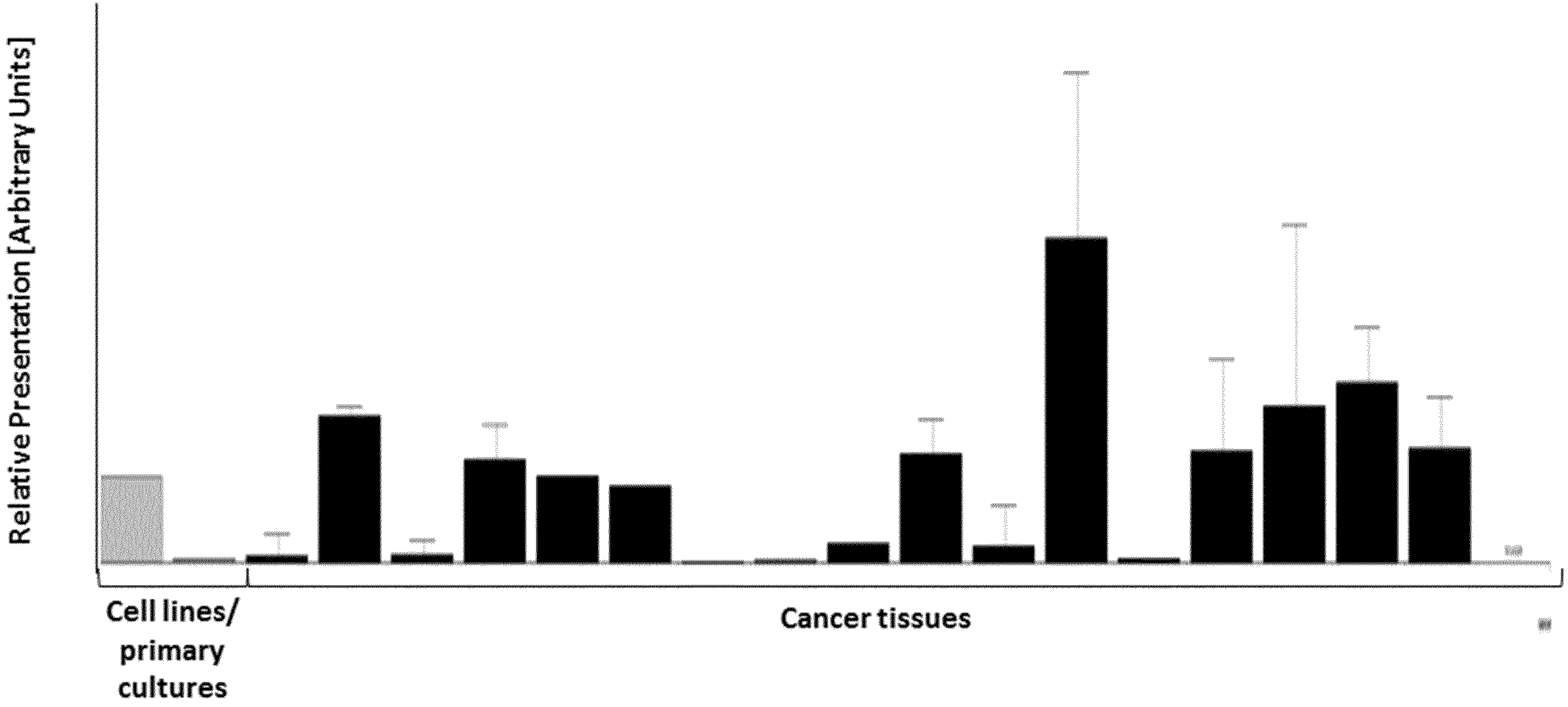


Peptide detected on
16 cancer tissues (10 leukocytic leukemia cancers, 6 lymph node cancers) (from left to right)

Figure 1P

Peptide: YIQEYLTLL (A*02)

SEQ ID NO: 192



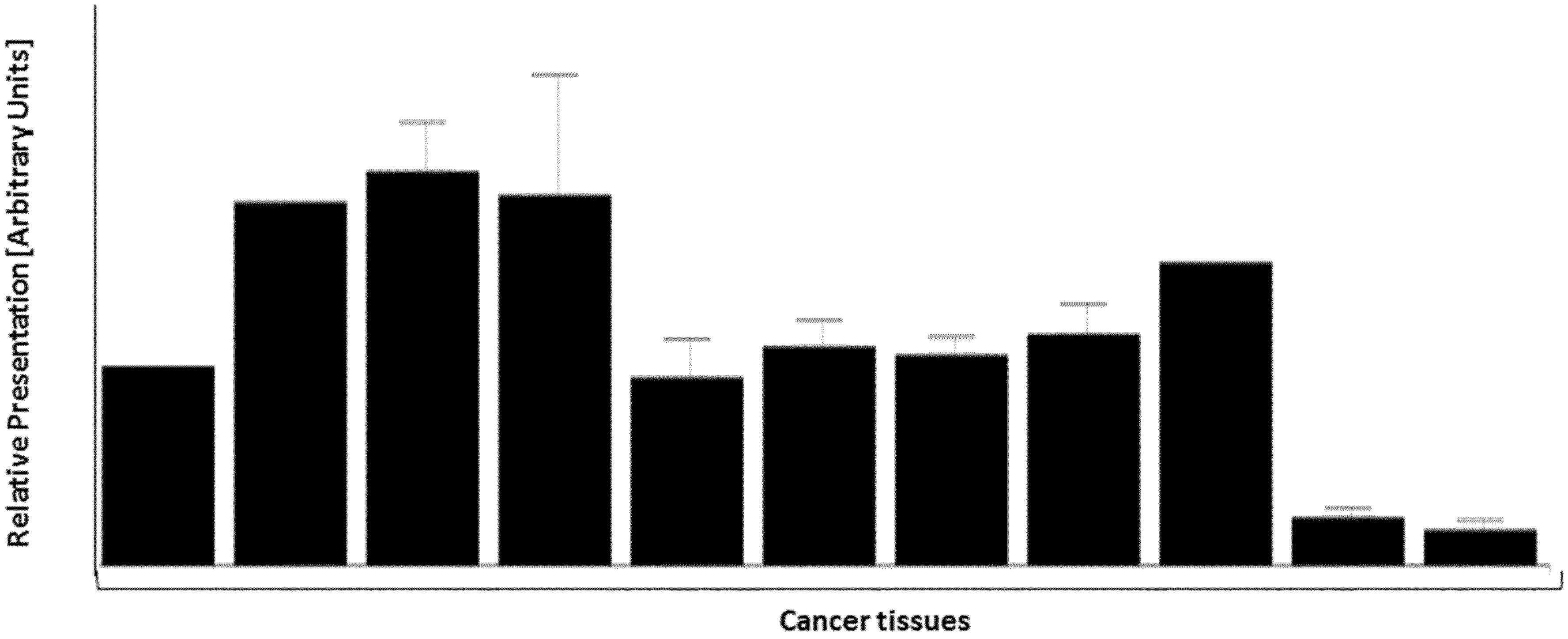
Peptide detected on

2 cell lines, 18 cancer tissues (1 colon cancer, 1 head and neck cancer, 1 kidney cancer, 4 leukocytic leukemia cancers, 5 lung cancers, 3 lymph node cancers, 1 myeloid cell cancer, 1 stomach cancer, 1 urinary bladder cancer) (from left to right)

Figure 1Q

Peptide: YLQEVPILTL (A*02)

SEQ ID NO: 197

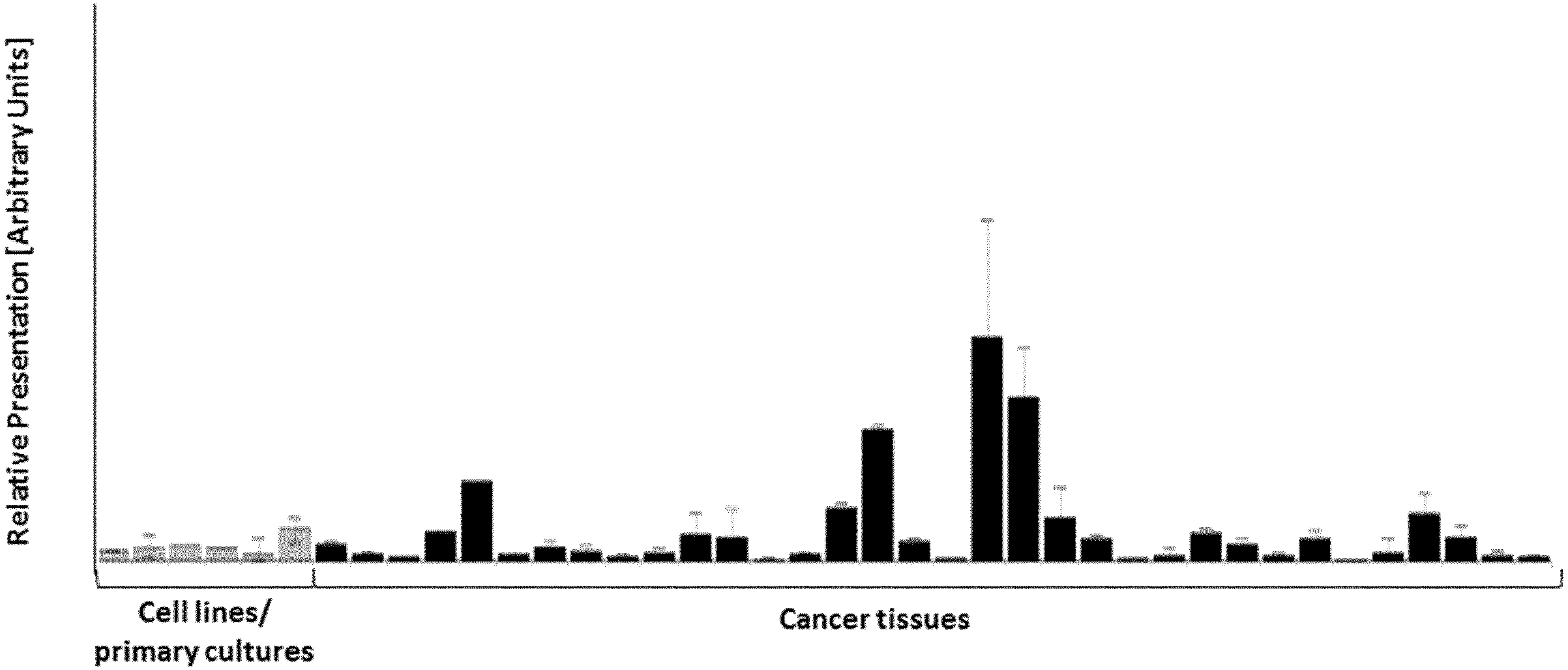


Peptide detected on
11 cancer tissues (5 leukocytic leukemia cancers, 4 lymph node cancers, 1 skin cancer, 1 uterus cancer) (from left to right)

Figure 1R

Peptide: YMFEEVPIVI (A*02)

SEQ ID NO: 200

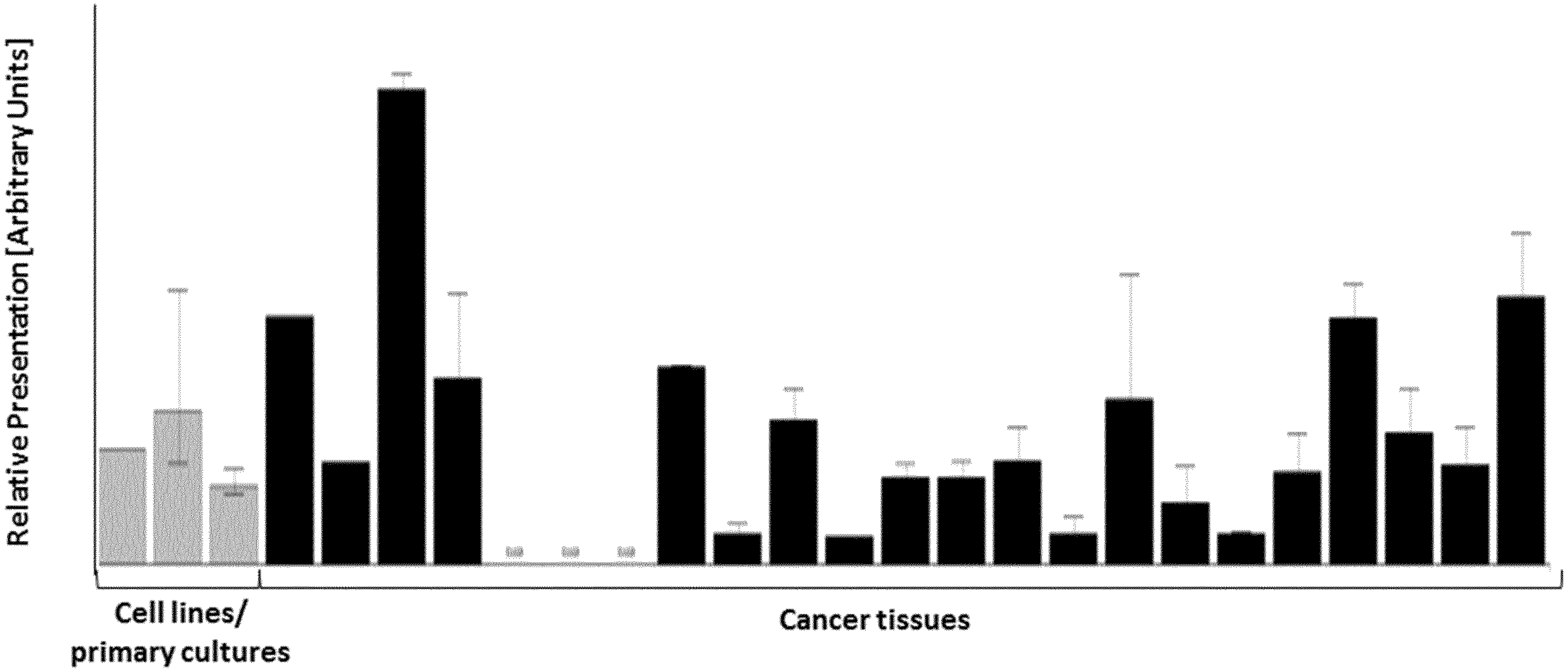


Peptide detected on
4 cell lines, 2 primary cultures, 34 cancer tissues (1 bile duct cancer, 2 breast cancers, 1 colon cancer, 1 esophageal cancer, 1 gallbladder cancer, 5 head and neck cancers, 2 leukocytic leukemia cancers, 2 liver cancers, 9 lung cancers, 4 lymph node cancers, 2 ovarian cancers, 1 rectum cancer, 2 skin cancers, 1 urinary bladder cancer) (from left to right)

Figure 1S

Peptide: LIDVKPLGV (A*02)

SEQ ID NO: 211

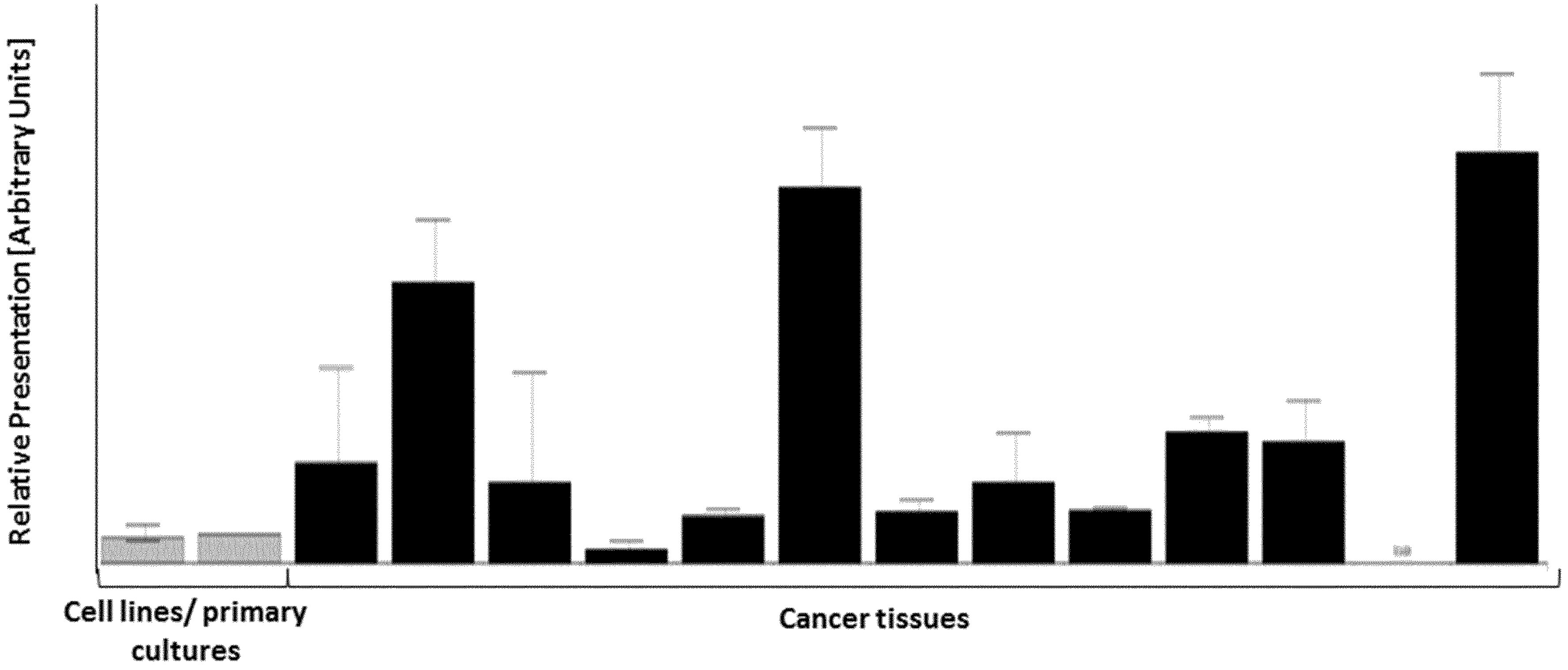


Peptide detected on
3 cell lines, 23 cancer tissues (5 brain cancers, 1 breast cancer, 1 esophageal cancer, 1 head and neck cancer, 1 kidney cancer, 3 leukocytic leukemia cancers, 2 liver cancers, 2 lung cancers, 4 lymph node cancers, 1 ovarian cancer, 1 prostate cancer, 1 skin cancer) (from left to right)

Figure 1T

Peptide: QLLPVSNVVSV (A*02)

SEQ ID NO: 256



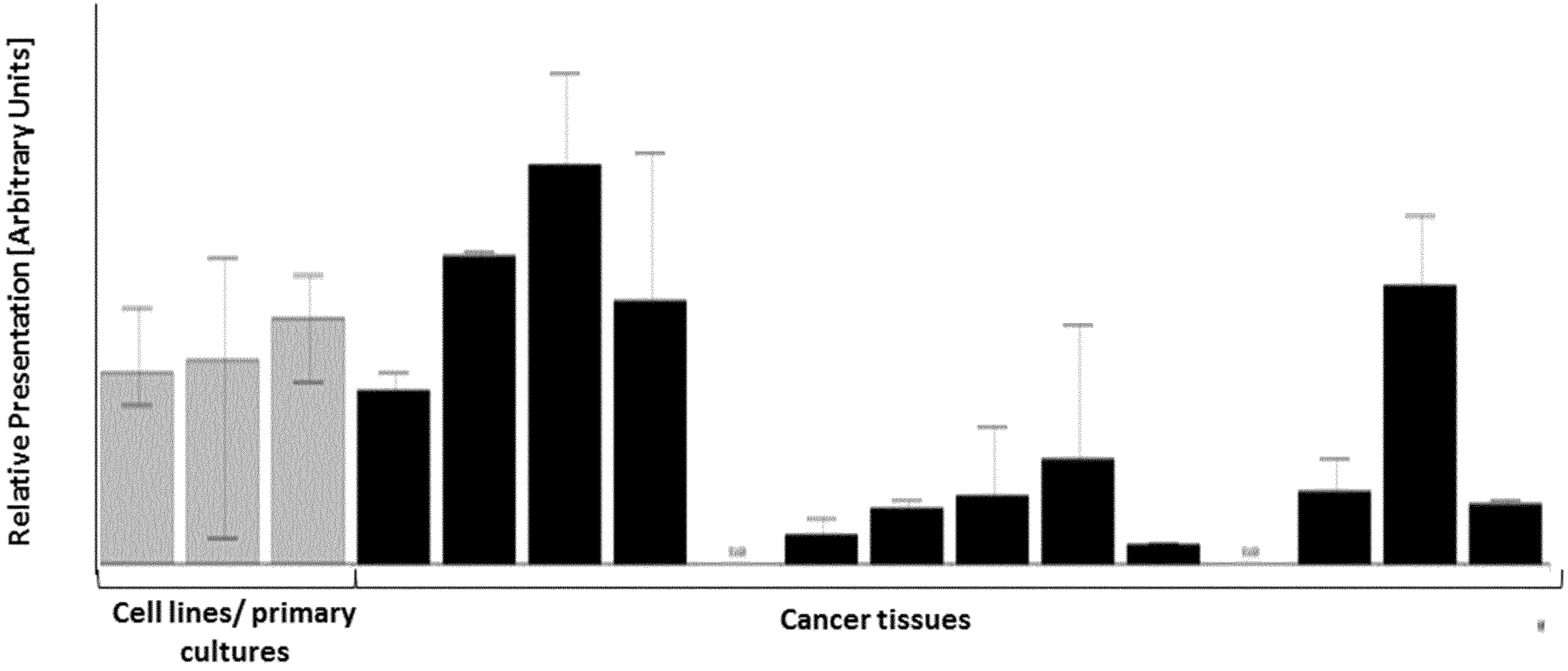
Peptide detected on

2 primary cultures, 13 cancer tissues (2 head and neck cancers, 3 leukocytic leukemia cancers, 1 liver cancer, 2 lung cancers, 4 lymph node cancers, 1 ovarian cancer) (from left to right)

Figure 1U

Peptide: LLYNSTDPTL (A*02)

SEQ ID NO: 312

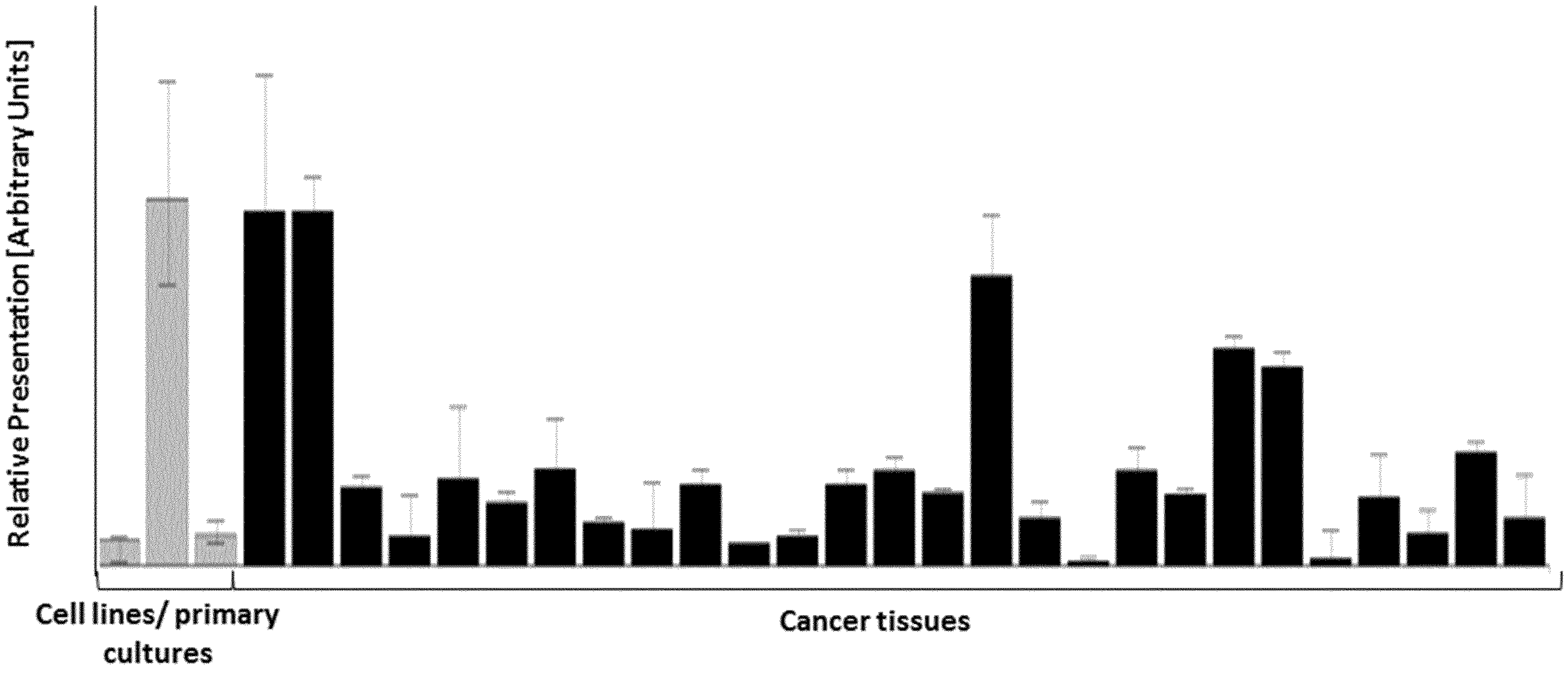


Peptide detected on
1 cell line, 2 primary cultures, 14 cancer tissues (1 colon cancer, 4 leukocytic leukemia cancers, 2 lung cancers, 5 lymph node cancers, 1 ovarian cancer, 1 urinary bladder cancer) (from left to right)

Figure 1V

Peptide: VLLPQETAEIHL (A*02)

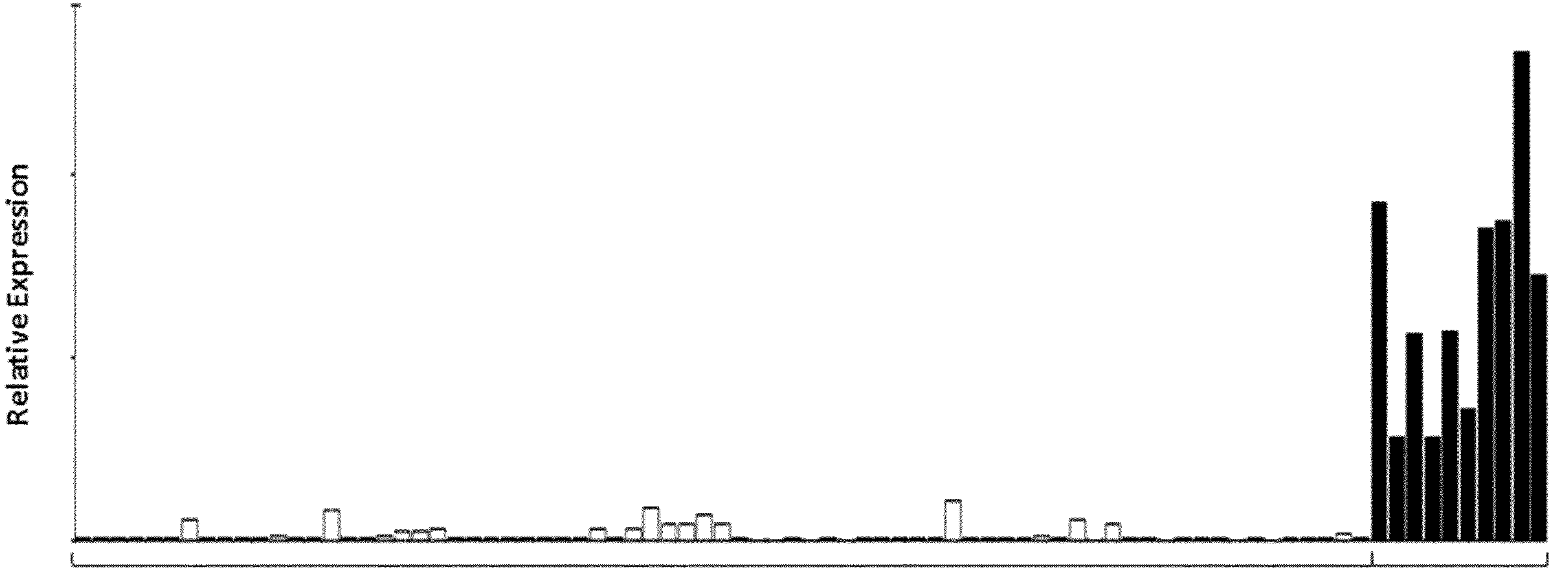
SEQ ID NO: 328



Peptide detected on
3 primary cultures, 27 cancer tissues (1 breast cancer, 1 colon cancer, 5 leukocytic leukemia cancers, 5 lung cancers, 7 lymph node cancers, 2 ovarian cancers, 2 skin cancers, 1 stomach cancer, 3 urinary bladder cancers) (from left to right)

Figure 2A

Gene: FCER2
(Peptide: ALWAGLLTL, SEQ ID NO: 21)

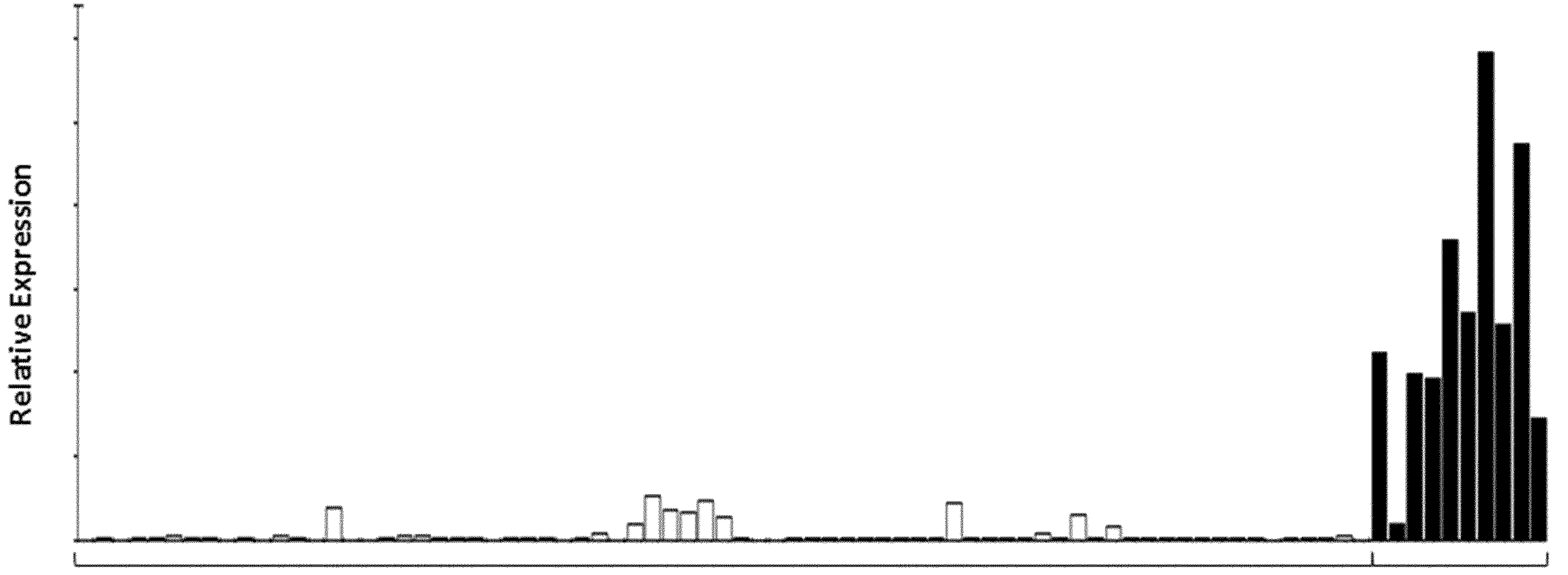


Normal tissue samples (each sample represents a pool of several donors): 6 arteries, 1 blood cells, 1 brain, 1 heart, 2 livers, 2 lungs, 2 veins, 1 adipose tissue, 1 adrenal gland, 6 bone marrows, 1 cartilage, 1 colon, 1 esophagus, 2 eyes, 2 gallbladders, 1 kidney, 6 lymph nodes, 5 pancreases, 2 pituitary glands, 1 rectum, 1 salivary gland, 1 skeletal muscle, 1 skin, 1 small intestine, 1 spleen, 1 stomach, 1 thyroid gland, 7 tracheas, 1 urinary bladder, 1 breast, 5 ovaries, 3 placentas, 1 prostate, 1 testis, 1 thymus, 1 uterus (from left to right)

10 CLL samples

Figure 2B

Gene: CLEC17A
(Peptide: GLKHDIARV, SEQ ID NO: 71)

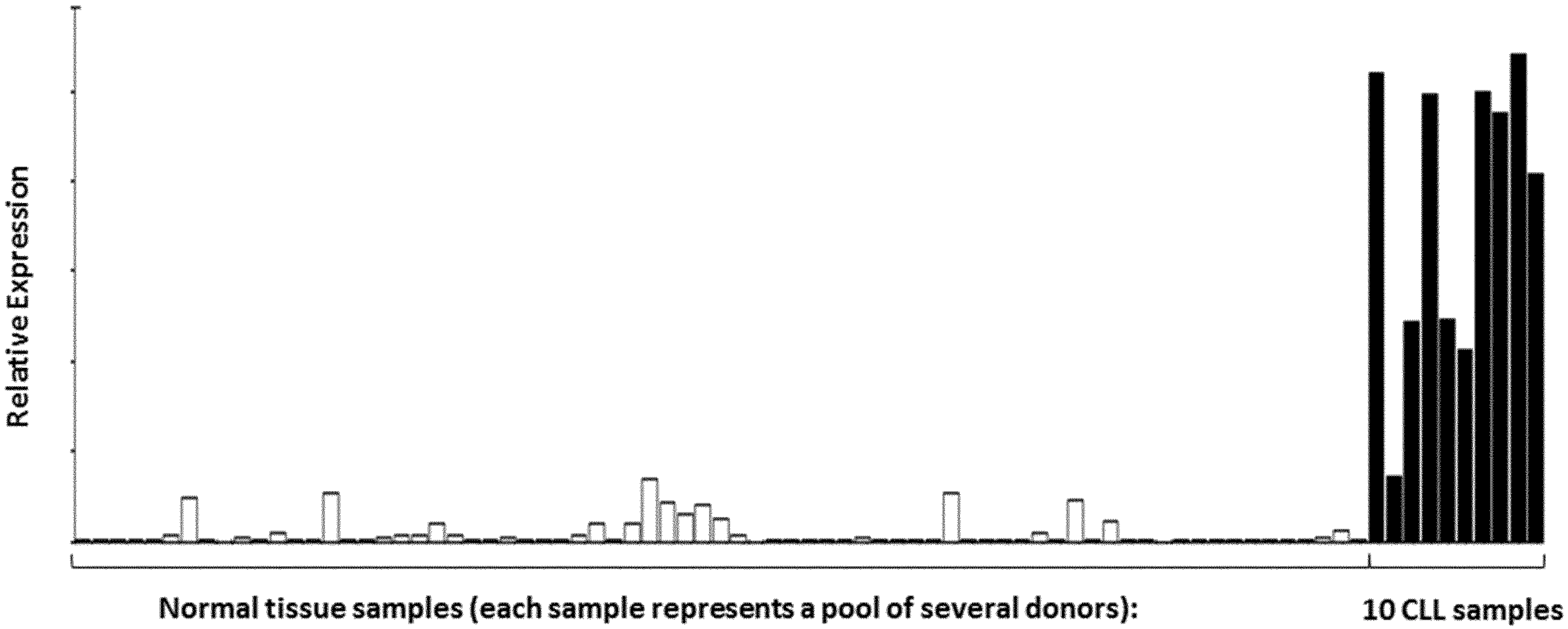


Normal tissue samples (each sample represents a pool of several donors): 6 arteries, 1 blood cells, 1 brain, 1 heart, 2 livers, 2 lungs, 2 veins, 1 adipose tissue, 1 adrenal gland, 6 bone marrows, 1 cartilage, 1 colon, 1 esophagus, 2 eyes, 2 gallbladders, 1 kidney, 6 lymph nodes, 5 pancreases, 2 pituitary glands, 1 rectum, 1 salivary gland, 1 skeletal muscle, 1 skin, 1 small intestine, 1 spleen, 1 stomach, 1 thyroid gland, 7 tracheas, 1 urinary bladder, 1 breast, 5 ovaries, 3 placentas, 1 prostate, 1 testis, 1 thymus, 1 uterus (from left to right)

10 CLL samples

Figure 2C

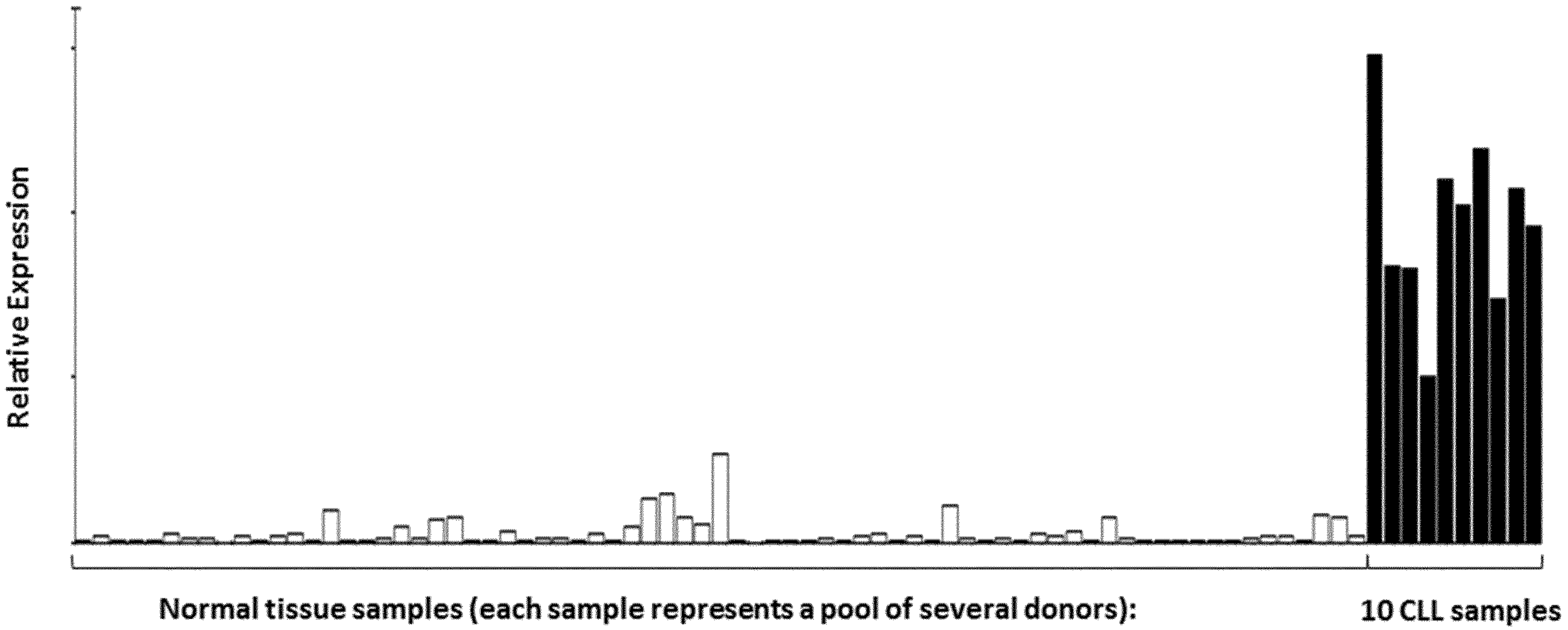
Gene: PAX5
(Peptide: GLDDMKANL, SEQ ID NO: 65)



Normal tissue samples (each sample represents a pool of several donors): 6 arteries, 1 blood cells, 1 brain, 1 heart, 2 livers, 2 lungs, 2 veins, 1 adipose tissue, 1 adrenal gland, 6 bone marrows, 1 cartilage, 1 colon, 1 esophagus, 2 eyes, 2 gallbladders, 1 kidney, 6 lymph nodes, 5 pancreases, 2 pituitary glands, 1 rectum, 1 salivary gland, 1 skeletal muscle, 1 skin, 1 small intestine, 1 spleen, 1 stomach, 1 thyroid gland, 7 tracheas, 1 urinary bladder, 1 breast, 5 ovaries, 3 placentas, 1 prostate, 1 testis, 1 thymus, 1 uterus (from left to right)

Figure 2D

Gene: KIAA0226L
(Peptide: GIIDGSPRL, SEQ ID NO: 62)



Normal tissue samples (each sample represents a pool of several donors): 6 arteries, 1 blood cells, 1 brain, 1 heart, 2 livers, 2 lungs, 2 veins, 1 adipose tissue, 1 adrenal gland, 6 bone marrows, 1 cartilage, 1 colon, 1 esophagus, 2 eyes, 2 gallbladders, 1 kidney, 6 lymph nodes, 5 pancreases, 2 pituitary glands, 1 rectum, 1 salivary gland, 1 skeletal muscle, 1 skin, 1 small intestine, 1 spleen, 1 stomach, 1 thyroid gland, 7 tracheas, 1 urinary bladder, 1 breast, 5 ovaries, 3 placentas, 1 prostate, 1 testis, 1 thymus, 1 uterus (from left to right)

Figure 3

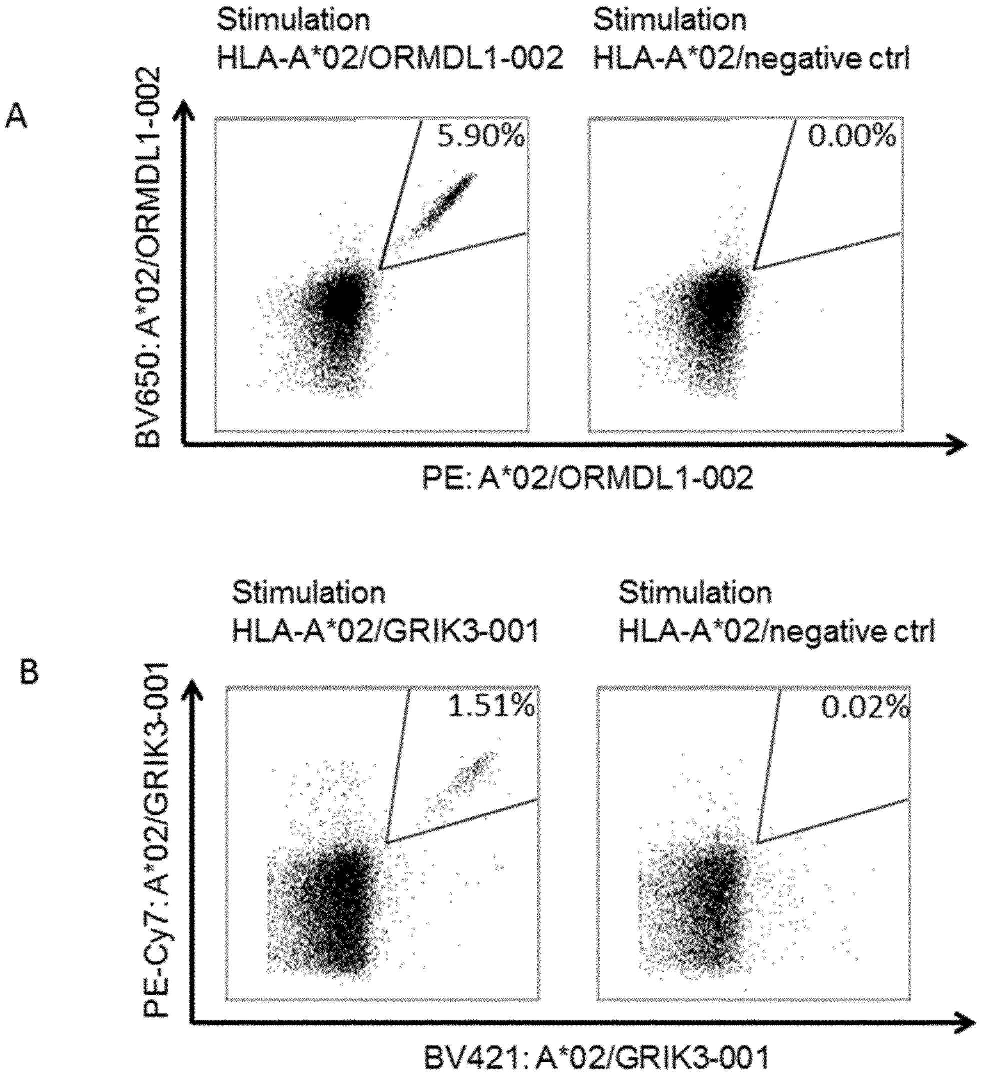

A
Stimulation
HLA-A*02/ORMDL1-002
Stimulation
HLA-A*02/negative ctrl
BV650: A*02/ORMDL1-002
5.90%
0.00%
PE: A*02/ORMDL1-002
B
Stimulation
HLA-A*02/GRIK3-001
Stimulation
HLA-A*02/negative ctrl
PE-Cy7: A*02/GRIK3-001
1.51%
0.02%
BV421: A*02/GRIK3-001

Figure 3

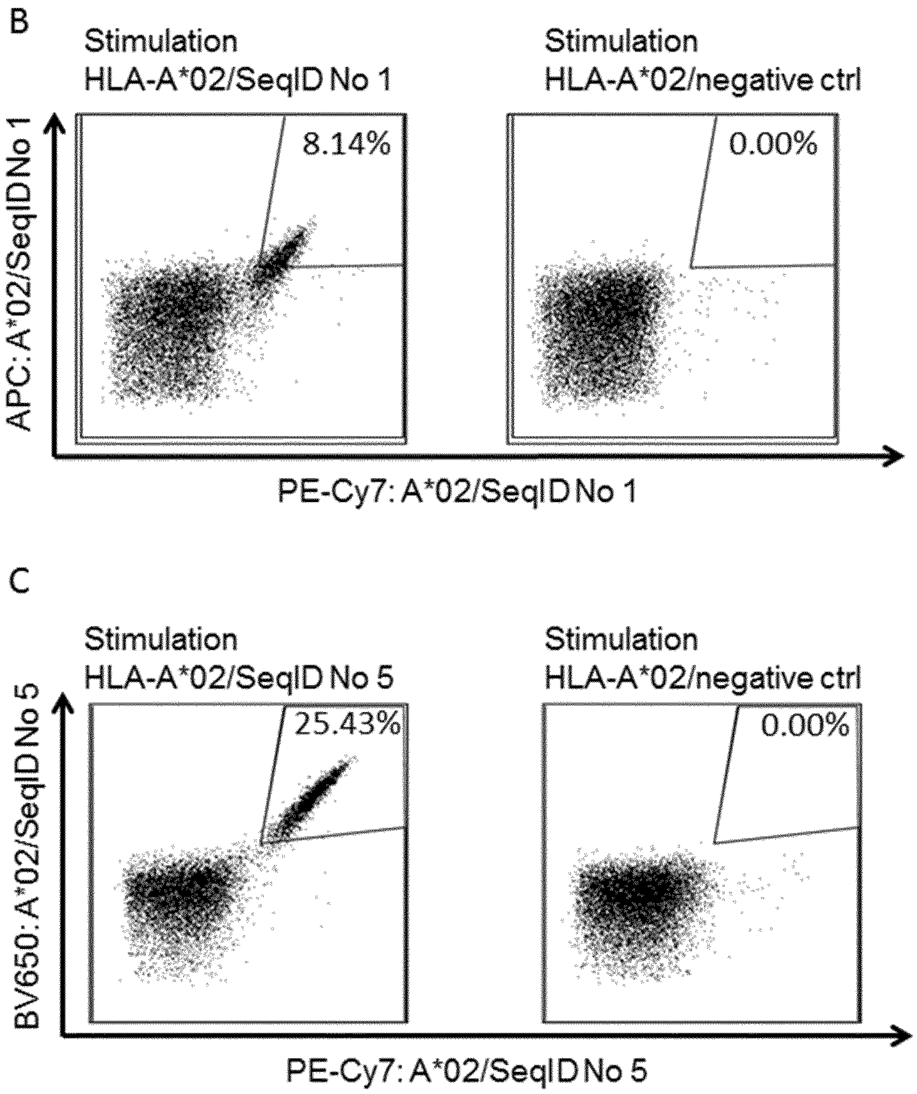
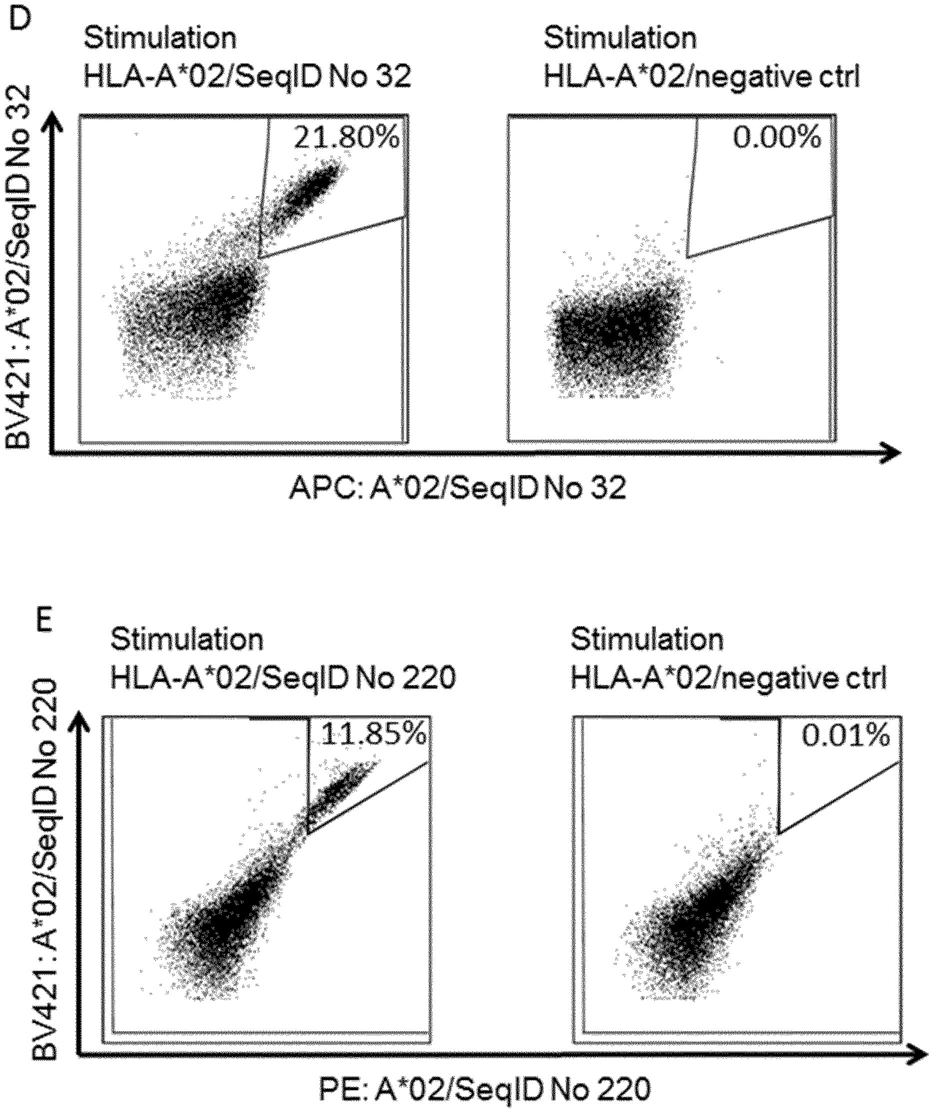

B
Stimulation
HLA-A*02/SeqID No 1
Stimulation
HLA-A*02/negative ctrl
8.14%
0.00%
APC: A*02/SeqID No 1
PE-Cy7: A*02/SeqID No 1
C
Stimulation
HLA-A*02/SeqID No 5
Stimulation
HLA-A*02/negative ctrl
25.43%
0.00%
BV650: A*02/SeqID No 5
PE-Cy7: A*02/SeqID No 5

Figure 3


D
Stimulation
HLA-A*02/SeqID No 32
Stimulation
HLA-A*02/negative ctrl
21.80%
0.00%
BV421: A*02/SeqID No 32
APC: A*02/SeqID No 32
E
Stimulation
HLA-A*02/SeqID No 220
Stimulation
HLA-A*02/negative ctrl
11.85%
0.01%
BV421: A*02/SeqID No 220
PE: A*02/SeqID No 220

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/078718

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

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

EPO-Internal, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/073905 A1 (KONINKL PHILIPS ELECTRONICS NV [NL]; HOFFMANN RALF [DE]) 23 June 2011 (2011-06-23) paragraphs [0201], [0338], [0345] - [0353]; claims 1, 3 -----	1-20, 30-39
X	US 2012/183552 A1 (JOSELOFF ELIZABETH [US] ET AL) 19 July 2012 (2012-07-19) page 117, line 32 - page 120, line 2 -----	1-20, 30-39
A	EP 2 330 122 A1 (ASKLEPIOS KLINIKEN HAMBURG GMBH [DE]) 8 June 2011 (2011-06-08) the whole document ----- -/--	1-20, 30-39

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

☒ See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2017	05/04/2017

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer
Jacques, Patrice

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International application No.
PCT/EP2016/078718

INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-20, 30-39

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

International application No
PCT/EP2016/078718

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/063302 A2 (IMMATICS BIOTECHNOLOGIES GMBH [DE]) 7 May 2015 (2015-05-07) the whole document -----	1-20, 30-39
A	WO 2015/018805 A1 (IMMATICS BIOTECHNOLOGIES GMBH [DE]) 12 February 2015 (2015-02-12) the whole document -----	1-20, 30-39

2

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011073905 A1	23-06-2011	NONE	
US 2012183552 A1	19-07-2012	US 7608413 B1	27-10-2009
		US 2010239581 A1	23-09-2010
		US 2012183552 A1	19-07-2012
		US 2014314770 A1	23-10-2014
		US 2016362461 A1	15-12-2016
EP 2330122 A1	08-06-2011	EP 2330122 A1	08-06-2011
		EP 2507260 A1	10-10-2012
		WO 2011067175 A1	09-06-2011
WO 2015063302 A2	07-05-2015	AU 2014343599 A1	26-05-2016
		CA 2929445 A1	07-05-2015
		CN 105793280 A	20-07-2016
		CU 20160060 A7	02-02-2017
		EA 201690868 A1	31-08-2016
		EP 3066115 A2	14-09-2016
		JP 2017502076 A	19-01-2017
		KR 20160079102 A	05-07-2016
		SG 11201603299P A	30-05-2016
		US 2015125477 A1	07-05-2015
		WO 2015063302 A2	07-05-2015
WO 2015018805 A1	12-02-2015	AU 2014304544 A1	03-12-2015
		CA 2912500 A1	12-02-2015
		CN 105377290 A	02-03-2016
		EA 201690016 A1	31-05-2016
		EP 3030255 A1	15-06-2016
		JP 2016527293 A	08-09-2016
		KR 20160038889 A	07-04-2016
		PH 12015502638 A1	07-03-2016
		SG 11201510580U A	26-02-2016
		TW 201506043 A	16-02-2015
		US 2016168200 A1	16-06-2016
		WO 2015018805 A1	12-02-2015

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-20, 30-39

The subject-matter of all said claims 1-20, 30-39 when relating to a peptide comprising an amino acid sequence consisiting of SEQ ID No. 1 and variant sequences thereof as defined in claim 1.

2-385. claims: 1-20, 30-39

the subject-matter of all said claims 1-20, 30-39 when relating respectively to a peptide comprising an amino acid sequence consisiting of SEQ ID No. 2 to 385, and variant sequences thereof as defined in claim 1, each said peptide representing a different invention.

---

386. claims: 21-29

A method for producing a personalized anti-cancer vaccine or a compound-based and/or cellular therapy for an individual patient as defined in claim 21.

摘要:　本發明涉及用於免疫治療方法的肽、蛋白、核酸和細胞。特別是，本發明涉及癌症的免疫療法。本發明還涉及單獨使用或與其他腫瘤相關肽(能夠例如作為刺激抗腫瘤免疫反應或體外刺激 T 細胞並轉入患者的疫苗組合物的活性藥物成分)聯合使用的腫瘤相關 T 細胞(CTL)肽表位。與主要組織兼容性複合體(MHC)分子結合的肽或與此同類的肽也可以是抗體、可溶性 T 細胞受體和其他結合分子的靶標。