



(51) International Patent Classification:

A61K 39/00 (2006.01) G01N 33/50 (2006.01)

C07K 14/47 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/EP2018/059114

(22) International Filing Date:

10 April 2018 (10.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/483,690 10 April 2017 (10.04.2017) US

10 2017 107 710.3

10 April 2017 (10.04.2017) DE

(71) Applicant: IMMATICS BIOTECHNOLOGIES GMBH

[DE/DE]; Paul-Ehrlich-Straße 15, 72076 Tuebingen (DE).

(72) Inventors: WALZ, Juliane Sarah; Gartenstrasse 71,

72076 Tuebingen (DE). KOWALEWSKI, Daniel; Den-

zenbergstrasse 29, 72074 Tuebingen (DE). LÖFFLER,

Markus; Sandgrabenstr. 20, 72119 Ammerbuch (DE). DI

MARCO, Moreno; Memmingerstr. 18/1, 72072 Tuebingen

(DE). TRAUTWEIN, Nico; Pelargusstr. 2, 70180 Stuttgart

(DE). NELDE, Annika; Viktor-Renner-Straße 27, 72074

Tuebingen (DE). STEVANOVIC, Stefan; Auf der Burg

3, 72074 Tuebingen (DE). RAMMENSEE, Hans-Georg;

Sommerhalde 3, 72070 Unterjesingen (DE). HAEN, Sebas-

tian; Brombergstr. 15, 72070 Tuebingen (DE).

(74) Agent: KRAUSS, Jan B.; Boehmert & Boehmert, Pet-

tenkoferstrasse 20 - 22, 80336 Munich (DE).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,

MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,

TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: PEPTIDES AND COMBINATION OF PEPTIDES FOR USE IN IMMUNOTHERAPY AGAINST LEUKEMIAS 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.



Peptides and combination of peptides for use in immunotherapy against leukemias 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

According to the World Health Organization (WHO), cancer ranged among the four major non-communicable deadly diseases worldwide in 2012. For the same year, colorectal cancer, breast cancer and respiratory tract cancers were listed within the top 10 causes of death in high income countries (<http://www.who.int/mediacentre/factsheets/fs310/en/>).

Epidemiology

In 2012, 14.1 million new cancer cases, 32.6 million patients suffering from cancer (within 5 years of diagnosis) and 8.2 million cancer deaths were estimated worldwide (Ferlay et al., 2013; Bray et al., 2013).

- 2 -

Within the group of leukemia, the current invention specifically focuses on chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML) and acute myeloid leukemia (AML).

CLL is the most common leukemia in the Western world where it comprises about one third of all leukemia. 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%.

AML is the second most common type of leukemia diagnosed in both adults and children. Estimated new cases in the United States are about 21,000 per year. The five-year survival rate of people with AML is approximately 25%.

CML accounts for 15-25% of adult leukemias with incidences ranging at 1.2/100,000 in Europe and 1.75/100,000 in the US (Hoglund et al., 2015). The number of estimated new CML cases in the US are 8,220 for the year 2016 with an estimated number of 1,070 deaths (National Cancer Institute, 2015). Since the introduction of imatinib in the year 2000, annual mortality in CML has decreased from 10-20% down to 1-2%. As a consequence, prevalence of CML in the US has increased from an estimated 25,000-30,000 cases in 2000 to 80,000-100,000 in 2015 (Huang et al., 2012).

Therapy

Chronic lymphocytic leukemia - 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, chimeric antigen-receptors (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 deletions at chromosome 17p (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) compared to the combination fludarabine/cyclophosphamide and has become the preferred treatment option (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-tetulumab) 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 tumor associated antigen (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 and 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 over-expressed 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 (OFALRP) (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).

Chronic myeloid leukemia - CML is a myeloproliferative neoplasm characterized by the (9;22)(q34;q11.2) chromosomal translocation resulting in *BCR-ABL1* gene fusion. The resultant BCR-ABL1 fusion protein shows dysregulated tyrosine kinase activity and

plays a central role in the pathogenesis and maintenance of CML (Lugo et al., 1990). The discovery of this molecular mechanism of CML leukemogenesis led to the development and successful clinical application of the BCR-ABL1 specific tyrosine kinase inhibitors (TKI), which was spearheaded by approval of imatinib for the first-line treatment of newly diagnosed CML in 2002 (Johnson et al., 2003). The introduction of TKIs drastically altered patient management and clinical outcome in CML and led to a vastly improved life expectancy of CML patients (Schmidt, 2016). However, TKI therapy has to be maintained lifelong which increases the risk of developing secondary resistance (Khorashad et al., 2013) and comes at significant cost (Kantarjian et al., 2013). Furthermore, albeit generally well tolerated, distinct toxicity profiles may prohibit application of certain TKI in patients with comorbidities and may lead to rare but severe adverse events (Jabbour and Kantarjian, 2016). Currently, the only curative therapy for CML is allogeneic stem cell transplantation, which due to significant morbidity and mortality is confined to patients diagnosed in advanced phase or used as a salvage option for patients that failed multiple TKI (Horowitz et al., 1996; Radich, 2010).

CML is classified into three clinical phases of chronic phase, accelerated phase and blast crisis based on the frequency of CML blasts in blood and bone marrow. Around 90% of patients are diagnosed in the initial chronic phase, with around 50% of all newly diagnosed patients being asymptomatic (Jabbour and Kantarjian, 2016). Before the introduction of TKI, CML drug therapy was limited to unspecific agents such as combination chemotherapy with the alkylating agent busulfan and the ribonucleotide reductase inhibitor hydroxyurea or treatment with interferon-alfa (IFN- α). In these settings, the chronic phase had a median duration of 3 to 5 years followed by an accelerated phase of 3-6 months, which generally terminated fatally (Silver et al., 1999). Although IFN- α induced disease regression and improved survival in a subset of patients it was hindered by significant toxicity and lack of efficacy (Kujawski and Talpaz, 2007) and was ultimately replaced by TKI therapy. The advent of targeted therapy for CML using TKI drastically altered the course of disease and increased the 10-year survival rate from approximately 20% to 80-90% (Jabbour and Kantarjian, 2016).

Currently five TKIs are approved in the US for the treatment of CML patients. The standard first-line therapy of chronic phase CML usually consists treatment with either the first-generation TKI Imatinib (O'Brien et al., 2003) or one of the second generation TKIs nilotinib (Saglio et al., 2010) or dasatinib (Kantarjian et al., 2010). Bosutinib (Cortes et al., 2012) and ponatinib are indicated for patients with intolerance or resistance to prior TKI.

Despite high response rates and deep responses, primary and secondary resistance to TKI therapy has been observed in CML patients. The best characterized resistance mechanisms are genomic amplification of *BCR-ABL1* or mutations to the BCR-ABL1 kinase domain (Khorashad et al., 2013). Mutation analysis is typically performed after progression under TKI therapy and may guide the selection of subsequent TKIs (Soverini et al., 2011). For patients showing resistance or intolerance to two or more TKIs, the unspecific protein translation inhibitor omacetaxine is approved in the US (Gandhi et al., 2014), although TKI are generally the preferred option (Soverini et al., 2011). Omacetaxine is a BCR-ABL1 independent drug which has been shown to induce apoptosis in primary CML stem cells through downregulation of the anti-apoptotic Mcl1-1 protein (Allan et al., 2011).

Cessation of imatinib treatment has been investigated in the Stop Imatinib (STIM) trial, which enrolled patients with complete molecular responses (CMR) of more than two years (Mahon et al., 2010). In a recent update, 61% of patients had experienced a molecular relapse, with 95% of events occurring within 7 months of stopping imatinib. Of note, almost all patients remained sensitive to imatinib and achieved CMR once imatinib was restarted. Similar observations were made in the TWISTER trial (Ross et al., 2013). However, while these studies demonstrate that stopping TKI therapy is feasible and some patients may be cured, TKI discontinuation still should only be performed under within the confines of clinical trials and requires rigorous patient monitoring (Jabbour and Kantarjian, 2016). The main reason for molecular relapse after discontinuation of TKI therapy is the presence of minimal residual disease (MRD), i.e. the persistence of residual CML stem cells in the bone marrow (Bhatia et al., 2003). These CML stem cells

have been shown to persist independently of BCR-ABL1 activity and thus cannot effectively be eliminated by TKI therapy (Corbin et al., 2011).

Together, this calls for novel therapeutic strategies targeting the BCR-ABL1 independent pool of CML stem cells to achieve eradication of MRD. The fact that allogeneic stem cell transplantation and unspecific immunotherapy with IFN- α can induce long-term remissions in a subset of patients indicates that immunological targeting of CML is a viable therapeutic option. Antigen-specific immunotherapy targeting BCR-ABL junction peptides has been shown to induce of peptide-specific T cell responses followed by antitumor effects in a substantial subset of patients in two independent clinical phase II vaccination trials (Bocchia et al., 2005; Cathcart et al., 2004). However, targeting of these CML-specific targets is confined to a subset of the patient collective expressing the appropriate HLA allotypes. Other targets of CML immunotherapy include overexpressed antigens such as the zinc finger transcription factor WT1, which has been shown to induce cytotoxic CD8 T cells capable of killing WT1⁺ in clinical vaccination trials of myeloid malignancies (Rezvani et al., 2008; Keilholz et al., 2009). Furthermore, a T cell receptor-like antibody specific for the WT1-derived HLA-A*02 peptide RMFPNAPYL has been shown to mediate antibody-dependent cellular cytotoxicity in human leukemia xenografts (Dao et al., 2013). Further leukemia-associated antigens described in CML include the Receptor for hyaluronan acid-mediated motility (RHAMM) (Greiner et al., 2002), PPP2R5C (Zheng et al., 2011), PR1, PR3, PPP2R5C, ELA2, PRAME (Smahel, 2011) as well as an epitope derived from the M-phase phosphoprotein 11 protein (MPP11) (Al et al., 2010). The antigens described in these studies were typically identified using reverse immunology approaches and predictions and lack direct evidence of CML-associated presentation by HLA molecules.

Immunostimulatory treatment of CML is being revisited in the age of TKI therapy as exemplified by studies assessing IFN- α in combination with TKI in patients with the multi-TKI resistant T315I mutation (Itonaga et al., 2012). One case report found induction of a sustained deep molecular response in a single patient, who could discontinue therapy (Ilander et al., 2014). Furthermore, ongoing trials are investigating

the efficacy of immune checkpoint inhibitors such as α -CTLA4 (ipilimumab) and α -PD1 (nivolumab). Antigen-specific (combination) therapy thus represents a promising avenue for the eradication of MRD in CML as well as for the treatment of multi-TKI resistant clones.

Acute myeloid leukemia - AML treatment is divided into two phases: induction therapy and post-remission/ "consolidation therapy". Induction therapy is administered to induce remission and consists of combinational chemotherapy. Consolidation therapy consists of additional chemotherapy or hematopoietic cell transplantation (HCT) (Showel and Levis, 2014).

The most common chemotherapeutic drugs used to treat AML are cytarabine, daunorubicin, idarubicin and mitoxantrone followed by cladribine, fludarabine and diverse others. Azacytidine and decitabine (DNA hypomethylating agents) are now used for treatment of MDS/AML. Treatment for APL/ AML M3 includes all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) (National Cancer Institute, 2015).

"Standard treatment" for AML is considered as "3+7": 3 days of idarubicin or daunorubicin and 7 days of cytarabine, followed by several similar courses to achieve complete remission (CR) (Estey, 2014). The decision between standard therapy and clinical trial is based on the risk stratification.

AML cases with intermediate-risk karyotype show either no karyotypic abnormalities or only one or two abnormalities not categorized as high- or low-risk.

FLT3 mutations are associated with an aggressive type of AML and a poor prognosis. They often occur together with NPM1 and DNMT3a (DNA methyltransferase 3A) mutations. NPM1 (nucleophosmin) mutations are a favorable prognostic indicator, if not found together with FLT3 mutations. CEPBA (CCAAT-enhancer-binding protein alpha/ C/EBP α) mutations confer a survival advantage in the case of double or homozygous CEPBA mutations without wild-type expression. Altered methylation patterns in a variety

of genes are caused by mutations in isocitrate dehydrogenase (IDH1 and IDH2) and DNMT3A. These mutations are associated with poor survival.

AML cases with favorable-risk karyotype consist of APL (acute promyelocytic leukemia) and CBF (core-binding factor) leukemias. APL cases are associated with the fusion of the myeloid transcription factor PML to the retinoic acid receptor subunit alpha (RARA). The PML/RARA translocation is a favorable prognostic mutation. CBF leukemia cases show translocations involving a subunit of CBF. In t(8;21) CBF alpha is fused to the ETO gene. In inv(16) CBF beta is fused to the smooth muscle myosin heavy chain. CBF translocations are very favorable prognostic mutations.

AML cases with unfavorable-risk karyotype are characterized by a complex karyotype including chromosomal aberrations such as translocations, unbalanced rearrangements and gains/losses of whole chromosomes. They are associated with a poor prognosis.

MDS/AML cases evolve from myelodysplastic syndromes and carry a worse prognosis than other AML sub-groups (Showel and Levis, 2014).

Besides the above-listed prognostic factors, additional molecular markers or marker combinations can be used to judge the prognosis in specific cytogenetic subsets:

TP53 mutations are the most unfavorable genetic alteration in AML. NPM1 mutated and FLT3 WT together with a mutation in IDH1 or IDH2 is seen as favorable. Unfavorable factors include a partial tandem duplication in the MLL gene, a mutated TET2 gene, FLT3 ITD + together with a mutation in DNMT3a and CEBPA, FLT3 ITD – together with a mutation in ASXL1 or PHF6, and CD25 expression (stem cell-like “signature” and poorer outcome). The presence of CKIT mutations converts the prognosis of patients with a favorable inv(16) or t(8;21) into a more intermediate range. SPARC is up-regulated in NK (normal karyotype) patients with unfavorable gene expression signature and down-regulated in association with the favorable NPM1 mutation. miR-155 over-expression conveys a poor prognosis in NK AML. Differentially methylated regions

(DMRs) are prognostic when found in association with several genes (FLT3 mutation, NPM1 mutation). In this case, a lower expression is associated with a better prognosis (Estey, 2014).

Post-treatment information/ information about minimal residual disease (MRD) should be included into following treatment decisions. These include the response to induction therapy, PCR of fusion transcripts, mutated genes and over-expressed genes to detect MRD and multi-parameter flow cytometry for observation of aberrant expression of surface antigens.

Clinical trials are recommended for patients who belong to the prognostic groups unfavorable and intermediate-2. Treatment options include hypomethylating agents (HMAs) as Azacytidine or decitabine, CPX-351, which is a liposomal formulation of daunorubicin and cytarabine in a 1:5 “optimal” molar ratio, and volasertib, which is an inhibitor of polo kinases. Volasertib is given in combination with LDAC (low-dose cytarabine). Several different FLT3 inhibitors can be administered in case of FLT3 mutations. These include sorafenib, which is given in combination with 3+7, quizartinib, a more selective inhibitor of FLT3 ITD that also inhibits CKIT, crenolanib, and midostaurin, an unselective FLT3 ITD inhibitor. Another treatment option is targeting CD33 with antibody-drug conjugates (anti-CD33 + calechiamicin, SGN-CD33a, anti-CD33 + actinium-225), bispecific antibodies (recognition of CD33 + CD3 (AMG 330) or CD33 + CD16) and chimeric antigen receptors (CARs) (Estey, 2014).

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia in particular. There is also a need to identify factors representing biomarkers for cancer in general and chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 immense 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).

Longer (elongated) 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 (IFN γ) (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 and 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.

SUMMARY OF THE INVENTION

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: 279 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: 279, 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: 279 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: 279, 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. In Table 1, peptides with SEQ ID NO: 1 to SEQ ID NO: 16 bind to HLA-A*01, peptides with SEQ ID NO: 17 to SEQ ID NO: 27 bind to HLA-A*02, peptides with SEQ ID NO: 28 to SEQ ID NO: 52 bind to HLA-A*03, peptides with SEQ ID NO: 53 to SEQ ID NO: 76 bind to HLA-A*24, peptides with SEQ ID NO: 77 to SEQ ID NO: 106 bind to HLA-B*07, peptides with SEQ ID NO: 107 to SEQ ID NO: 121 bind to HLA-B*08, peptides with SEQ ID NO: 122 to SEQ ID NO: 187 bind to HLA-B*44. 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. In Table 2, peptides with SEQ ID NO: 188 to SEQ ID NO: 196 bind to HLA-A*01, peptides with SEQ ID NO: 197 to SEQ ID NO: 207 bind to HLA-A*02, peptides with SEQ ID NO: 208 to SEQ ID NO: 221 bind to HLA-A*03, peptides with SEQ ID NO: 222 to SEQ ID NO: 224 bind to HLA-A*24, peptides with SEQ ID NO: 225 to SEQ ID NO: 255 bind to HLA-B*07, peptide with SEQ ID NO: 256 binds to HLA-B*08, peptides with SEQ ID NO: 257 to SEQ ID NO: 277 bind to HLA-B*44. The peptides in Table 3 are additional peptides that may be useful in particular in combination with the other peptides of the invention. In Table 3, peptide with SEQ ID NO: 278 binds to HLA-A*02, peptide with SEQ ID NO: 279 binds to HLA-A*24.

Table 1: Peptides according to the present invention.

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
1	LTEGHSGNYY	FCRL5	A*01
2	TMIRIFHRY	S100Z	A*01/B*15
3	YINPAKLTPY	CARD11	A*01/A*03

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
4	ALDQNKMHY	CPA3	A*01
5	GTDVLSTRY	CPA3	A*01
6	VTEGVAQTSFY	HLA-DOA	A*01
7	FMDSESFYY	INPP5F	A*01
8	STDSAGSSY	PAX5	A*01
9	YSH PQYSSY	PAX5	A*01/B*15
10	YSDIGHLL	SESN3	A*01
11	AAADHHS LY	SOX4	A*01/A*32
12	ATDIVDSQY	T	A*01
13	ITDIHIKY	VPS13C	A*01
14	TFDLTVVSY	VPS13C	A*01
15	SVADIRNAY	WDFY4	A*01/A*32
16	WIGDKSFEY	ZNF121	A*01/A*29
17	KAYNRVIFV	MARCH1	A*02
18	YLLPSVLL	AGPAT5	A*02
19	SLFEGIYTI	FLT3	A*02
20	FSLEDLVRI	KIAA0226L	A*02
21	FLFDKLLLI	PLEKHG1	A*02
22	ILHAQTLKI	RALGPS2	A*02/B*13
23	FAFSGVLRA	RREB1	A*02
24	KLGPVAVSI	SIPA1L3	A*02/B*13
25	YLNEKSLQL	ST8SIA6	A*02
26	SLYVQQLKI	SYNE2	A*02/B*13
27	RLIAKEMNI	WDFY4	A*02/B*13
28	VILESIFLK	BTK	A*03/A*11
29	RIYDEILQSK	CD84	A*03
30	RTYGFVLTF	DENND5B	A*03/A*32
31	ATFNKLVS Y	DNMT3B	A*03/A*32
32	KTSNIVKIK	FCRL2	A*03
33	SVFEGDSIVLK	FCRL2	A*03/A*11
34	SVYSETSNMDK	GSAP	A*03/A*11
35	ATKSPAKPK	HIST1H1B	A*03
36	KAKAAAKPK	HIST1H1B	A*03
37	KAKKPAGAAK	HIST1H1E	A*03
38	KARKSAGAAK	HIST1H1E	A*03
39	IVIQLRAQK	HLA-DOB	A*03
40	RSKEYIRKK	KBTBD8	A*03
41	SVAHLLSKY	MAP3K1	A*03/B*15
42	SVSSSTHFTR	MAP3K1	A*03/A*11
43	KLMETSMGF	MGA	A*03/A*32

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
44	KVYDPVSEY	MTMR1	A*03/B*15
45	VVFPFPV NK	MYCN	A*03
46	RVFPSPMRI	PLCL2	A*03
47	SVLDLSVHK	PRDM2	A*03/A*11
48	RIKPPGPTAVPK	SCML2	A*03
49	GLLEEALFY	SMYD3	A*03/A*29
50	GVFNTLISY	TARBP1	A*03
51	ASTTVLALK	WDFY4	A*03/A*11
52	KAFNQSSTLTK	ZNF431, ZNF714, ZNF92, ZNF93	A*03
53	KYIEYYLVL	ADAM28	A*24
54	QQALNFTRF	AKAP13	A*24/B*15
55	IFVARLYYF	APOBEC3G	A*24
56	KYSSGFRNI	ATM	A*24
57	RFPPTPLF	BCL11A	A*24
58	KYLADLPTL	CEP85L	A*24
59	GLYEGTGRLF	DNMT3A, DNMT3B	A*24
60	TQDPHVNAFF	DOCK10	A*24/B*38
61	IFKEHNFSF	FLT3	A*24
62	YYLSHLERI	GNA15	A*24
63	IYFSNTHFF	GPR114	A*24
64	SFQSKATVF	HOXA9	A*24
65	AYLKQVLLF	INPP5F	A*24
66	SQPAVATSF	MYB	A*24/B*15
67	VFLPSEGFNF	N4BP2	A*24
68	LYQDRFDYL	NLRP3	A*24
69	EYNTIKDKF	PARP15	A*24
70	LYSDIGHLL	SESN3	A*24
71	RYLGKNWSF	SPNS3	A*24
72	TYVENLRLL	SYNE2	A*24
73	TYPQLEGFKF	WDFY4	A*24
74	SYADNILSF	WDR11	A*24
75	RFYLLTEHF	ZNF121	A*24
76	KAFSWSSAF	ZNF92	A*24/A*32
77	RPNGNSLFTSA	AFF3	B*07
78	RPRGLALVL	CASP2	B*07
79	SPVPSHWMVA	CD79B	B*07
80	KPLFKVSTF	DOCK10	B*07
81	SESPWLHAPSL	FAIM3	B*07/B*40
82	APFGFLGMQSL	FAM129C	B*07
83	IPVSRPIL	FCRL1	B*07

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
84	SPKLQIAAM	FCRL1	B*07
85	IPVSHPV	FCRL3	B*07
86	IPASHPV	FCRL5	B*07
87	FPAPILRAV	FCRLA	B*07
88	MPDPHLYHQM	FCRLA	B*07
89	FPETVNNLL	HEATR5B	B*07
90	KPKAAKPKA	HIST1H1B	B*07
91	KPKAAKPKAA	HIST1H1B	B*07
92	KAKKPAGAA	HIST1H1E	B*07
93	KARKSAGAA	HIST1H1E	B*07
94	KPKAAKPKKAAA	HIST1H1E	B*07
95	KPKAAKPKTA	HIST1H1E	B*07
96	KPKKAPKSPA	HIST1H1E	B*07
97	LPFGKIPIL	HPGDS	B*07/B*51
98	YPIALTRAEM	IKZF3	B*07/B*35
99	SPRAINNLVL	KLHL14	B*07
100	YPYQERVFL	PIK3R6	B*07/B*35
101	NPRYPNYMF	ROR1	B*07
102	LPLSMEAKI	RREB1	B*07/B*35
103	IPANTEKASF	SCIMP	B*07/B*50
104	RPMTPTQIGPSL	TCL1A	B*07
105	NPLTKLLAI	TFEC	B*07/B*08
106	KAFKWFSAL	ZNF736	B*07
107	QAAQRTAL	AFF3	B*08
108	ILAIRQNAL	AGPAT5	B*08
109	LGHVRYVL	AGPAT5	B*08
110	FGLARIYSF	CDK6	B*08
111	VTLIKYQEL	CLEC17A	B*08/A*02
112	APLLRHWEL	HLA-DOA	B*08/B*07
113	DANSRTSQL	MAP3K1	B*08
114	HNALRILTF	NUP210	B*08
115	ELYQRIYAF	PIK3R6	B*08
116	TLKIRAEVL	RALGPS2	B*08
117	YIKTAKKL	RALGPS2	B*08
118	FEKEKKESL	SESN3	B*08
119	DLRTKEVVF	SIPA1L3	B*08
120	VPPKKHLL	TRRAP	B*08
121	RPKKVNTL	ZBTB24	B*08
122	KELPGVKKY	ADAM28	B*44
123	EENPGKFLF	ARHGAP24	B*44

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
124	SESLPKEAF	ATF7IP	B*44
125	SESTFDRTF	ATF7IP	B*44
126	EENKPGIVY	BTLA	B*44
127	TEYPVFVY	CACNA2D4	B*44
128	GENDRLNHTY	CCDC88A	B*44
129	GEGAYGKVF	CDK6	B*44
130	EEEHGKGREY	CHD1	B*44
131	EEFETIERF	CHD1	B*44
132	GELPAVRDL	CIITA	B*44/B*40
133	AEHNFVAKA	CLEC17A	B*44/B*50
134	SEYADTHYF	CLNK	B*44
135	NEIKVYITF	CPA3	B*44
136	AEYKGRVTL	FAIM3	B*44/B*40/B*49
137	GELGGSVTI	FAIM3	B*44/B*49
138	SQAPAARAF	FAM129C	B*44/B*15
139	RENQVLGSGW	FCRL2	B*44
140	EYDLKWEF	FLT3	B*44/A*23
141	REYEYDLKWEF	FLT3	B*44
142	TEIFKEHNF	FLT3	B*44
143	YEYDLKWEF	FLT3	B*44
144	TEGKRYFTW	GANC	B*44
145	AEPLVGQRW	GDF7	B*44
146	SESKTVVTY	ICOSLG	B*44
147	KEVPRSYEL	IKZF3	B*44/B*40
148	REYNEYENI	IKZF3	B*44/B*49
149	SEKETVAYF	INPP5F	B*44
150	EEVTDRSQL	IRF8	B*44/B*40
151	EVDASIFKAW	IRF8	B*44
152	AELLAKELY	KIAA0226L	B*44
153	KEFEQVPGHL	KIAA0226L	B*44/B*40
154	AEPGPVITW	LILRA4	B*44
155	NEFPVIVRL	LRRK1	B*44
156	FEVESLFQKY	MAP3K1	B*44
157	VEIAEAIQL	MAP3K1	B*44/B*40
158	GENEDNRIGL	MCOLN2	B*44/B*40
159	GELLGRQSF	MDM4	B*44
160	EEETILHFF	NEK8	B*44
161	EEGDTLLHLF	NFKBID	B*44
162	DEAQARAAF	NLRP3	B*44
163	EEWMGLLEY	NLRP3	B*44

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
164	SEYSHLTRV	NPR3	B*44/B*49
165	VELDLQRSV	PIK3R6	B*44
166	NEVLASKY	PLCL2	B*44/B*18
167	KEIGAAVQAL	PLEKHA2	B*44/B*40
168	QEIQSLLTNW	PLEKHG1	B*44
169	EENGEVKEL	PRDM2	B*44
170	SENEQRRMF	PYHIN1	B*44
171	SEDLAVHLY	RALGPS2	B*44
172	VEDGLFHEF	RBM15	B*44
173	KEYDFGTQL	RNF220	B*44/B*49
174	TDKSFPNAY	RNGTT	B*44/B*47
175	HEIDGKALFL	SCML2	B*44
176	AENAVSNLSF	SLAMF6	B*44
177	QENMQIQSF	SPG11	B*44
178	REYEHYWTEL	STAP1	B*44/B*50
179	AEIKQTEEKY	VAV3	B*44
180	EPPAFNVSY	VOPP1	B*44
181	GEIKEPLEI	VPS13C	B*44/B*40/B*49
182	AQNLSIIQY	WDFY4	B*44/B*15
183	GESQDSTTAL	WDFY4	B*44/B*40
184	RMPPFTQAF	WDFY4	B*44/B*15
185	SEGDNVESW	ZCCHC7	B*44
186	NEQKIVRF	ZNF699	B*44/B*18
187	SDAQRPFSS	ZNF831	B*44/B*37

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

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
188	YVDAGTPMY	AGPAT5	A*01
189	VTEEPQRLFY	BMF	A*01
190	HVDQDLTTY	CDK6	A*01
191	ISEAGKDILY	CNTRL	A*01
192	RSDPGGGGLAY	MEX3B	A*01
193	LTDSEKGNSY	RALGPS2	A*01
194	YTDKKSIIY	STAP1	A*01
195	YSDKEFAGSY	TBC1D9	A*01
196	FTDIDGQVY	WDR11	A*01
197	SLADVHIEV	BTAF1	A*02
198	KLLGYDVHV	CASP2	A*02

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
199	AMPDSPA EV	CBFA2T3	A*02
200	VMLQINPKL	CCDC88A	A*02
201	ILAAVETRL	FBXO28	A*02
202	MVALPMVLV	ITGB7	A*02
203	FLLPKVQSI	KIAA0922	A*02
204	FLLPKVQSIQL	KIAA0922	A*02
205	FLINTNSEL	PDE4A, PDE4B, PDE4C, PDE4D	A*02
206	SLMDLQERL	STIM2	A*02
207	KLSDNILKL	SYNE2	A*02/B*13
208	KLNPQQAPLY	AKAP13	A*03
209	KTLPAMLGTGK	BTLA	A*03/A*11
210	RMYSQLKTLQK	DNMBP	A*03
211	ATYNKQPMYR	DNMT3A	A*03
212	LLWHWDTTQSLK	FCER2	A*03
213	RVYNIYIRR	GPR114	A*03/A*32
214	ATGAATPKK	HIST1H1E	A*03/A*11
215	KATGAATPK	HIST1H1E	A*03
216	RIKAPSRNTIQK	MAP3K1	A*03
217	TTVPHVFSK	MAP3K1	A*03/A*11
218	RVLTGVFTK	PARP15	A*03
219	HSYSSPSTK	RBM15	A*03
220	SISNLVFTY	SOX4	A*03/A*29
221	LLNRHILAH	ZNF669	A*03
222	RYLDEINLL	GNA15	A*24
223	RRMYPPPLI	VOPP1	A*24/B*27
224	VYEYVVERF	ZCCHC11	A*24
225	LPARFYQAL	AGPAT5	B*07
226	YLNRLHHTW	BCL2	B*07/A*32
227	APINKAGSFL	BLK	B*07
228	SPRITFPSL	BLK	B*07
229	SPLGSLARSSL	CARD11	B*07
230	KPMKSVLVV	CCR7	B*07
231	MPLSTIREV	CDK6	B*07/B*51
232	APRPAGSYL	CIITA	B*07
233	SPRVYWLGL	CLEC17A	B*07
234	SPKESENAL	DEPDC5	B*07
235	SPSLPSRTL	DEPDC5	B*07
236	RPSNKAPLL	EHMT1	B*07
237	SPWLHAPSL	FAIM3	B*07
238	SPRSWIQVQI	FCRL5	B*07

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
239	APSKTSLIM	FOXP1	B*07
240	SPSLPNITL	HDAC4, HDAC9	B*07
241	APAPAEKTPV	HIST1H1E	B*07
242	SPFSFHHVL	ITGB7	B*07/B*35
243	LPKVQSIQL	KIAA0922	B*07
244	MPSSDTTVTF	MAP3K1	B*07/B*35
245	SPLSHHSQL	MAP3K1	B*07
246	YPGWHSTTI	MYB	B*07/B*51
247	QPSPARAPAE	PMAIP1	B*07
248	LPYDSKHQI	PTPN22	B*07/B*51
249	SPADHRGYASL	SOX4	B*07
250	VPNLQTVSV	SP4	B*07/B*51
251	QRLFTMDL	TRRAP	B*07
252	RPHIPISKL	UBASH3B	B*07
253	RPFADLLGTAF	WDFY4	B*07
254	SPRNLPQRAAL	WDFY4	B*07
255	YPGSDRIML	WDFY4	B*07
256	SPYKKLKEAL	TRAPPC10	B*08
257	KEFFFVKVF	AIM2	B*44
258	EELFRDGVNW	BCL2	B*44
259	EENTLVQNY	BTAF1	B*44
260	AEIGEGAYGKVF	CDK6	B*44
261	NEIEHIPVW	CNTRL	B*44
262	QENQAETHAW	CXCR5	B*44
263	REAGFQVKAY	FCRLA	B*44
264	SEDHSGSYW	FCRLA	B*44
265	QEVDAIFKAW	IRF8	B*44
266	VDASIFKAW	IRF8	B*44
267	KEKFPINGW	MTMR1	B*44
268	NEDKGTKAW	MYSM1	B*44
269	KELEDLNKW	PDE4B	B*44
270	AESEDLAVHL	RALGPS2	B*44/B*40
271	AESEDLAVHLY	RALGPS2	B*44
272	KEFELRSSW	STIM2	B*44
273	AEIEIVKEEF	SYNE2	B*44
274	GEAVTDHPDRLW	TCL1A	B*44
275	TENPLTKLL	TFEC	B*44
276	EEEGNLLRSW	WDFY4	B*44
277	EEGNLLRSW	WDFY4	B*44

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

Seq ID No	Sequence	Official Gene Symbol(s)	HLA allotype
278	YLDRKLLTL	SYK	A*02
279	LYIDRPLPYL	FAM21A, FAM21B, FAM21C	A*24

The present invention furthermore generally relates to the peptides according to the present invention for use in the treatment of proliferative diseases, such as, lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) for example as well as myeloid neoplasms, such as , primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, esophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma.

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: 279. More preferred are the peptides – alone or in combination - selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 187 (see Table 1), and their uses in the immunotherapy of chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and other lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) as well as other myeloid neoplasms, such as primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, esophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma, and preferably chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia.

- 27 -

A particularly preferred combination of the peptides according to the present invention includes peptides presented by the seven most common HLA-A and -B allotypes (see tables above), which allows for a medicament providing a (genetic) coverage of >92% of the European collective of patients.

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia, and other lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) as well as other myeloid neoplasms, such as primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, esophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma.

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: 279.

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.: 279, preferably containing SEQ ID No. 1 to SEQ ID No. 187, 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

- 30 -

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia, and other lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) as well as other myeloid neoplasms, such as primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, esophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma, and preferably chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia. 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.

DETAILED DESCRIPTION OF THE INVENTION

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).

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, or 12 or longer, and in case of MHC class II peptides (elongated variants of the peptides of the invention) they can be as long as 13, 14, 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.

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 4: Expression frequencies F of HLA-A*02, HLA-A*01, HLA-A*03, HLA-A*24, HLA-B*07, HLA-B*08 and HLA-B*44 serotypes. Haplotype frequencies Gf are derived from a study which used HLA-typing data from a registry of more than 6.5 million volunteer donors in the U.S. (Gragert et al., 2013). The haplotype frequency is the frequency of a distinct allele on an individual chromosome. Due to the diploid set of chromosomes within mammalian cells, the frequency of genotypic occurrence of this allele is higher and can be calculated employing the Hardy-Weinberg principle ($F = 1 - (1-Gf)^2$).

Allele	Population	Calculated phenotype from allele frequency (F)
--------	------------	--

A*02	African (N=28557)	32.3%
	European Caucasian (N=1242890)	49.3%
	Japanese (N=24582)	42.7%
	Hispanic, S + Cent Amer. (N=146714)	46.1%
	Southeast Asian (N=27978)	30.4%
A*01	African (N=28557)	10.2%
	European Caucasian (N=1242890)	30.2%
	Japanese (N=24582)	1.8%
	Hispanic, S + Cent Amer. (N=146714)	14.0%
	Southeast Asian (N=27978)	21.0%
A*03	African (N=28557)	14.8%
	European Caucasian (N=1242890)	26.4%
	Japanese (N=24582)	1.8%
	Hispanic, S + Cent Amer. (N=146714)	14.4%
	Southeast Asian (N=27978)	10.6%
A*24	African (N=28557)	2.0%
	European Caucasian (N=1242890)	8.6%
	Japanese (N=24582)	35.5%
	Hispanic, S + Cent Amer. (N=146714)	13.6%
	Southeast Asian (N=27978)	16.9%
B*07	African (N=28557)	14.7%
	European Caucasian (N=1242890)	25.0%
	Japanese (N=24582)	11.4%
	Hispanic, S + Cent Amer. (N=146714)	12.2%
	Southeast Asian (N=27978)	10.4%
B*08	African (N=28557)	6.0%
	European Caucasian (N=1242890)	21.6%
	Japanese (N=24582)	1.0%
	Hispanic, S + Cent Amer. (N=146714)	7.6%
	Southeast Asian (N=27978)	6.2%

B*44	African (N=28557)	10.6%
	European Caucasian (N=1242890)	26.9%
	Japanese (N=24582)	13.0%
	Hispanic, S + Cent Amer. (N=146714)	18.2%
	Southeast Asian (N=27978)	13.1%

The peptides of the invention, preferably when included into a vaccine of the invention as described herein bind to A*02, A*01, A*03, A*24, B*07, B*08 or B*44. 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-, A*01-, A*03-, A*24-, B*07-, B*08- or B*44-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.allele frequencies.net).

Table 5: HLA alleles coverage in European Caucasian population (calculated from (Gragert et al., 2013)).

	coverage (at least one A- allele)	combined with B*07	combined with B*44	combined with B*07 and B*44
A*02 / A*01	70%	78%	78%	84%
A*02 / A*03	68%	76%	76%	83%
A*02 / A*24	61%	71%	71%	80%
A*01 / A*03	52%	64%	65%	75%
A*01 / A*24	44%	58%	59%	71%

	coverage (at least one A- allele)	combined with B*07	combined with B*44	combined with B*07 and B*44
A*03 / A*24	40%	55%	56%	69%
A*02 / A*01 / A*03	84%	88%	88%	91%
A*02 / A*01 / A*24	79%	84%	84%	89%
A*02 / A*03 / A*24	77%	82%	83%	88%
A*01 / A*03 / A*24	63%	72%	73%	81%
A*02 / A*01 / A*03 / A*24	90%	92%	93%	95%

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:

$$\text{percent identity} = 100 [1 - (C/R)]$$

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

- 40 -

- (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: 279 or a variant thereof which is 88% homologous to SEQ ID NO: 1 to SEQ ID NO: 279, 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: 279. 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 279, 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 acid whose incorporation does not substantially affect T-cell reactivity and does not eliminate binding to the

[illegible]

- 46 -

		L									Y
		L									R
		L									F
		I									
		I									Y
		I									R
		I									F
		M									
		M									Y
		M									R
		M									F
											Y
											R
											F
		T									
		T									Y
		T									R
		T									F
Position	1	2	3	4	5	6	7	8	9	10	11
SEQ ID No 210	R	M	Y	S	Q	L	K	T	L	Q	K
Variant		L									
		L									Y
		L									R
		L									F
		I									
		I									Y
		I									R
		I									F
											Y
											R
											F
		V									
		V									Y
		V									R
		V									F
		T									
		T									Y
		T									R
		T									F
Position	1	2	3	4	5	6	7	8	9		

[illegible]

- 49 -

									M		
									F		
					R						
					R				V		
					R				I		
					R				M		
					R				F		
					H						
					H				V		
					H				I		
					H				M		
					H				F		
			R								
			R						V		
			R						I		
			R						M		
			R						F		
			R		R						
			R		R				V		
			R		R				I		
			R		R				M		
			R		R				F		
			R		H						
			R		H				V		
			R		H				I		
			R		H				M		
			R		H				F		
			L								
			L						V		
			L						I		
			L						M		
			L						F		
			L		R						
			L		R				V		
			L		R				I		
			L		R				M		
			L		R				F		
			L		H						
			L		H				V		
			L		H				I		
			L		H				M		

- 50 -

			L		H				F		
Position	1	2	3	4	5	6	7	8	9		
SEQ ID No											
134	S	E	Y	A	D	T	H	Y	F		
Variant									W		
									Y		
									L		
		D									
		D							W		
		D							Y		
		D							L		
Position	1	2	3	4	5	6	7	8	9		
SEQ ID No											
148	R	E	Y	N	E	Y	E	N	I		
Variant									F		
									W		
									Y		
									L		
		D							F		
		D							W		
		D							Y		
		D							L		

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

C-terminus	N-terminus
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 μ M, 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: 279.

“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 279 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, -CH₂-NH, -CH₂S-, -CH₂CH₂-, -CH=CH-, -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-. US 4,897,445 provides a method for the solid phase synthesis of non-peptide bonds (-CH₂-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 NaCNBH₃.

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 carbobenzoxy, 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'-ethyl carbodiimide 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, Bromo ethylamine, and chloramine T.

Tetranitromethane and N-acetyl imidazole 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: 279 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 $(\text{NH}_4)_3\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{SO}_4$, $\text{NH}_4\text{CH}_3\text{COO}$, 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 , $\text{Rb}_4\text{CH}_3\text{COO}$, 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 , $\text{Mg}_3(\text{PO}_4)_2$, Mg_2HPO_4 , $\text{Mg}(\text{H}_2\text{PO}_4)_2$, Mg_2SO_4 , $\text{Mg}(\text{CH}_3\text{COO})_2$, MgCl_2 , MgBr_2 , $\text{Mg}(\text{NO}_3)_2$, $\text{Mg}(\text{ClO}_4)_2$, MgI_2 , $\text{Mg}(\text{SCN})_2$, MnCl_2 , $\text{Ca}_3(\text{PO}_4)_2$, Ca_2HPO_4 , $\text{Ca}(\text{H}_2\text{PO}_4)_2$, CaSO_4 , $\text{Ca}(\text{CH}_3\text{COO})_2$, CaCl_2 , CaBr_2 , $\text{Ca}(\text{NO}_3)_2$, $\text{Ca}(\text{ClO}_4)_2$, CaI_2 , $\text{Ca}(\text{SCN})_2$, $\text{Ba}_3(\text{PO}_4)_2$, Ba_2HPO_4 , $\text{Ba}(\text{H}_2\text{PO}_4)_2$, BaSO_4 , $\text{Ba}(\text{CH}_3\text{COO})_2$, BaCl_2 , BaBr_2 , $\text{Ba}(\text{NO}_3)_2$, $\text{Ba}(\text{ClO}_4)_2$, BaI_2 , and $\text{Ba}(\text{SCN})_2$. 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 dimethyl acrylamide (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-dicyclohexylcarbodiimide/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.

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 chronic lymphocytic leukemia (N = 35 samples), chronic myeloid leukemia (N = 16 samples) and acute myeloid leukemia (N = 32 samples) 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 83 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia patients (cf. Example 1).

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.

HLA-peptide complexes from chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia tissue samples were purified and HLA-associated peptides were isolated and analyzed by LC-MS (see example 1). All TUMAPs contained in the present application were identified with this approach on primary chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia samples confirming their presentation on primary chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia.

Besides 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 1). 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 peripheral blood mononuclear cells (PBMC) 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 presented on tumor tissue – “tumor tissue” in relation to this invention shall mean a sample from a patient suffering from chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia.

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. chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 (see also below). 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 fragments of the TCRs according to the invention that are capable of binding to a peptide antigen according to the present invention when presented by an HLA molecule. The term particularly relates to soluble TCR fragments, for example TCRs missing the transmembrane parts and/or constant regions, single chain TCRs, and fusions thereof to, for example, with Ig.

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 a peptide-HLA molecule complex with a binding affinity (KD) of about 100 μ M or less, about 50 μ M or less, about 25 μ M or less, or about 10 μ M or less. More preferred are high affinity TCRs having binding affinities of about 1 μ M 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 μ M 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 peptides 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 fluorescence 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 TCR $\alpha\beta$ 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 bicistronic constructs in a single vector, which has been shown to be capable of overcoming 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 CD3 ζ (CD3 ζ 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 $-NH_2$ 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. 279, 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.,

- 70 -

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 derivatives 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 derivatives, poly-(I:C) and derivatives, 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 another 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, and 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 279, 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 (preferably a peptide according to the present invention), 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 thus 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: 279, or a variant thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 279 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: 279 or a variant thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 279, 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: 279.

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia.

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 said peptide containing SEQ ID NO: 1 to SEQ ID NO: 279 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia cells or other solid or hematological tumor cells such as other lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) as well as other myeloid neoplasms, such as primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, esophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma.

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia. 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia marker (poly)peptide, delivery of a toxin to a chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia cell expressing a cancer marker gene at an increased level, and/or inhibiting the activity of a chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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: 279 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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, immunohistochemically 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

- 90 -

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 (μ g/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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia, 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 occur 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), and 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 , ^3H , ^{32}P or ^{35}S) so that the tumor can be localized using immunoscintigraphy. 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 (K_d) is less than $1 \times 10\mu\text{M}$.

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: 279, 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, and 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 279.

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia, the medicament of the invention is preferably used to treat chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia.

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia tissues, the warehouse may contain HLA-A*02, HLA-A*01, HLA-A*03, HLA-A*24, HLA-B*07, HLA-B*08 and HLA-B*44 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, chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 (chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia) compared with a range of normal organs and tissues

- 100 -

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 an 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

- 102 -

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia. 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 or 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

Figures 1A through 1W show exemplary expression profile of source genes of the present invention that are over-expressed in different cancer samples. Tumor (black dots) and normal (grey dots) samples are grouped according to organ of origin, and box-and-whisker plots represent median, 25th and 75th percentile (box), and minimum and maximum (whiskers) RPKM values. Normal organs are ordered according to risk categories. RPKM = reads per kilobase per million mapped reads. Normal samples: blood cells; blood vessel; brain; heart; liver; lung; adipose: adipose tissue; adren.gl.: adrenal gland; bile duct; bladder; BM: bone marrow; cartilage; esoph: esophagus; eye; gallb: gallbladder; head and neck; kidney; large_int: large intestine; LN: lymph node; nerve; pancreas; parathyr: parathyroid; perit: peritoneum; pituit: pituitary; skel.mus: skeletal muscle; skin; small_int: small intestine; spleen; stomach; thyroid; trachea; ureter; breast; ovary; placenta; prostate; testis; thymus; uterus. Tumor samples: AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; NHL: non-hodgkin lymphoma. Figure 1A) Gene symbol: S100Z, Peptide: TMIRIFHRY (SEQ ID No.: 2), Figure 1B) Gene symbol: PAX5, Peptide: YSHPQYSSY (SEQ ID No.: 9), 1C) Gene symbol: FLT3, Peptide: SLFEGIYTI (SEQ ID No.: 19), 1D) Gene symbol: RALGPS2, Peptide: ILHAQTLKI (SEQ ID No.: 22), 1E) Gene symbol: FCRL2, Peptide: KTSNIVKIK (SEQ ID No.: 32), 1F) Gene symbol: KBTBD8, Peptide: RSKEYIRKK (SEQ ID No.: 40), 1G) Gene symbols: ZNF92, Peptide: KAFNQSSTLTK (SEQ ID No.: 52), 1H) Gene symbol: ADAM28, Peptide: KYIEYYLVL (SEQ ID No.: 53), 1I) Gene symbol: FLT3, Peptide: IFKEHNFSF (SEQ ID No.: 61), 1J) Gene symbol: ZNF92, Peptide: KAFSWSSAF (SEQ ID No.: 76), 1K) Gene symbol: FCRL3, Peptide: IPVSHPV (SEQ ID No.: 85), 1L) Gene symbol: CDK6, Peptide: FGLARIYSF (SEQ ID No.: 110), 1M) Gene symbol: CLEC17A, Peptide: VTLIKYQEL (SEQ ID No.: 111), 1N) Gene symbol: RALGPS2, Peptide: YIKTAKKL (SEQ ID No.: 117), 1O) Gene symbol: CDK6, Peptide: GEGAYGKVF (SEQ ID No.: 129), 1P) Gene symbol: FCRL2, Peptide: RENQVLGSGW (SEQ ID No.: 139), 1Q) Gene symbol: FLT3, Peptide: REYEYDLKWEF (SEQ ID No.: 141), 1R) Gene symbol: BMF, Peptide: VTEEPQRLFY (SEQ ID No.: 189), 1S) Gene symbol: FCER2, Peptide: LLWHWDTTQSLK (SEQ ID No.: 212), 1T) Gene symbol: CDK6, Peptide: MPLSTIREV (SEQ ID No.: 231), 1U) Gene symbol: CLEC17A, Peptide: SPRVYWLGL (SEQ ID No.: 233), 1V) Gene symbol: PMAIP1, Peptide:

- 106 -

QPSPARAPAEI (SEQ ID No.: 247), 1W) Gene symbol: CDK6, Peptide: AEIGEGAYGKVF (SEQ ID No.: 260).

Figure 2 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*02⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*02 in complex with SeqID No 278 peptide (YLDRKLLTL, Seq ID NO: 278) (A, left panel). After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*02/SeqID No 278 (A). Right panel (B) 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.

Figure 3 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*24⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*24 in complex with SeqID No 279 peptide (A, left panel). After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*24/SeqID No 279 (LYIDRPLPYL, Seq ID NO: 279) (A). Right panel (B) shows control staining of cells stimulated with irrelevant A*24/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.

Figure 4 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*01⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*01 in complex with Seq ID NO: 12 peptide (ATDIVDSQY, Seq ID NO: 12; A, left panel) and Seq ID NO: 192 peptide (RSDPGGGGLAY, Seq ID NO: 192; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*01/Seq ID NO: 12

(A) or A*01/Seq ID NO: 192 (B). Right panels (A and B) show control staining of cells stimulated with irrelevant A*01/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.

Figure 5 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*02⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*02 in complex with Seq ID NO: 19 peptide (SLFEGITYI, Seq ID NO: 19; A, left panel) and Seq ID NO: 26 peptide (SLYVQQLKI, Seq ID NO: 26; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*02/Seq ID NO: 19 (A) or A*02/Seq ID NO: 26 (B). Right panels (A and B) 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.

Figure 6 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*03⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*03 in complex with Seq ID NO: 45 peptide (VVFPPVNVK, Seq ID NO: 45; A, left panel) and SeqID No 215 peptide (KATGAATPK, Seq ID NO: 215; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*03/Seq ID NO: 45 (A) or A*03/Seq ID NO: 215 (B). Right panels (A and B) show control staining of cells stimulated with irrelevant A*03/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.

Figure 7 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-A*24⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-A*24 in complex with Seq ID NO: 53 peptide (KYIEYYLVL, Seq ID NO: 53; A, left panel) and Seq ID NO: 68 peptide (LYQDRFDYL, Seq ID NO: 68; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with A*02/Seq ID NO: 53 (A) or A*24/Seq ID NO: 68 (B). Right panels (A and B) show control staining of cells stimulated with irrelevant A*24/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.

Figure 8 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-B*07⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-B*07 in complex with SeqID No 233 peptide (SPRVYWLGL, Seq ID NO: 233; A, left panel) and Seq ID NO: 84 peptide (SPKLQIAAM, Seq ID NO: 84; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with B*07/Seq ID NO: 233 (A) or B*07/Seq ID NO: 84 (B). Right panels (A and B) show control staining of cells stimulated with irrelevant B*07/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.

Figure 9 shows exemplary results of peptide-specific in vitro CD8⁺ T cell responses of a healthy HLA-B*44⁺ donor. CD8⁺ T cells were primed using artificial APCs coated with anti-CD28 mAb and HLA-B*44 in complex with SEQ ID NO: 145 peptide (AEPLVGQRW, SEQ ID NO: 145; A, left panel) and SEQ ID NO: 171 peptide (SEDLAVHLY, SEQ ID NO: 171; B, left panel), respectively. After three cycles of stimulation, the detection of peptide-reactive cells was performed by 2D multimer staining with B*44/SEQ ID NO: 145 (A) or B*44/SEQ ID NO: 171 (B). Right panels (A

and B) show control staining of cells stimulated with irrelevant B*44/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 of tumor associated peptides presented on the cell surface

Tissue samples

Patients' tumor samples and normal tissues were obtained from the University Hospital Tübingen (Tübingen, Germany). Written informed consents of all patients had been given before blood draw. PBMC were isolated from blood samples using Ficoll-Hypaque density gradient centrifugation immediately after blood draw. PBMC pellets were shock-frozen immediately after purification and stored until isolation of TUMAPs at -70°C or below.

Isolation of HLA peptides from tissue samples

HLA peptide pools from shock-frozen samples were obtained by immune precipitation 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 (Ultimate 3000 RSLC Nano UHPLC System, Dionex) and the eluting peptides were analyzed in LTQ-Orbitrap and Fusion Lumos hybrid mass spectrometers (ThermoElectron) equipped with an ESI source. Peptide samples were loaded with 3% of solvent B (20% H₂O, 80% acetonitrile and 0.04% formic acid) on a 2 cm PepMap 100 C18 Nanotrap column (Dionex) at a flowrate of 4 µl/min for 10 min.

- 110 -

Separation was performed on either 25 cm or 50 cm PepMap C18 columns with a particle size of 2 μm (Dionex) mounted in a column oven running at 50°C. The applied gradient ranged from 3 to 40% solvent B within 90 min at a flow rate of 300 nl/min (for 25 cm columns) or 140 min at a flow rate of 175 nl/min (for 50 cm columns). (Solvent A: 99% H_2O , 1% ACN and 0.1% formic acid; Solvent B: 20% H_2O , 80% ACN and 0.1% formic acid).

Mass spectrometry analysis was performed in data dependent acquisition mode employing a top five method (i.e. during each survey scan the five most abundant precursor ions were selected for fragmentation). Alternatively, a TopSpeed method was employed for analysis on Fusion Lumos instruments.

Survey scans were recorded in the Orbitrap at a resolution of 60,000 (for Orbitrap XL) or 120,000 (for Orbitrap Fusion Lumos). MS/MS analysis was performed by collision induced dissociation (CID, normalized collision energy 35%, activation time 30 ms, isolation width 1.3 m/z) with subsequent analysis in the linear trap quadrupole (LTQ). Mass range for HLA class I ligands was limited to 400-650 m/z with charge states 2+ and 3+ selected for fragmentation. For HLA class II mass range was set to 300-1500 m/z allowing for fragmentation for all positive charge states ≥ 2 .

Tandem mass spectra were interpreted by MASCOT or SEQUEST database search at a fixed Percolator false discovery rate ($q \leq 0.05$) and additional manual control. In cases where the identified peptide sequence was uncertain it was additionally validated by comparison of the generated natural peptide fragmentation pattern with the fragmentation pattern of a synthetic sequence-identical reference peptide.

Table 8a and 8b show the presentation on various cancer entities for selected peptides, and thus the particular relevance of the peptides as mentioned for the diagnosis and/or treatment of the cancers as indicated (e.g. peptide SEQ ID No. 3 for AML, CML (Table 8a) and for GBC, MEL, NHL, NSCLC and UBC (Table 8b), peptide SEQ ID No. 4 for CLL (Table 8a) and for BRCA, CCC, CRC, GBC, GC, GEJC, HCC, HNSCC, MEL,

NSCLCadenos, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, SCLC, UBC, UEC (Table 8b)).

Table 8a: Overview of presentation of selected tumor-associated peptides of the present invention across entities (diseases).

CLL = chronic lymphocytic leukemia, AML = acute myeloid leukemia, CML = Chronic myeloid leukemia.

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
1	LTEGHSGNYY	CLL
2	TMIRIFHRY	AML
3	YINPAKLTPY	CLL
4	ALDQNKMHY	AML
5	GTDVLSTRY	AML, CML
6	VTEGVAQTSFY	CLL
7	FMDSESFYY	CLL
8	STDSAGSSY	CLL
9	YSH PQYSSY	CLL
10	YSDIGHLL	CLL
11	AAADHHSLY	AML
12	ATDIVDSQY	CLL
13	ITDIHIKY	CLL
14	TFDLTVVSY	CLL
15	SVADIRNAY	CLL
16	WIGDKSFY	AML
17	KAYNRVIFV	CLL
18	YLLPSVLL	CLL
19	SLFEGIYTI	AML
20	FSLEDLVRI	CLL
21	FLFDKLLLI	CLL
22	ILHAQTLKI	CLL
23	FAFSGVLRA	AML
24	KLGPVAVSI	CLL
25	YLNEKSLQL	AML, CML
26	SLYVQQLKI	CLL
27	RLIAKEMNI	CLL
28	VILESIFLK	CLL
29	RIYDEILQSK	AML
30	RTYGFVLTF	CLL

- 112 -

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
31	ATFNKLVS Y	AML
32	KTSNIVKIK	CLL
33	SVFEGDSIVLK	CLL
34	SVYSETSNMDK	CLL
35	ATKSPAKPK	CLL
36	KAKAAAKPK	CLL
37	KAKKPAGAAK	CLL
38	KARKSAGAAK	CLL
39	IVIQLRAQK	CLL
40	RSKEYIRKK	CLL
41	SVAHLLSKY	CLL
42	SVSSSTHFTR	CLL
43	KLMETSMGF	CLL
44	KVYDPVSEY	CLL
45	VVFPFPV NK	AML, CML
46	RVFPSPMRI	CLL
47	SVLDLSVHK	CLL
48	RIKPPGPTAVPK	AML
49	GLLEEALFY	AML
50	GVFNTLISY	AML
51	ASTTVLALK	CLL
52	KAFNQSSTLTK	CLL
53	KYIEYYLVL	CLL
54	QQALNFTRF	CLL
55	IFVARLYYF	CLL
56	KYSSGFRNI	CLL
57	RFPPTPPLF	CLL
58	KYLADLPTL	CLL
59	GLYEGTGRLF	AML
60	TQDPHVNAFF	CLL
61	IFKEHNFSF	AML
62	YYLSHLERI	AML
63	IYFSNTHFF	AML
64	SFQSKATVF	AML
65	AYLKQVLLF	CLL
66	SQPAVATSF	AML
67	VFLPSEGFNF	AML
68	LYQDRFDYL	AML
69	EYNTIKDKF	CLL

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
70	LYSDIGHLL	CLL
71	RYLGKNWSF	AML
72	TYVENLRLL	CLL
73	TYPQLEGFKF	CLL
74	SYADNILSF	CLL
75	RFYLLTEHF	AML
76	KAFSWSSAF	CLL
77	RPNGNSLFTSA	CLL
78	RPRGLALVL	AML, CML
79	SPVPSHWMVA	CLL
80	KPLFKVSTF	CLL
81	SESPWLHAPSL	CLL
82	APFGFLGMQSL	CLL
83	IPVSRPIL	CLL
84	SPKLQIAAM	CLL
85	IPVSHPVL	CLL
86	IPASHPVL	CLL
87	FPAPILRAV	CLL
88	MPDPHLYHQM	CLL
89	FPETVNNLL	CLL
90	KPKAAKPKA	CLL
91	KPKAAKPKAA	CLL
92	KAKKPAGAA	CLL
93	KARKSAGAA	CLL
94	KPKAAKPKKAAA	CLL
95	KPKAAKPKTA	CLL
96	KPKKAPKSPA	CLL
97	LPFGKIPIL	AML, CML
98	YPIALTRAEM	CLL
99	SPRAINNLVL	CLL
100	YPYQERVFL	CML
101	NPRYPNYMF	CLL
102	LPLSMEAKI	CML
103	IPANTEKASF	CLL
104	RPMTPTQIGPSL	CLL
105	NPLTKLLAI	AML
106	KAFKWFSAL	CLL
107	QAAQRTAL	CLL
108	ILAIRQNAL	CLL

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
109	LGHVRYVL	CLL
110	FGLARIYSF	AML, CML
111	VTLIKQEL	CLL
112	APLLRHWEL	CLL
113	DANSRTSQL	CLL
114	HNALRILTF	AML
115	ELYQRIYAF	AML
116	TLKIRAEVL	CLL
117	YIKTAKKL	CLL
118	FEKEKKESL	CLL
119	DLRTKEVVF	CLL
120	VPPKKHLL	CLL
121	RPKKVNTL	CLL
122	KELPGVKKY	CLL
123	EENPGKFLF	CLL
124	SESLPKEAF	CLL
125	SESTFDRTF	CLL
126	EENKPGIVY	CLL
127	TEYPVFVY	AML
128	GENDRLNHTY	CLL
129	GEGAYGKVF	AML
130	EEEHGKGREY	CLL
131	EEFETIERF	CLL
132	GELPAVRDL	CLL
133	AEHNFVAKA	CLL
134	SEYADTHYF	CLL
135	NEIKVYITF	AML, CML
136	AEYKGRVTL	CLL
137	GELGGSVTI	CLL
138	SQAPAARAF	CLL
139	RENQVLGSGW	CLL
140	EYDLKWEF	AML
141	REYEYDLKWEF	AML
142	TEIFKEHNF	AML
143	YEYDLKWEF	AML
144	TEGKRYFTW	CLL
145	AEPLVGQRW	CLL
146	SESKTVVTY	CLL
147	KEVPRSYEL	CLL

- 115 -

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
148	REYNEYENI	CLL
149	SEKETVAYF	CLL
150	EEVTDRSQL	CLL
151	EVDASIFKAW	CLL
152	AELLAKELY	CLL
153	KEFEQVPGHL	CLL
154	AEPGPVITW	CLL
155	NEFPVIVRL	CLL
156	FEVESLFQKY	CLL
157	VEIAEAIQL	CLL
158	GENEDNRIGL	CLL
159	GELLGRQSF	CLL
160	EEETILHFF	CLL
161	EEGDTLLHLF	CLL
162	DEAQARAAF	AML
163	EEWMGLLEY	AML
164	SEYSHLTRV	AML
165	VELDLQRSV	AML
166	NEVLASKY	CLL
167	KEIGAAVQAL	CLL
168	QEIQSLLTNW	CLL
169	EENGEVKEL	CLL
170	SENEQRRMF	CLL
171	SEDLAVHLY	CLL
172	VEDGLFHEF	CLL
173	KEYDFGTQL	AML
174	TDKSFPNAY	CLL
175	HEIDGKALFL	AML
176	AENAVSNLSF	CLL
177	QENMQIQSF	CLL
178	REYEHYWTEL	CLL
179	AEIKQTEEKY	AML
180	EPAFNVSY	CLL
181	GEIKEPLEI	CLL
182	AQNLSIIQY	CLL
183	GESQDSTTAL	CLL
184	RMPPFTQAF	CLL
185	SEGDNVESW	CLL
186	NEQKIVRF	CLL

- 116 -

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
187	SDAQRPSF	CLL
188	YVDAGTPMY	CLL
189	VTEEPQRLFY	CLL
190	HVDQDLTTY	AML
191	ISEAGKDLY	AML, CML, CLL
192	RSDPGGGGLAY	AML
193	LTDSEKGNSY	CLL
194	YTDKKSIIY	CLL
195	YSDKEFAGSY	CLL
196	FTDIDGQVY	CLL
197	SLADVHIEV	CLL
198	KLLGYDVHV	AML
199	AMPDSPAEV	AML
200	VMLQINPKL	CLL
201	ILAAVETRL	CLL
202	MVALPMVLV	CLL
203	FLLPKVQSI	CLL
204	FLLPKVQSIQL	CLL
205	FLINTNSEL	CLL
206	SLMDLQERL	CLL
207	KLSDNILKL	CLL
208	KLNPQQAPLY	CLL
209	KTLPAMLGTGK	CLL
210	RMYSQKTLQK	CLL
211	ATYNKQPMYR	CML
212	LLWHWDTTQSLK	CLL
213	RVYNIYIRR	AML
214	ATGAATPKK	CLL
215	KATGAATPK	CLL
216	RIKAPSRNTIQK	CLL
217	TTVPHVFSK	CLL
218	RVLTGVFTK	CLL
219	HSYSSPSTK	CLL
220	SISNLVFTY	AML
221	LLNRHILAH	CLL
222	RYLDEINLL	AML
223	RRMYPPPLI	CLL
224	VYEYVVERF	CLL
225	LPAFYQAL	CLL

- 117 -

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
226	YLNRLHTW	CLL
227	APINKAGSFL	CLL
228	SPRITFPSL	CLL
229	SPLGSLARSSL	CLL
230	KPMKSVLVV	CLL
231	MPLSTIREV	AML, CML
232	APRPAGSYL	CLL
233	SPRVYWLGL	CLL
234	SPKESENAL	CLL
235	SPSLPSRTL	CLL
236	RPSNKAPLL	CLL
237	SPWLHAPSL	CLL
238	SPRSWIQVQI	CLL
239	APSKTSLIM	CLL
240	SPSLPNITL	CLL
241	APAPAEKTPV	CLL
242	SPFSFHHVL	CLL
243	LPKVQSIQL	CLL
244	MPSSDTTVTF	CLL
245	SPLSHHSQL	CLL
246	YPGWHSTTI	AML
247	QPSPARAPAEI	CLL
248	LPYDSKHQI	CLL
249	SPADHRGYASL	AML
250	VPNLQTVSV	CLL
251	QPRLFTMDL	CLL
252	RPHIISKL	CLL
253	RPFADLLGTAF	CLL
254	SPRNLQPQRAAL	CLL
255	YPGSDRIML	CLL
256	SPYKKLKEAL	CLL
257	KEFFFVKVF	CLL
258	EELFRDGVNW	CLL
259	EENTLVQNY	CLL
260	AEIGEGAYGKVF	AML
261	NEIEHIPVW	CLL
262	QENQAETHAW	CLL
263	REAGFQVKAY	CLL
264	SEDHSGSYW	CLL

SEQ ID No.	Sequence	Peptide Presentation on cancer entities
265	QEVDASIFKAW	CLL
266	VDASIFKAW	CLL
267	KEKFPINGW	CLL
268	NEDKGTKAW	CLL
269	KELEDLNKW	CLL
270	AESEDLAVHL	CLL
271	AESEDLAVHLY	CLL
272	KEFELRSSW	CLL
273	AEIEIVKEEF	CLL
274	GEAVTDHPDRLW	CLL
275	TENPLTKLL	AML
276	EEEGNLLRSW	CLL
277	EEGNLLRSW	CLL

Table 8b: Overview of presentation of selected tumor-associated peptides of the present invention across entities (diseases).

BRCA = breast cancer, CCC = bile duct cancer, GBM = brain cancer, CRC = colorectal carcinoma, OSCAR = esophageal cancer, GBC = gallbladder adenocarcinoma, GC = gastric cancer, HNSCC = head and neck squamous cell carcinoma, HCC = hepatocellular carcinoma, MEL = melanoma, NHL = non-Hodgkin lymphoma, NSCLCadenoc = non-small cell lung cancer adenocarcinoma, NSCLCother = NSCLC samples that could not unambiguously be assigned to NSCLCadenoc or NSCLCsquam, NSCLCsquam = squamous cell non-small cell lung cancer, OC = ovarian cancer, PACA = pancreatic cancer, PRCA = prostate cancer and benign prostate hyperplasia, RCC = renal cell carcinoma, SCLC = small cell lung cancer, UBC = urinary bladder cancer, UEC = uterine cancer.

Seq ID No	Sequence	Peptide Presentation on tumor types
1	LTEGHSGNYY	NHL
3	YINPAKLTPY	GBC, MEL, NHL, NSCLCother, UBC
4	ALDQNKMHY	BRCA, CCC, CRC, GBC, GC, GEJC, HCC, HNSCC, MEL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, SCLC, UBC, UEC
5	GTDVLSTRY	BRCA, CRC, GBC, GC, GEJC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, RCC,

Seq ID No	Sequence	Peptide Presentation on tumor types
		SCLC, UBC, UEC
6	VTEGVAQTSFY	BRCA, CCC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
7	FMDSESFYY	BRCA, GBC, GC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam
8	STDSAGSSY	NHL
10	YSDIGHLL	BRCA, CCC, GC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, PACA, RCC, UEC
11	AAADHHSLY	GC, SCLC
13	ITDIHIKY	BRCA, CRC, GBC, GBM, GC, GEJC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, RCC, SCLC, UBC
14	TFDLTVVSY	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
15	SVADIRNAY	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
16	WIGDKSFEY	BRCA, CRC, GBC, GBM, GC, HCC, MEL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, PRCA, RCC
17	KAYNRVIFV	BRCA, CCC, CRC, GBC, GBM, GEJC, HCC, HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, SCLC, UBC, UEC
18	YLLPSVLL	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
20	FSLEDLVRI	NHL
21	FLFDKLLLI	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
22	ILHAQTLKI	HCC, NHL, NSCLCsquam, PRCA
23	FAFSGVLRA	BRCA, CRC, GBC, GEJC, HCC, HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PRCA, SCLC, UBC, UEC
24	KLGPVAVSI	HCC, HNSCC, NHL, NSCLCsquam, OSCAR, PRCA
25	YLNEKSLQL	BRCA, CCC, CRC, GEJC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, PRCA, RCC, SCLC, UBC, UEC
26	SLYVQQLKI	HNSCC, NHL, OC, PRCA
27	RLIAKEMNI	HCC, NHL
28	VILESIFLK	GBC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC,

Seq ID No	Sequence	Peptide Presentation on tumor types
		OSCAR, PRCA, UEC
29	RIYDEILQSK	RCC
31	ATFNKLVSY	CRC, SCLC, UBC
33	SVFEGDSIVL K	NHL, NSCLCadeno, NSCLCother, OC
34	SVYSETSNMD K	BRCA, MEL, NHL, NSCLCsquam, OC, OSCAR, RCC, UEC
41	SVAHLLSKY	UEC
42	SVSSSTHFTR	GC, HNSCC, NHL, NSCLCadeno, NSCLCsquam, UEC
43	KLMETSMGF	BRCA, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
44	KVYDPVSEY	BRCA, CCC, GBC, GBM, HCC, MEL, NHL, NSCLCadeno, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, RCC, SCLC, UBC, UEC
45	VVFPFPVVK	GBM, NHL, NSCLCother, OC, SCLC, UEC
46	RVFPSPMRI	MEL, NHL, NSCLCsquam, OSCAR, SCLC
47	SVLDLSVHK	CCC, HCC, NHL, OSCAR, PRCA, SCLC, UEC
48	RIKPPGPTAV PK	BRCA, MEL
49	GLLEEALFY	BRCA, CRC, GBC, GC, HCC, MEL, NSCLCadeno, NSCLCsquam, OSCAR, PACA, PRCA, RCC
50	GVFNTLISY	MEL, PRCA
51	ASTTVLALK	NHL
53	KYIEYYLVL	GC, NSCLCadeno, NSCLCsquam, UEC
54	QQALNFTRF	GBC, MEL, NHL, NSCLCadeno, NSCLCsquam, OC, RCC, SCLC
55	IFVARLYYF	BRCA, CRC, GBC, GC, HCC, MEL, NHL, NSCLCadeno, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
56	KYSSGFRNI	BRCA, CCC, CRC, GBC, GBM, GC, HCC, MEL, NHL, NSCLCadeno, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
57	RFPPTPLF	HNSCC, NHL, NSCLCsquam, OSCAR, UEC
58	KYLADLPTL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadeno, NSCLCother, NSCLCsquam, OC, OSCAR, PRCA, RCC, SCLC, UEC
60	TQDPHVNAFF	BRCA, CRC, GBC, GEJC, HCC, HNSCC, NHL, NSCLCadeno, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
62	YYLSHLERI	GBC, HNSCC, NSCLCadeno, NSCLCother, NSCLCsquam,

Seq ID No	Sequence	Peptide Presentation on tumor types
		UBC
65	AYLKQVLLF	BRCA, CCC, CRC, GBC, GBM, GC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
66	SQPAVATSF	CRC, SCLC
67	VFLPSEGFNF	MEL, NSCLCadenoc, SCLC
68	LYQDRFDYL	GC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, PRCA, SCLC, UEC
69	EYNTIKDKF	GBC, GC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, SCLC, UEC
70	LYSDIGHLL	CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PRCA, RCC, UBC, UEC
71	RYLGKNWSF	GBC, OC, RCC
72	TYVENLRLL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
73	TYPQLEGFKF	BRCA, CRC, GBC, GC, GEJC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
74	SYADNILSF	BRCA, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
75	RFYLLTEHF	CRC, GBC, HCC, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, SCLC
76	KAFSWSSAF	BRCA, GBM, NHL, NSCLCsquam, PRCA, SCLC, UBC
77	RPNGNSLFTS A	HCC, MEL, NHL, PRCA, SCLC, UEC
78	RPRGLALVL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
79	SPVPSHWMV A	CCC, MEL, NHL, PRCA, SCLC, UBC
80	KPLFKVSTF	NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, RCC, UEC
84	SPKLQIAAM	NHL, OC
85	IPVSHPV	NHL
86	IPASHPV	GBC, MEL, NHL, NSCLCsquam, OSCAR, PACA, SCLC
87	FPAPILRAV	GBC, GC, MEL, NHL, NSCLCother, UBC
88	MPDPHLYHQ M	MEL, NHL, NSCLCsquam
89	FPETVNNLL	NSCLCadenoc, NSCLCother, NSCLCsquam, PACA

Seq ID No	Sequence	Peptide Presentation on tumor types
97	LPFGKIPIL	BRCA, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
98	YPIALTRAEM	BRCA, CCC, GBC, GC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
99	SPRAINNLVL	NHL
100	YPYQERVFL	NHL, NSCLCadenoc, NSCLCsquam
101	NPRYPNYMF	NHL
104	RPMTPTQIGP SL	NHL
105	NPLTKLLAI	CCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC
106	KAFKWFSAL	NHL
108	ILAIRQNAL	CCC, NHL, OC
109	LGHVRYVL	CCC, GBM, NHL, UBC
110	FGLARIYSF	BRCA, CCC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
112	APLLRHWEL	BRCA, CCC, CRC, GBC, HCC, HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, SCLC
113	DANSRTSQL	MEL, NHL, NSCLCadenoc, NSCLCother, UEC
114	HNALRILTF	BRCA, NHL, NSCLCother, NSCLCsquam
115	ELYQRIYAF	HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, PRCA, SCLC
116	TLKIRAEVL	CCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PRCA, SCLC
118	FEKEKKESL	OC
119	DLRTKEVVF	GC, NHL
122	KELPGVKKY	CCC, CRC, GC, HCC, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, RCC, UEC
123	EENPGKFLF	HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PRCA
124	SESLPKEAF	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
126	EENKPGIVY	CRC, HCC, NHL
128	GENDRLNHTY	NHL
129	GEGAYGKVF	HCC, HNSCC, NSCLCadenoc, OC, OSCAR, RCC
131	EEFETIERF	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC,

Seq ID No	Sequence	Peptide Presentation on tumor types
		OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
132	GELPAVRDL	CRC, GBC, GBM, GC, HCC, HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA
133	AEHNFVAKA	BRCA, GBM, HNSCC, MEL, NHL, NSCLCadenoc, OC, OSCAR
134	SEYADTHYF	NHL
135	NEIKVYITF	BRCA, CRC, HNSCC, NSCLCadenoc, NSCLCsquam, PRCA, RCC
136	AEYKGRVTL	NHL, OSCAR
138	SQAPAARAF	NHL, NSCLCadenoc
139	RENQVLGSGW	NHL
143	YEYDLKWEF	NHL
144	TEGKRYFTW	MEL, NHL
145	AEPLVGQRW	NHL, PRCA, UBC
146	SESKTVVTY	CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
147	KEVPRSYEL	HNSCC, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, RCC
148	REYNEYENI	NHL, NSCLCadenoc, OSCAR
149	SEKETVAYF	MEL, NHL
150	EEVTDERSQL	GBC, GC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC
151	EVDASIFKAW	BRCA, CCC, GBC, GBM, HCC, HNSCC, NHL, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
152	AELLAKELY	CCC, CRC, NHL, NSCLCsquam, UEC
154	AEPGPVITW	NHL
155	NEFPVIVRL	BRCA, CRC, GBC, GBM, GC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
156	FEVESLFQKY	BRCA, CRC, GBC, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
157	VEIAEAIQL	CCC, CRC, HNSCC, NSCLCadenoc, NSCLCother, NSCLCsquam, OC
159	GELLGRQSF	BRCA, CCC, CRC, MEL, NHL, NSCLCadenoc, OC, PACA, RCC, UEC
160	EEETILHFF	NHL
161	EEGDTLLHLF	CRC, HNSCC, NHL, NSCLCsquam, OSCAR, PACA, UBC, UEC
164	SEYSHLTRV	RCC

Seq ID No	Sequence	Peptide Presentation on tumor types
166	NEVLASKY	BRCA, CCC, CRC, GBC, GC, HCC, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PRCA, SCLC, UBC, UEC
167	KEIGAAVQAL	NSCLCadenoc, NSCLCother, OC
168	QEIQSLLTNW	CCC, CRC, GBM, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, RCC, SCLC, UEC
169	EENGEVKEL	NHL
171	SEDLAVHLY	BRCA, CRC, GC, HCC, HNSCC, NHL, OSCAR, PACA, PRCA, UBC
172	VEDGLFHEF	BRCA, CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, UBC, UEC
174	TDKSFPNAY	PRCA, SCLC
176	AENAVSNLSF	NHL
177	QENMQIQSF	BRCA, CCC, CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, SCLC, UBC, UEC
178	REYEHYWTEL	MEL, NHL, NSCLCadenoc, NSCLCother, OSCAR
179	AEIKQTEEKY	HCC
180	EETAFNVSY	BRCA, CCC, GBC, NHL, NSCLCadenoc, OC, OSCAR
181	GEIKEPLEI	BRCA, CCC, CRC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, RCC, UEC
182	AQNLSIIQY	BRCA, GBC, GC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PRCA
183	GESQDSTTAL	CCC, HNSCC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC
184	RMPPFTQAF	BRCA, GBC, GC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, PACA, PRCA, RCC, SCLC
185	SEGDNVESW	NHL
187	SDAQRPSF	NSCLCother, RCC
188	YVDAGTPMY	GBC, GBM, GC, NSCLCadenoc, NSCLCsquam
189	VTEEPQRLFY	BRCA, CCC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
190	HVDQDLTTY	GBM, GC, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, PACA, PRCA
191	ISEAGKDLLY	BRCA, CCC, GEJC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, PRCA, SCLC, UEC
192	RSDPGGGGLAY	BRCA, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
193	LTDSEKGNYSY	GBC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, PRCA

Seq ID No	Sequence	Peptide Presentation on tumor types
195	YSDKEFAGSY	BRCA, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
196	FTDIDGQVY	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
197	SLADVHIEV	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
198	KLLGYDVHV	BRCA, CRC, NHL, NSCLCother, OC, RCC, UEC
199	AMPDSPAIEV	HNSCC, NHL, NSCLCadenoc
200	VMLQINPKL	BRCA, GBC, GBM, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, RCC, SCLC, UEC
201	ILAAVETRL	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
203	FLLPKVQSI	BRCA, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR
204	FLLPKVQSIQL	MEL, NSCLCadenoc, NSCLCother, NSCLCsquam
205	FLINTNSEL	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
206	SLMDLQERL	BRCA, CCC, CRC, GBC, GBM, GEJC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
207	KLSDNLIK	CRC, GBC, MEL, NHL, PRCA
208	KLNPQQAPLY	MEL, NHL, OSCAR, UEC
209	KTLPAMLGTG K	NHL, OC
210	RMYSQKTLQ K	MEL, NHL
211	ATYNKQPMY R	CCC, CRC, GBC, GC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, RCC, SCLC, UEC
213	RVYNIYIRR	NHL, NSCLCadenoc, OC
215	KATGAATPK	NSCLCsquam
216	RIKAPSRNTIQ K	NHL
217	TTVPHVFSK	BRCA, CCC, CRC, GBC, GC, HCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UEC
218	RVLTGVFTK	NHL
220	SISNLVFTY	BRCA, CRC, GBC, GC, HCC, HNSCC, MEL, NSCLCadenoc,

Seq ID No	Sequence	Peptide Presentation on tumor types
		NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
221	LLNRHILAH	MEL, UEC
222	RYLDEINLL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
223	RRMYPPPLI	HCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, SCLC, UEC
224	VYEYVVERF	BRCA, CCC, CRC, GBC, GBM, GC, GEJC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
225	LPARFYQAL	BRCA, OSCAR
226	YLNRLHTW	NHL, NSCLCsquam
227	APINKAGSFL	MEL, NHL, OC
229	SPLGSLARSSL	GBC, NHL, OC, OSCAR
230	KPMKSVLVV	BRCA, GC, NHL, NSCLCaden, OC, OSCAR, UBC
231	MPLSTIREV	BRCA, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
232	APRPAGSYL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
233	SPRVYWLGL	NHL
234	SPKESENAL	NHL, NSCLCsquam
237	SPWLHAPSL	GBC, NHL, NSCLCaden, OC
238	SPRSWIQVQI	BRCA, CRC, GBC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, RCC, SCLC, UEC
239	APSKTSLIM	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
240	SPSLPNITL	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
241	APAPAEKTPV	BRCA, CRC, GBC, GBM, GC, HNSCC, MEL, NHL, NSCLCaden, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
242	SPFSFHHLV	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCaden, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
243	LPKVQSIQL	CCC, HCC, NSCLCaden, NSCLCsquam, OC, OSCAR, RCC

Seq ID No	Sequence	Peptide Presentation on tumor types
244	MPSSDTTVTF	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
245	SPLSHHSQ	GC, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR
246	YPGWHSTTI	CRC, HNSCC, NHL
247	QPSPARAPAE L	GBC, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, SCLC
248	LPYDSKHQI	BRCA, CRC, GBC, GBM, GC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
249	SPADHRGYA SL	BRCA, CRC, GBC, HCC, HNSCC, MEL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, RCC, SCLC, UBC, UEC
250	VPNLQTVSV	BRCA, CCC, CRC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
251	QPRFLTMDL	BRCA, CRC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, RCC, SCLC, UEC
252	RPHIPISKL	BRCA, CRC, GBC, GBM, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, RCC, SCLC, UBC, UEC
253	RPFADLLGTA F	BRCA, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OC, OSCAR, PACA, SCLC, UBC, UEC
254	SPRNLQPQR AAL	CRC, GBC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, RCC, SCLC, UBC
255	YPGSDRIML	MEL, NHL, NSCLCadenoc
256	SPYKKLKEAL	BRCA, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OSCAR, PRCA, RCC, SCLC, UBC
257	KEFFFVKVF	BRCA, CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, OSCAR, PACA, RCC, SCLC, UBC, UEC
258	EELFRDGVN W	CCC, CRC, HCC, MEL, NHL, NSCLCadenoc, NSCLCsquam, PACA, PRCA, UEC
259	EENTLVQNY	BRCA, CRC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, SCLC, UBC, UEC
260	AEIGEGAYGK VF	BRCA, CCC, CRC, GBM, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquam, OC, OSCAR, PACA, PRCA, UBC, UEC
261	NEIEHIPVW	NHL, OSCAR
262	QENQAETHA	NHL

Seq ID No	Sequence	Peptide Presentation on tumor types
	W	
263	REAGFQVKAY	MEL, NHL
264	SEDHSGSYW	CRC, GC, MEL, NHL, NSCLCadenoc, NSCLCsquamous
265	QEVDAIFKA W	BRCA, CCC, CRC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquamous, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
266	VDASIFKAW	BRCA, CRC, GBC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquamous, OC, OSCAR, PACA, RCC, SCLC, UBC, UEC
267	KEKFPINGW	CRC, HNSCC, NHL, PACA
269	KELEDLNKW	BRCA, CRC, GBC, GBM, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquamous, OC, OSCAR, PACA, PRCA, RCC, UEC
270	AESEDLAVHL	MEL, NSCLCsquamous
271	AESEDLAVHL Y	CRC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquamous, OSCAR, PACA
272	KEFELRSSW	BRCA, CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquamous, OSCAR, PACA, PRCA, RCC, UEC
273	AEIEIVKEEF	BRCA, CCC, CRC, GBC, GBM, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCsquamous, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
274	GEAVTDHPD RLW	NHL
275	TENPLTKLL	BRCA, CRC, HCC, NHL
276	EEEGNLLRS W	BRCA, CCC, CRC, GBC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquamous, OC, OSCAR, PACA, PRCA, RCC, SCLC, UBC, UEC
277	EEGNLLRSW	BRCA, CRC, GC, HCC, HNSCC, MEL, NHL, NSCLCadenoc, NSCLCother, NSCLCsquamous, OSCAR, PACA, RCC, UBC, UEC

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); Bio-Options Inc. (Brea, CA, USA); Geneticist Inc. (Glendale, CA, USA); ProteoGenex Inc. (Culver City, CA, USA); Tissue Solutions Ltd (Glasgow, UK). Total RNA from tumor 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). 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 equimolar and sequenced on the Illumina HiSeq 2500 sequencer according to the manufacturer's instructions, generating 50 bp single end

- 130 -

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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia are shown in Figure 1. 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: blood cells, blood vessels, brain, heart, liver, lung, adipose tissue, adrenal gland, bile duct, bladder, bone marrow, cartilage, esophagus, eye, gallbladder, head&neck, kidney, large intestine, lymph node, nerve, pancreas, parathyroid, peritoneum, pituitary, pleura, skeletal muscle, skin, small intestine, spleen, stomach, thyroid gland, trachea, ureter. 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	
		AML	CLL
1	LTEGHSGNYY		+++
2	TMIRIFHRY	+++	
3	YINPAKLTPY		+
4	ALDQNKMHY	+	
5	GTDVLSTRY	+	
6	VTEGVAQTSFY		+
7	FMDSESFYY		++
8	STDSAGSSY		++
9	YSHQPQYSSY		+++

- 131 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
10	YSDIGHLL		+
11	AAADHHSly	+	
12	ATDIVDSQY		++
13	ITDIHIKY		+
14	TFDLTVVSY		+
15	SVADIRNAY		+
16	WIGDKSFEY	+	
17	KAYNRVIFV		+
18	YLLPSVLL		+
19	SLFEGIYTI	+++	
20	FSLEDLVRI		+
21	FLFDKLLLI		+
22	ILHAQTLKI		++
23	FAFSGVLRA	+	
24	KLGPVAVSI		+
25	YLNEKSLQL	+	
26	SLYVQQDKI		+
27	RLIAKEMNI		+
28	VILESIFLK		+
29	RIYDEILQSK	+	+
30	RTYGFVLTf		+
31	ATFNKLVSY	+	
32	KTSNIVKIK		++
33	SVFEGDSIVLK		++
34	SVYSETSNMDK		+
35	ATKSPAKPK		++
36	KAKAAAKPK		++
37	KAKKPAGAAK		+
38	KARKSAGAAK		+
39	IVIQLRAQK		+
40	RSKEYIRKK		++
41	SVAHLLSKY		+
42	SVSSSTHFTR		+
43	KLMETSMGF		+
44	KVYDPVSEY		+
45	VVFPFPVnk	++	
46	RVFPSPMRI		+
47	SVLDLSVHK		+
48	RIKPPGPTAVPK	+	

- 132 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
49	GLLEEALFY	+	
50	GVFNTLISY	+	
51	ASTTVLALK		+
52	KAFNQSSTLTK		+++
53	KYIEYYLVL		++
54	QQALNFTRF		+
55	IFVARLYYF		+
56	KYSSGFRNI		+
57	RFPPTPPLF		+
58	KYLADLPTL		+
59	GLYEGTGRLF	+	
60	TQDPHVNAFF		+
61	IFKEHNFSF	+++	
62	YYLSHLERI	+	
63	IYFSNTHFF	+	
64	SFQSKATVF	+++	
65	AYLKQVLLF		++
66	SQPAVATSF	+	
67	VFLPSEGFNF	+	+
68	LYQDRFDYL	++	
69	EYNTIKDKF		+
70	LYSDIGHLL		+
71	RYLGKNWSF	++	
72	TYVENLRLL		+
73	TYPQLEGFKF		++
74	SYADNILSF		+
75	RFYLLTEHF	+	
76	KAFSWSSAF		+++
77	RPNGNSLFTSA		+
78	RPRGLALVL	+	
79	SPVPSHWMVA		+
80	KPLFKVSTF		+
81	SESPWLHAPSL		+
82	APFGFLGMQSL		+
83	IPVSRPIL		++
84	SPKLQIAAM		++
85	IPVSHPVL		+++
86	IPASHPVL		+++
87	FPAPILRAV		+

- 133 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
88	MPDPHLYHQM		+
89	FPETVNNLL	+	+
90	KPKAAKPKA		++
91	KPKAAKPKAA		++
92	KAKKPAGAA		+
93	KARKSAGAA		+
94	KPKAAKPKKAAA		+
95	KPKAAKPKTA		+
96	KPKKAPKSPA		+
97	LPFGKIPIL	+	
98	YPIALTRAEM		+
99	SPRAINNLVL		++
100	YPYQERVFL	+	
101	NPRYPNYMF		+
102	LPLSMEAKI	+	
103	IPANTEKASF		+
104	RPMTPTQIGPSL		++
105	NPLTKLLAI	++	+
106	KAFKWFSAL		+
107	QAAQRTAL		+
108	ILAIRQNAL		+
109	LGHVRYVL		+
110	FGLARIYSF	++	
111	VTLIKYQEL		++
112	APLLRHWEL		+
113	DANSRTSQL		+
114	HNALRILTF	+	
115	ELYQRIYAF	+	
116	TLKIRAEVL		++
117	YIKTAKKL		++
118	FEKEKKESL		+
119	DLRTKEVVF		+
120	VPPKKHLL		+
121	RPKKVNTL		+
122	KELPGVKKY		++
123	EENPGKFLF		++
124	SESLPKEAF		+
125	SESTFDRTF		+
126	EENKPGIVY		+

- 134 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
127	TEYPVFVY	+	
128	GENDRLNHTY		+
129	GEGAYGKVF	+++	
130	EEEHGKGREY		+
131	EEFETIERF		+
132	GELPAVRDL		+
133	AEHNFVAKA		++
134	SEYADTHYF		+++
135	NEIKVYITF	+	
136	AEYKGRVTL		++
137	GELGGSVTI		++
138	SQAPAARAF		+
139	RENQVLGSGW		++
140	EYDLKWEF	+++	
141	REYEYDLKWEF	+++	
142	TEIFKEHNF	+++	
143	YEYDLKWEF	+++	
144	TEGKRYFTW		+
145	AEPLVGQRW		++
146	SESKTVVTY		++
147	KEVPRSYEL		+
148	REYNEYENI		+++
149	SEKETVAYF		++
150	EEVTDRSQL		+
151	EVDASIFKAW		+
152	AELLAKELY		++
153	KEFEQVPGHL		+
154	AEPGPVITW		++
155	NEFPVIVRL		+
156	FEVESLFQKY		+
157	VEIAEAIQL		+
158	GENEDNRIGL		++
159	GELLGRQSF		+
160	EEETILHFF		+
161	EEGDTLLHLF	+	+
162	DEAQARAAF	++	
163	EEWMGLLEY	++	
164	SEYSHLTRV	+	
165	VELDLQRSV	+	

- 135 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
166	NEVLASKY		+
167	KEIGAAVQAL		+
168	QEIQSLLTNW		+
169	EENGEVKEL		+
170	SENEQRRMF		+
171	SEDLAVHLY		++
172	VEDGLFHEF		+
173	KEYDFGTQL	+	
174	TDKSFPNAY		+
175	HEIDGKALFL	+	
176	AENAVSNLSF		++
177	QENMQIQSF		+
178	REYEHYWTEL		+
179	AEIKQTEEKY	+	
180	EPPAFNVSY		+
181	GEIKEPLEI		+
182	AQNLSIIQY		++
183	GESQDSTTAL		++
184	RMPPFTQAF		+
185	SEGDNVESW		+
186	NEQKIVRF		+
187	SDAQRPSF		+
188	YVDAGTPMY		+
189	VTEEPQRLFY		+++
190	HVDQDLTTY	++	
191	ISEAGKDLLY	+	+
192	RSDPGGGGLAY	++	
193	LTDSEKGNSY		++
194	YTDKKSIIY		+
195	YSDKEFAGSY		+
196	FTDIDGQVY		+
197	SLADVHIEV		+
198	KLLGYDVHV	+	
199	AMPDSPAEV	+	+
200	VMLQINPKL		+
201	ILAAVETRL		+
202	MVALPMVLV		+
203	FLLPKVQSI		+
204	FLLPKVQSIQL		+

- 136 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
205	FLINTNSEL		+
206	SLMDLQERL		+
207	KLSDNILKL		+
208	KLNPQQAPLY		+
209	KTLPAMLGTGK		++
210	RMYSQKTLQK		++
211	ATYNKQPMYR	+	
212	LLWHWDTTQSLK		++
213	RVYNIYIRR	+	
214	ATGAATPKK		+
215	KATGAATPK		+
216	RIKAPSRNTIQK		+
217	TTVPHVFSK		+
218	RVLTGVFTK		+
219	HSYSSPSTK		+
220	SISNLVFTY	+	
221	LLNRHILAH		+
222	RYLDEINLL	+	
223	RRMYPPPLI		+
224	VYEYVVERF		+
225	LPARFYQAL		+
226	YLNRLHTW		++
227	APINKAGSFL		+
228	SPRITFPSL		+
229	SPLGSLARSSL		+
230	KPMKSVLVV		+
231	MPLSTIREV	+++	
232	APRPAGSYL		+
233	SPRVYWLGL		++
234	SPKESENAL		+
235	SPSLPSRTL		+
236	RPSNKAPLL		+
237	SPWLHAPSL		+
238	SPRSWIQVQI		+++
239	APSKTSLIM		+
240	SPSLPNITL		+
241	APAPAEKTPV		+
242	SPFSFHHVL		+
243	LPKVQSIQL		+

- 137 -

SEQ ID No	Sequence	Gene Expression	
		AML	CLL
244	MPSSDTTVTF		+
245	SPLSHHSQL		+
246	YPGWHSTTI	+	
247	QPSPARAPAE	+	+++
248	LPYDSKHQI		+
249	SPADHRGYASL	+	
250	VPNLQTVSV		+
251	QPRLFTMDL		+
252	RPHIPISKL	+	+
253	RPFADLLGTAF		++
254	SPRNLPQRAAL		++
255	YPGSDRIML		+
256	SPYKKLKEAL		+
257	KEFFFVKVF		+
258	EELFRDGVNW		++
259	EENTLVQNY		+
260	AEIGEGAYGKVF	+++	
261	NEIEHIPVW		+
262	QENQAETHAW		+
263	REAGFQVKAY		+
264	SEDHSGSYW		+
265	QEVDAIFKAW		+
266	VDASIFKAW		+
267	KEKFPINGW		+
268	NEDKGTKAW		+
269	KELEDLNKW		+
270	AESEDLAVHL		++
271	AESEDLAVHLY		++
272	KEFELRSSW		+
273	AEIEIVKEEF		+
274	GEAVTDHPDRLW		++
275	TENPLTKLL	++	+
276	EEEGNLLRSW		++
277	EEGNLLRSW		++

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*02:01, HLA-A*24:02, HLA-A*01:01, HLA-A*03:01, HLA-B*07:02 and HLA-B*44:02 restricted TUMAPs of the invention, demonstrating that these peptides are T-cell epitopes against which CD8⁺ precursor T cells exist in humans (Table 10a and Table 10b).

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, HLA-A*24, HLA-A*01, HLA-A*03, HLA-B*07 or HLA-B*44 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. 280) from modified Melan-A/MART-1) and A*0201/DDX5-001 (YLLPAIVHI from DDX5, SEQ ID NO. 281), 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 1×10^6 CD8⁺ T cells with 2×10^5 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 chronic lymphocytic leukemia, chronic myeloid leukemia and acute myeloid leukemia 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 14 peptides of the invention are shown in Figures 2 to 9 together with corresponding negative controls. Results for 63 peptides from the invention are summarized in Table 10a and 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 No	Sequence	Wells positive [%]
278	YLDRKLLTL	++++
279	LYIDRPLPYL	++++

Table 10b: *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 No	Sequence	Wells positive [%]	HLA
1	LTEGHSGNYY	"+"	A*01
12	ATDIVDSQY	"++"	A*01
19	SLFEGITYI	"++++"	A*02
22	ILHAQTLKI	"+"	A*02
24	KLGPVAVSI	"+++"	A*02
25	YLNEKSLQL	"+"	A*02
26	SLYVQQLKI	"++"	A*02
27	RLIAKEMNI	"+"	A*02
29	RIYDEILQSK	"+"	A*03
45	VVFPFPVVK	"+"	A*03
47	SVLDLSVHK	"+"	A*03
51	ASTTVLALK	"+"	A*03
53	KYIEYYLVL	"++++"	A*24
55	IFVARLYYF	"+"	A*24

- 141 -

Seq ID No	Sequence	Wells positive [%]	HLA
57	RFPPTPPLF	"+"	A*24
68	LYQDRFDYL	"+"	A*24
73	TYPQLEGFKF	"+"	A*24
83	IPVSRPIL	"+"	B*07
84	SPKLQIAAM	"++"	B*07
87	FPAPILRAV	"+"	B*07
89	FPETVNNLL	"+"	B*07
96	KPKKAPKSPA	"+"	B*07
99	SPRAINNLVL	"+"	B*07
104	RPMTPTQIGPSL	"+"	B*07
122	KELPGVKKY	"++++"	B*44
123	EENPGKFLF	"++"	B*44
129	GEGAYGKVF	"++++"	B*44
133	AEHNFVAKA	"+"	B*44
134	SEYADTHYF	"++++"	B*44
136	AEYKGRVTL	"+"	B*44
137	GELGGSVTI	"++++"	B*44
139	RENQVLGSGW	"++++"	B*44
140	EYDLKWEF	"++++"	B*44
142	TEIFKEHNF	"++"	B*44
143	YEYDLKWEF	"+"	B*44
145	AEPLVGQRW	"++++"	B*44
146	SESKTVVTY	"++++"	B*44
149	SEKETVAYF	"+"	B*44
154	AEPGPVITW	"++++"	B*44
158	GENEDNRIGL	"++"	B*44
162	DEAQARAAF	"+"	B*44
171	SEDLAVHLY	"++++"	B*44
176	AENAVSNLSF	"++"	B*44
182	AQNLSIIQY	"++++"	B*44
192	RSDPGGGGLAY	"+"	A*01
197	SLADVHIEV	"++"	A*02
207	KLSDNILKL	"++"	A*02
210	RMYSQKTLQK	"+"	A*03
215	KATGAATPK	"++"	A*03
233	SPRVYWLGL	"++++"	B*07
238	SPRSWIQVQI	"+"	B*07
247	QPSPARAPAEI	"++"	B*07
253	RPFADLLGTAF	"++++"	B*07

Seq ID No	Sequence	Wells positive [%]	HLA
254	SPRNLQPQRAAL	"+"	B*07
258	EELFRDGVNW	"+"	B*44
260	AEIGEGAYGKVF	"++"	B*44
270	AESEDLAVHL	"+"	B*44
271	AESEDLAVHLY	"+"	B*44
275	TENPLTKLL	"+"	B*44
276	EEEGNLLRSW	"++"	B*44
277	EEGNLLRSW	"++"	B*44

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 lyophilizes (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 ($\beta 2m$) 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 for 1h 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*01:01 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

Seq ID No	Sequence	Peptide exchange
1	LTEGHSGNYY	"++++"
3	YINPAKLTPY	"++"
4	ALDQNKMHY	"++++"
5	GTDVLSTRY	"++++"
6	VTEGVAQTSFY	"++++"
7	FMDSESFYY	"+"
8	STDSAGSSY	"++++"
9	YSH PQYSSY	"++"
10	YSDIGHLL	"++"
11	AAADHHS LY	"++++"
12	ATDIVDSQY	"++++"
13	ITDIHIKY	"++++"
14	TFDLTVVSY	"++"
15	SVADIRNAY	"++"
16	WIGDKSFEY	"++"
188	YVDAGTPMY	"++++"
189	VTEEPQRLFY	"++++"
190	HVDQDLTTY	"++"
191	ISEAGKDLLY	"++++"
192	RSDPGGGGLAY	"++++"
193	LTDSEKGNSY	"++++"

- 144 -

Seq ID No	Sequence	Peptide exchange
194	YTDKKSIIY	"+++"
195	YSDKEFAGSY	"++++"
196	FTDIDGQVY	"++++"

Table 12: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-A*02:01 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

Seq ID No	Sequence	Peptide exchange
17	KAYNRVIFV	"+++"
18	YLLPSVLL	"++++"
19	SLFEGIYTI	"++++"
20	FSLEDLVRI	"++"
21	FLFDKLLLI	"++++"
22	ILHAQTLKI	"+++"
23	FAFSGVLRA	"++"
24	KLGPVAVSI	"++++"
25	YLNEKSLQL	"++++"
26	SLYVQQLKI	"++++"
27	RLIAKEMNI	"++++"
197	SLADVHIEV	"++++"
198	KLLGYDVHV	"+++"
199	AMPDSPAEV	"++++"
200	VMLQINPKL	"+++"
201	ILAAVETRL	"+++"
203	FLLPKVQSI	"++++"
204	FLLPKVQSIQL	"+++"
205	FLINTNSEL	"++++"
206	SLMDLQERL	"+++"
207	KLSDNILKL	"++++"

Table 13: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-A*03:01 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

Seq ID No	Sequence	Peptide exchange
28	VILESIFLK	"++"

Seq ID No	Sequence	Peptide exchange
29	RIYDEILQSK	"++"
30	RTYGFVLTF	"++"
32	KTSNIVKIK	"++"
33	SVFEGDSIVLK	"++"
34	SVYSETSNMDK	"+++"
35	ATKSPAKPK	"+++"
36	KAKAAAKPK	"++"
37	KAKKPAGAAK	"+++"
38	KARKSAGAAK	"+++"
40	RSKEYIRKK	"+"
41	SVAHLLSKY	"++"
42	SVSSSTHFTR	"++"
43	KLMETSMGF	"++"
44	KVYDPVSEY	"++"
45	VVFPFPVNK	"+++"
46	RVFPSPMRI	"++"
47	SVLDLSVHK	"++"
48	RIKPPGPTAVPK	"+++"
49	GLLEEALFY	"++"
51	ASTTVLALK	"++"
52	KAFNQSSTLTK	"+++"
208	KLNPQQAPLY	"++"
209	KTLPAMLGTGK	"++"
210	RMYSQKTLQK	"+++"
211	ATYNKQPMYR	"+++"
212	LLWHWDTTQSLK	"+"
213	RVYNIYIRR	"++"
214	ATGAATPKK	"+++"
215	KATGAATPK	"++"
216	RIKAPSRNTIQK	"++"
217	TTVPHVFSK	"++"
218	RVLTGVFTK	"++"
219	HSYSSPSTK	"+++"
220	SISNLVFTY	"++"
221	LLNRHILAH	"+++"

Table 14: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-A*24:02 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

- 146 -

Seq ID No	Sequence	Peptide exchange
53	KYIEYYLVL	"++++"
55	IFVARLYYF	"++++"
56	KYSSGFRNI	"+++"
57	RFPPTPPLF	"++++"
58	KYLADLPTL	"++++"
61	IFKEHNFSF	"++++"
62	YYLSHLERI	"++++"
63	IYFSNTHFF	"+++"
64	SFQSKATVF	"++"
67	VFLPSEGFNF	"+++"
68	LYQDRFDYL	"++++"
69	EYNTIKDKF	"+++"
70	LYSDIGHLL	"+++"
71	RYLGKNWSF	"++++"
72	TYVENLRLL	"+++"
73	TYPQLEGFKF	"++++"
74	SYADNILSF	"++++"
75	RFYLLTEHF	"+++"
222	RYLDEINLL	"+++"

Table 15: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-B*07:02 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

Seq ID No	Sequence	Peptide exchange
77	RPNGNSLFTSA	"+++"
78	RPRGLALVL	"+++"
79	SPVPSHWMVA	"+++"
80	KPLFKVSTF	"++"
81	SESPWLHAPSL	"+++"
82	APFGFLGMQSL	"+++"
83	IPVSRPIL	"+++"
84	SPKLQIAAM	"+++"
85	IPVSHPV	"++"
86	IPASHPV	"++"
87	FPAPILRAV	"+++"
88	MPDPHLYHQM	"++"
89	FPETVNNLL	"++"

- 147 -

Seq ID No	Sequence	Peptide exchange
90	KPKAAKPKA	"++"
91	KPKAAKPKAA	"++"
92	KAKKPAGAA	"++"
93	KARKSAGAA	"+++"
94	KPKAAKPKKAAA	"++"
95	KPKAAKPKTA	"++"
96	KPKKAPKSPA	"+++"
97	LPFGKIPIL	"++"
98	YPIALTRAEM	"+++"
99	SPRAINNLVL	"++++"
100	YPYQERVFL	"++"
101	NPRYPNYMF	"+++"
102	LPLSMEAKI	"++"
103	IPANTEKASF	"++"
104	RPMTPTQIGPSL	"+++"
105	NPLTKLLAI	"+++"
106	KAFKWFSAL	"++"
225	LPARFYQAL	"++++"
226	YLNRLHTW	"++"
227	APINKAGSFL	"++++"
228	SPRITFPSL	"++++"
229	SPLGSLARSSL	"++++"
230	KPMKSVLVV	"+++"
231	MPLSTIREV	"++"
232	APRPAGSYL	"+++"
233	SPRVYWLGL	"+++"
234	SPKESENAL	"++"
235	SPSLPSRTL	"++"
236	RPSNKAPLL	"+++"
237	SPWLHAPSL	"++"
238	SPRSWIQVQI	"+++"
239	APSKTSLIM	"++"
240	SPSLPNITL	"+++"
241	APAPAEKTPV	"+++"
242	SPFSFHHVL	"++"
243	LPKVQSIQL	"++"
244	MPSSDTTVTF	"+++"
245	SPLSHHSQL	"++"
246	YPGWHSTTI	"++"

- 148 -

Seq ID No	Sequence	Peptide exchange
247	QPSPARAPAEI	"++"
248	LPYDSKHQI	"++"
249	SPADHRGYASL	"++"
250	VPNLQTVSV	"++"
251	QPRLFTMDL	"+++"
252	RPHIPISKL	"++"
253	RPFADLLGTAF	"+++"
254	SPRNLPQRAAL	"+++"
255	YPGSDRIML	"++"

Table 16: MHC class I binding scores. Binding of HLA-class I restricted peptides to HLA-B*44:02 was ranged by peptide exchange yield: >10% = +; >20% = ++; >50 = +++; > 75% = ++++

Seq ID No	Sequence	Peptide exchange
122	KELPGVKKY	"++"
123	EENPGKFLF	"+++"
124	SESLPKEAF	"++"
125	SESTFDRTF	"+++"
126	EENKPGIVY	"++"
127	TEYPVFVY	"+"
128	GENDRLNHTY	"++"
129	GEGAYGKVF	"++++"
130	EEEHGKGREY	"++"
131	EEFETIERF	"++"
132	GELPAVRDL	"++"
133	AEHNFVAKA	"+++"
134	SEYADTHYF	"+++"
135	NEIKVYITF	"+++"
136	AEYKGRVTL	"++++"
137	GELGGSVTI	"+++"
138	SQAPAAARAF	"++"
139	RENQVLGSGW	"+++"
140	EYDLKWEF	"++"
141	REYEDLKWEF	"+++"
142	TEIFKEHNF	"++"
143	YEYDLKWEF	"+++"
144	TEGKRYFTW	"+++"

- 149 -

Seq ID No	Sequence	Peptide exchange
145	AEPLVGQRW	"+++"
146	SESKTVVTY	"+++"
147	KEVPRSYEL	"++"
148	REYNEYENI	"++"
149	SEKETVAYF	"+++"
150	EEVTDRSQL	"++"
151	EVDASIFKAW	"++"
152	AELLAKELY	"++"
153	KEFEQVPGHL	"++"
154	AEPGPVITW	"+++"
155	NEFPVIVRL	"+++"
156	FEVESLFQKY	"+++"
157	VEIAEAIQL	"+++"
158	GENEDNRIGL	"++"
159	GELLGRQSF	"+++"
160	EEETILHFF	"++"
161	EEGDTLLHLF	"+++"
162	DEAQARAAF	"++"
163	EEWMGLLEY	"++++"
164	SEYSHLTRV	"++"
165	VELDLQRSV	"++"
166	NEVLASKY	"+"
167	KEIGAAVQAL	"+++"
168	QEIQSLLTNW	"+++"
169	EENGVEVKEL	"++"
170	SENEQRRMF	"+++"
171	SEDLAVHLY	"++"
172	VEDGLFHEF	"+++"
173	KEYDFGTQL	"++"
174	TDKSFPNAY	"+"
175	HEIDGKALFL	"++"
176	AENAVSNLSF	"++"
177	QENMQIQSF	"+++"
178	REYEHYWTEL	"+++"
179	AEIKQTEEKY	"++"
180	EEPAFNVSY	"++"
181	GEIKEPLEI	"++"
182	AQNLSIIQY	"++"
183	GESQDSTTAL	"++"

- 150 -

Seq ID No	Sequence	Peptide exchange
184	RMPPFTQAF	"++"
185	SEGDNVESW	"+++"
186	NEQKIVRF	"+"
187	SDAQRPSF	"+"
257	KEFFVVKVF	"+++"
258	EELFRDGVNW	"+++"
259	EENTLVQNY	"++"
260	AEIGEGAYGKVF	"+++"
261	NEIEHIPVW	"+++"
262	QENQAETHAW	"+++"
263	REAGFQVKAY	"+++"
264	SEDHSGSYW	"+++"
265	QEVDAIFKAW	"+++"
266	VDASIFKAW	"++"
267	KEKFPINGW	"+++"
268	NEDKGTKAW	"++"
269	KELEDLNKW	"+++"
270	AESEDLAVHL	"++"
271	AESEDLAVHLY	"++"
272	KEFELRSSW	"+++"
273	AEIEIVKEEF	"++"
274	GEAVTDHPDRLW	"+++"
275	TENPLTKLL	"++++"
276	EEEGNLLRSW	"+++"
277	EEGNLLRSW	"+++"

EXAMPLE 6Peptide-MHC class I stability

The peptide-MHC stability for HLA-B*08:01 peptides was performed by ImmunAware (Copenhagen, Denmark). The data were obtained using a proximity based, homogenous, real-time assay to measure the dissociation of peptides from HLA class I molecules. First human recombinant HLA-B*08:01 and b2m were expressed in *E. coli* and purified in a series of liquid chromatography based steps (Ferre et al., 2003; Ostergaard et al., 2001). Afterwards, the stability of a peptide-MHC complex (pMHC) can be determined by measuring the amount of b2m associated with the MHC heavy chain over time at 37°C (Harndahl et al., 2012). The stability of each pMHC, expressed

as the half life of b2m associated with the respective heavy chain, was calculated by fitting the data to a one-phase dissociation equation.

The pMHC stability were measured in three independent experiments and the peptides in question, for HLA-B*08:01, were found to span the range from weak-binders (+) to very stable binders (++++). The mean half-life (T1/2) is shown in Table 17.

Table 17: Mean half-life (T1/2) based on three individual measurements. T1/2 > 2 h = +; T1/2 > 4 h = ++; T1/2 > 6 h = +++; T1/2 > 10 h = ++++

Seq ID No	Sequence	Mean Half-life (T1/2)
107	QAAQRTAL	++
108	ILAIRQNAL	++
109	LGHVRYVL	+
110	FGLARIYSF	+
112	APLLRHWEL	+
113	DANSRTSQL	+++
114	HNALRILTF	++
115	ELYQRIYAF	++
116	TLKIRAEVL	+++
117	YIKTAKKL	++
118	FEKEKKESL	+++
119	DLRTKEVVF	++
120	VPPKKHLL	+
121	RPKKVNTL	+

Reference List

- Al, Qudaihi G. et al., Hematol.Oncol Stem Cell Ther. **3** (2010): 24-33
- Allan, E. K. et al., Leukemia **25** (2011): 985-994
- Allison, J. P. et al., Science **270** (1995): 932-933
- Andersen, R. S. et al., Nat.Protoc. **7** (2012): 891-902
- Appay, V. et al., Eur.J Immunol. **36** (2006): 1805-1814
- Banchereau, J. et al., Cell **106** (2001): 271-274

- Beatty, G. et al., J Immunol **166** (2001): 2276-2282
- Beggs, J. D., Nature **275** (1978): 104-109
- Bhatia, R. et al., Blood **101** (2003): 4701-4707
- Bocchia, M. et al., Lancet **365** (2005): 657-662
- Boulter, J. M. et al., Protein Eng **16** (2003): 707-711
- Braumuller, H. et al., Nature (2013)
- Bray, F. et al., Int J Cancer **132** (2013): 1133-1145
- Brossart, P. et al., Blood **90** (1997): 1594-1599
- Bruckdorfer, T. et al., Curr.Pharm.Biotechnol. **5** (2004): 29-43
- Byrd, J. C. et al., N.Engl.J Med. **369** (2013): 32-42
- Carballido, E. et al., Cancer Control **19** (2012): 54-67
- Card, K. F. et al., Cancer Immunol Immunother. **53** (2004): 345-357
- Cathcart, K. et al., Blood **103** (2004): 1037-1042
- Cohen, C. J. et al., J Mol Recognit. **16** (2003a): 324-332
- Cohen, C. J. et al., J Immunol **170** (2003b): 4349-4361
- Cohen, S. N. et al., Proc.Natl.Acad.Sci.U.S.A **69** (1972): 2110-2114
- Coligan, J. E. et al., Current Protocols in Protein Science (1995)
- Colombetti, S. et al., J Immunol. **176** (2006): 2730-2738
- Corbin, A. S. et al., J Clin Invest **121** (2011): 396-409
- Cortes, J. E. et al., J Clin Oncol **30** (2012): 3486-3492
- Counter, C. M. et al., Blood **85** (1995): 2315-2320
- Dao, T. et al., Sci.Transl.Med **5** (2013): 176ra33
- Dengjel, J. et al., Clin Cancer Res **12** (2006): 4163-4170
- Denkberg, G. et al., J Immunol **171** (2003): 2197-2207
- Eichhorst, B. F. et al., Blood **107** (2006): 885-891

- Estey, E. H., Am.J Hematol. **89** (2014): 1063-1081
- Falk, K. et al., Nature **351** (1991): 290-296
- Ferlay et al., GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No.11 [Internet], (2013), <http://globocan.iarc.fr>
- Ferre, H. et al., Protein Sci. **12** (2003): 551-559
- Follenzi, A. et al., Nat Genet. **25** (2000): 217-222
- Fong, L. et al., Proc.Natl.Acad.Sci.U.S.A **98** (2001): 8809-8814
- Furman, R. R. et al., N.Engl.J Med. **370** (2014): 997-1007
- Gabrilovich, D. I. et al., Nat Med. **2** (1996): 1096-1103
- Gandhi, V. et al., Clin Cancer Res **20** (2014): 1735-1740
- Gattinoni, L. et al., Nat Rev.Immunol **6** (2006): 383-393
- Giannopoulos, K. et al., Leukemia **24** (2010): 798-805
- Giannopoulos, K. et al., Int.J Oncol **29** (2006): 95-103
- Gnjatic, S. et al., Proc Natl.Acad.Sci.U.S.A **100** (2003): 8862-8867
- Godkin, A. et al., Int.Immunol **9** (1997): 905-911
- Goede, V. et al., N.Engl.J Med. **370** (2014): 1101-1110
- Gragert, L. et al., Hum.Immunol. **74** (2013): 1313-1320
- Granziero, L. et al., Blood **97** (2001): 2777-2783
- Green, M. R. et al., Molecular Cloning, A Laboratory Manual **4th** (2012)
- Greenfield, E. A., Antibodies: A Laboratory Manual **2nd** (2014)
- Greiner, J. et al., Exp.Hematol. **30** (2002): 1029-1035
- Gunawardana, C. et al., Br.J Haematol. **142** (2008): 606-609
- Gustafsson, C. et al., Trends Biotechnol. **22** (2004): 346-353
- Hallek, Michael et al., ASH Annual Meeting Abstracts **112** (2008): 325
- Harig, S. et al., Blood **98** (2001): 2999-3005
- Harndahl, M. et al., Eur.J Immunol. **42** (2012): 1405-1416

- Hoglund, M. et al., Ann.Hematol. **94 Suppl 2** (2015): S241-S247
- Horowitz, M. M. et al., Bone Marrow Transplant. **17 Suppl 3** (1996): S5-S6
- Huang, X. et al., Cancer **118** (2012): 3123-3127
- Hus, I. et al., Oncol Rep. **20** (2008): 443-451
- Hwang, M. L. et al., J Immunol. **179** (2007): 5829-5838
- Ilander, M. et al., Leuk.Lymphoma **55** (2014): 934-937
- Itonaga, H. et al., Int.J Hematol. **95** (2012): 209-213
- Jabbour, E. et al., Am.J Hematol. **91** (2016): 252-265
- Johnson, J. R. et al., Clin Cancer Res **9** (2003): 1972-1979
- Jung, G. et al., Proc Natl Acad Sci U S A **84** (1987): 4611-4615
- Kalos, M. et al., Sci.Transl.Med. **3** (2011): 95ra73
- Kantarjian, H. et al., N.Engl.J Med **362** (2010): 2260-2270
- Kantarjian, H. M. et al., J Clin Oncol **31** (2013): 3600-3604
- Keilholz, U. et al., Blood **113** (2009): 6541-6548
- Khorashad, J. S. et al., Blood **121** (2013): 489-498
- Kibbe, A. H., Handbook of Pharmaceutical Excipients **rd** (2000)
- Krackhardt, A. M. et al., Blood **100** (2002): 2123-2131
- Krieg, A. M., Nat Rev.Drug Discov. **5** (2006): 471-484
- Kronenberger, K. et al., J Immunother. **31** (2008): 723-730
- Kuball, J. et al., Blood **109** (2007): 2331-2338
- Kujawski, L. A. et al., Cytokine Growth Factor Rev **18** (2007): 459-471
- Liddy, N. et al., Nat Med. **18** (2012): 980-987
- Ljunggren, H. G. et al., J Exp.Med. **162** (1985): 1745-1759
- Longenecker, B. M. et al., Ann N.Y.Acad.Sci. **690** (1993): 276-291
- Lugo, T. G. et al., Science **247** (1990): 1079-1082

- Lukas, T. J. et al., Proc.Natl.Acad.Sci.U.S.A **78** (1981): 2791-2795
- Lundblad, R. L., Chemical Reagents for Protein Modification **3rd** (2004)
- Mahon, F. X. et al., Lancet Oncol **11** (2010): 1029-1035
- Maus, M. V. et al., Blood **123** (2014): 2625-2635
- Mayr, C. et al., Exp.Hematol. **34** (2006): 44-53
- Mayr, C. et al., Blood **105** (2005): 1566-1573
- Meziere, C. et al., J Immunol **159** (1997): 3230-3237
- Morgan, R. A. et al., Science **314** (2006): 126-129
- Mortara, L. et al., Clin Cancer Res. **12** (2006): 3435-3443
- Muller, M. R. et al., Blood **103** (2004): 1763-1769
- Mumberg, D. et al., Proc.Natl.Acad.Sci.U.S.A **96** (1999): 8633-8638
- National Cancer Institute, (5-6-2015), www.cancer.gov
- O'Brien, S. et al., Lancet Oncol **15** (2014): 48-58
- O'Brien, S. G. et al., N.Engl.J Med **348** (2003): 994-1004
- Ostergaard, Pedersen L. et al., Eur.J Immunol. **31** (2001): 2986-2996
- Palma, M. et al., Cancer Immunol Immunother. **57** (2008): 1705-1710
- Parikh, S. A. et al., Blood **118** (2011): 2062-2068
- Plebanski, M. et al., Eur.J Immunol **25** (1995): 1783-1787
- Porta, C. et al., Virology **202** (1994): 949-955
- Porter, D. L. et al., N.Engl.J Med. **365** (2011): 725-733
- Radich, J., Semin.Hematol. **47** (2010): 354-361
- Rammensee, H. et al., Immunogenetics **50** (1999): 213-219
- Rezvani, K. et al., Blood **111** (2008): 236-242
- Richards, S. et al., J Natl.Cancer Inst. **91** (1999): 861-868
- Rini, B. I. et al., Cancer **107** (2006): 67-74

- Robak, T. et al., *Expert.Opin.Biol.Ther* **14** (2014): 651-661
- Rock, K. L. et al., *Science* **249** (1990): 918-921
- Rodenko, B. et al., *Nat Protoc.* **1** (2006): 1120-1132
- Ross, D. M. et al., *Blood* **122** (2013): 515-522
- Saglio, G. et al., *N.Engl.J Med* **362** (2010): 2251-2259
- Saiki, R. K. et al., *Science* **239** (1988): 487-491
- Schettelig, J. et al., *J Clin Oncol* **26** (2008): 5094-5100
- Schmidt, S., *Memo.* **9** (2016): 157-162
- Schmidt, S. M. et al., *Cancer Res* **64** (2004): 1164-1170
- Schmitt, T. M. et al., *Hum.Gene Ther.* **20** (2009): 1240-1248
- Scholten, K. B. et al., *Clin Immunol.* **119** (2006): 135-145
- Seeger, F. H. et al., *Immunogenetics* **49** (1999): 571-576
- Sherman, F. et al., *Laboratory Course Manual for Methods in Yeast Genetics* (1986)
- Showel, M. M. et al., *F1000Prime.Rep.* **6** (2014): 96
- Siegel, S. et al., *Blood* **102** (2003): 4416-4423
- Silver, R. T. et al., *Blood* **94** (1999): 1517-1536
- Singh-Jasuja, H. et al., *Cancer Immunol.Immunother.* **53** (2004): 187-195
- Smahel, M., *Cancer Immunol.Immunother.* **60** (2011): 1655-1668
- Small, E. J. et al., *J Clin Oncol.* **24** (2006): 3089-3094
- Soverini, S. et al., *Blood* **118** (2011): 1208-1215
- Spaner, D. E. et al., *Cancer Immunol Immunother.* **54** (2005): 635-646
- Teufel, R. et al., *Cell Mol Life Sci.* **62** (2005): 1755-1762
- Tran, E. et al., *Science* **344** (2014): 641-645
- Walter, S. et al., *J Immunol* **171** (2003): 4974-4978
- Walter, S. et al., *Nat Med.* **18** (2012): 1254-1261

Wierda, W. G. et al., Blood **118** (2011): 5126-5129

Willcox, B. E. et al., Protein Sci. **8** (1999): 2418-2423

Zaremba, S. et al., Cancer Res. **57** (1997): 4570-4577

Zheng, Y. et al., Clin Biochem. **44** (2011): 1405-1411

Zufferey, R. et al., J Virol. **73** (1999): 2886-2892

CLAIMS

1. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 279, and variant sequences thereof which are at least 88% homologous to SEQ ID No. 1 to SEQ ID No. 279, 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. 279.
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. 279, preferably of an amino acid sequence according to any of SEQ ID No. 1 to SEQ ID No. 187.
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).

- 159 -

7. An antibody, in particular a soluble or membrane-bound antibody, preferably a monoclonal antibody or fragment thereof, 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.
8. A T-cell receptor, preferably soluble or membrane-bound, or a fragment thereof, that is reactive with an HLA ligand, wherein said ligand is 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.
9. The T-cell receptor according to claim 8, wherein said ligand amino acid sequence is at least 88% identical to any one of SEQ ID No. 1 to SEQ ID No. 279, or wherein said ligand amino acid sequence consists of any one of SEQ ID No. 1 to SEQ ID No. 279.
10. The T-cell receptor according to claim 8 or 9, 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.
11. 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.
12. A nucleic acid, encoding for a peptide or variant thereof according to any one of claims 1 to 5, an antibody or fragment thereof according to claim 7, a T-cell receptor or fragment thereof according to claim 8 or 9, optionally linked to a heterologous promoter sequence, or an expression vector expressing said nucleic acid.
13. A recombinant host cell comprising the peptide according to any one of claims 1 to 6, the antibody or fragment thereof according to claim 7, the T-cell receptor or fragment thereof according to claim 8 or 9 or the nucleic acid or the expression

- 160 -

vector according to claim 12, wherein said host cell preferably is selected from an antigen presenting cell, such as a dendritic cell, a T cell or an NK cell.

14. 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.
15. An activated T lymphocyte, produced by the method according to claim 14, that selectively recognizes a cell which presents a polypeptide comprising an amino acid sequence given in any one of claims 1 to 4.
16. A pharmaceutical composition comprising at least one active ingredient selected from the group consisting of the peptide according to any one of claims 1 to 6, the antibody or fragment thereof according to claim 7, the T-cell receptor or fragment thereof according to claim 8 or 9, the aptamer according to claim 11, the nucleic acid or the expression vector according to claim 12, the host cell according to claim 13, or the activated T lymphocyte according to claim 15, or a conjugated or labelled active ingredient, and a pharmaceutically acceptable carrier, and optionally, pharmaceutically acceptable excipients and/or stabilizers.
17. A method for producing the peptide or variant thereof according to any of claims 1 to 6, the antibody or fragment thereof according to claim 7, or the T-cell receptor or fragment thereof according to claim 8 or 9, the method comprising culturing the host cell according to claim 13, and isolating the peptide or variant thereof, the antibody or fragment thereof or the T cell receptor or fragment thereof from said host cell and/or its culture medium.
18. The peptide according to any one of claims 1 to 6, the antibody or fragment thereof according to claim 7, the T-cell receptor or fragment thereof according to claim 8 or

- 161 -

- 9, the aptamer according to claim 11, the nucleic acid or the expression vector according to claim 12, the host cell according to claim 13, or the activated T lymphocyte according to claim 15 for use in medicine.
19. 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 15.
20. The peptide according to any one of claims 1 to 6, the antibody or fragment thereof according to claim 7, the T-cell receptor or fragment thereof according to claim 8 or 9, the aptamer according to claim 11, the nucleic acid or the expression vector according to claim 12, the host cell according to claim 13, or the activated T lymphocyte according to claim 15 for use in diagnosis and/or treatment of cancer, or for use in the manufacture of a medicament against cancer.
21. The use according to claim 20, wherein said cancer is selected from the group of chronic lymphocytic leukaemia (CLL), acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML) and other lymphoid neoplasms, for example, Non-Hodgkin lymphoma, post-transplant lymphoproliferative disorders (PTLD) as well as other myeloid neoplasms, such as primary myelofibrosis, essential thrombocytopenia, polycythemia vera, as well as other neoplasms such as hepatocellular carcinoma, colorectal carcinoma, glioblastoma, gastric cancer, oesophageal cancer, non-small cell lung cancer, small cell lung cancer, pancreatic cancer, renal cell carcinoma, prostate cancer, melanoma, breast cancer, gallbladder cancer and cholangiocarcinoma, urinary bladder cancer, uterine cancer, head and neck squamous cell carcinoma, mesothelioma and other tumors that show an overexpression of a protein from which a peptide SEQ ID No. 1 to SEQ ID No. 279 is derived from.
22. A kit comprising:

- 162 -

- (a) a container comprising a pharmaceutical composition containing the peptide(s) or the variant according to any one of claims 1 to 6, the antibody or fragment thereof according to claim 7, the T-cell receptor or fragment thereof according to claim 8 or 9, the aptamer according to claim 11, the nucleic acid or the expression vector according to claim 12, the host cell according to claim 13, or the activated T lymphocyte 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. 279, and
 - (d) optionally, instructions for (i) use of the solution or (ii) reconstitution and/or use of the lyophilized formulation.
23. The kit according to claim 22, further comprising one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, or (v) a syringe.
24. 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).
25. The method according to claim 24, 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

- 163 -

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.

26. The method according to claim 24 or 25, 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.
27. The method according to any of claims 24 to 26, wherein the normal tissue corresponding to the tissue type of the tumor sample is obtained from the same patient.
28. The method according to any of claims 24 to 27, 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;

- 164 -

- 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.
29. The method according to any of claims 24 to 28, 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.
30. The method according to any of claims 24 to 29, wherein said warehouse comprises a plurality of peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 279.
31. The method according to any of claims 24 to 30, 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.
32. The method according to claim 31, wherein said at least one mutation is identified by whole genome sequencing.

Figure 1A

Gene: S100Z

Peptide: TMIRIFHRY

SEQ ID No: 2

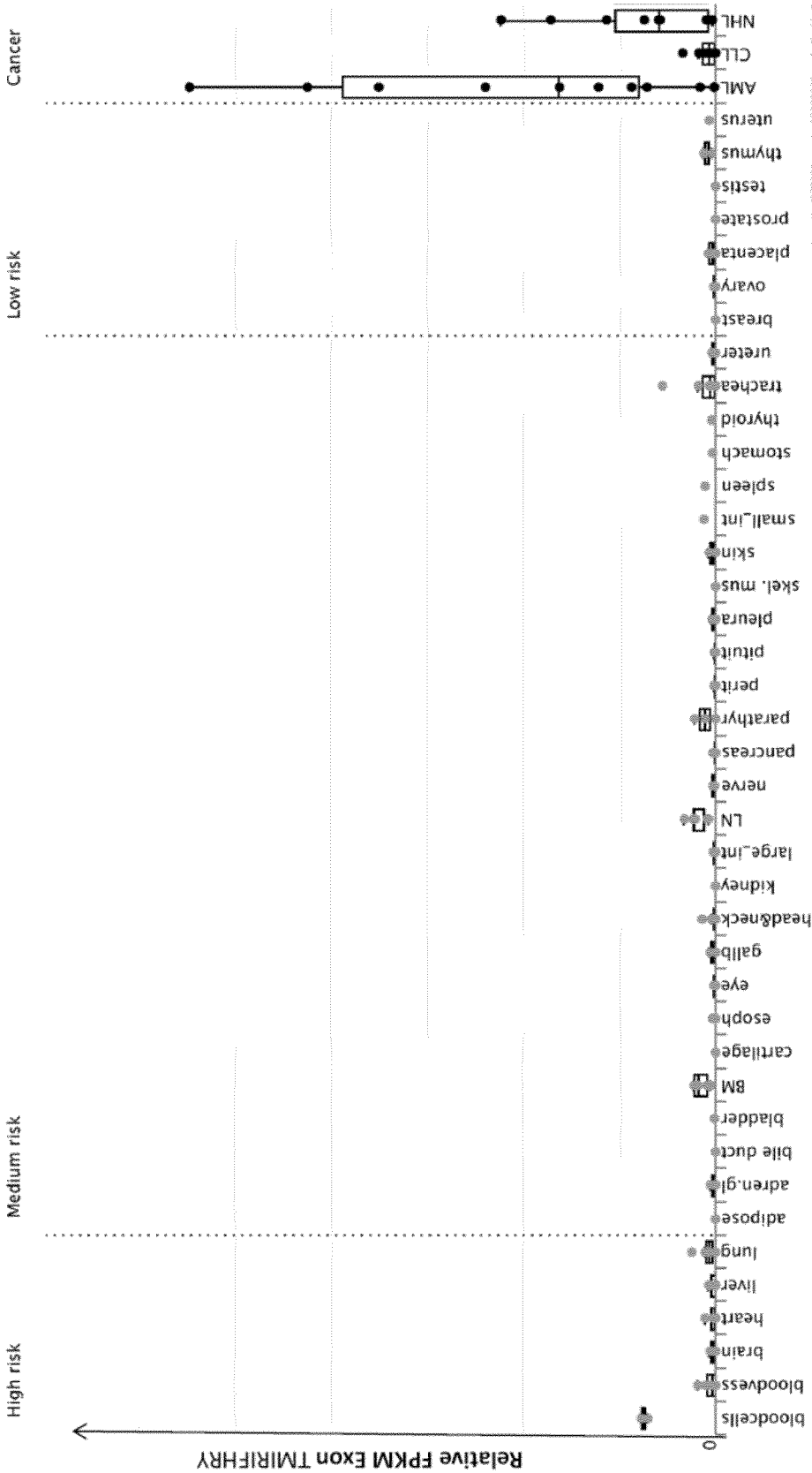


Figure 1B

Gene: PAX5

Peptide: YSHPQYSSY

SEQ ID No: 9

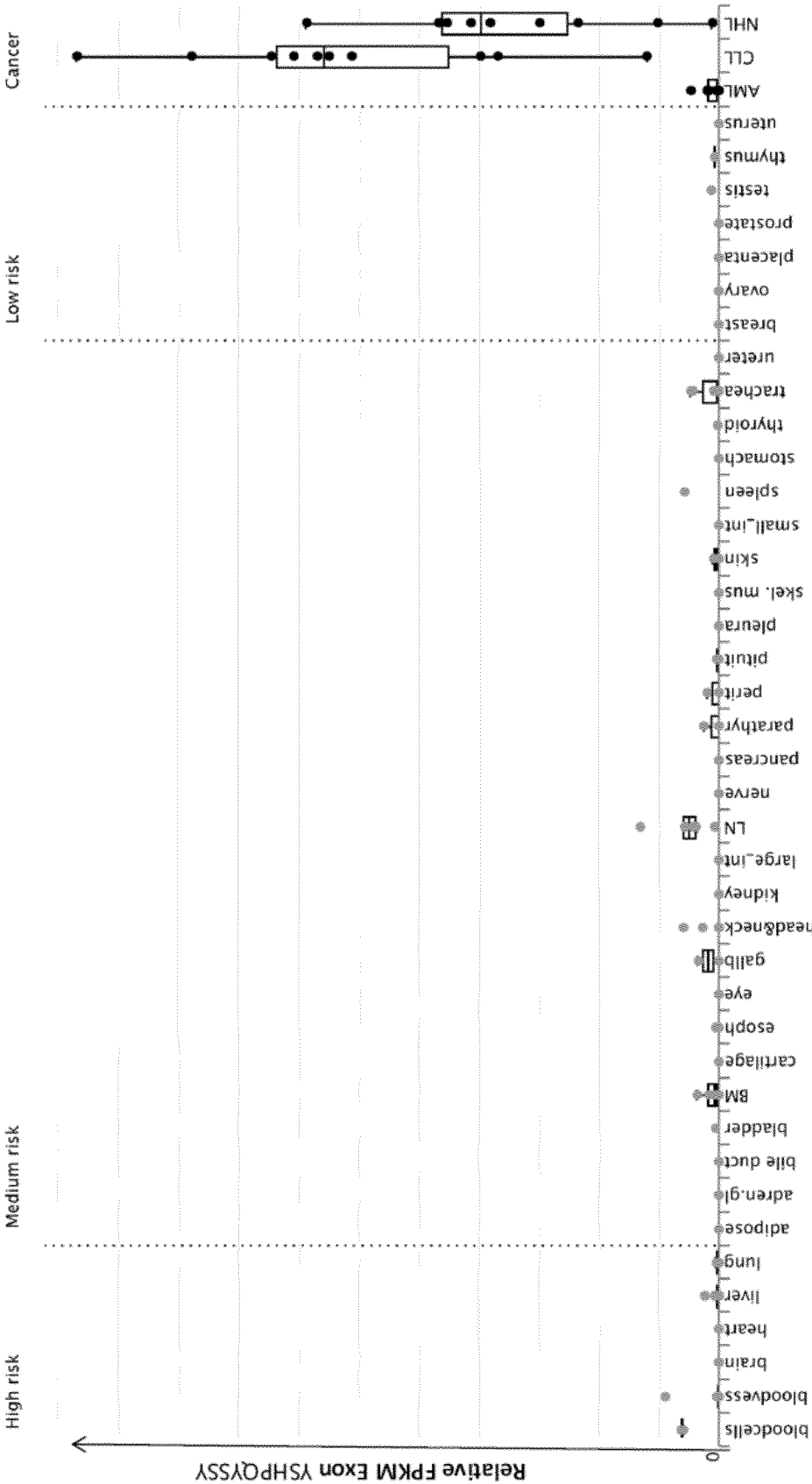


Figure 1C

Gene: FLT3
Peptide: SLFEGIYTI
SEQ ID No: 31



Figure 1D

Gene: RALGPS2

Peptide: ILHAQTLKI

SEQ ID No: 22

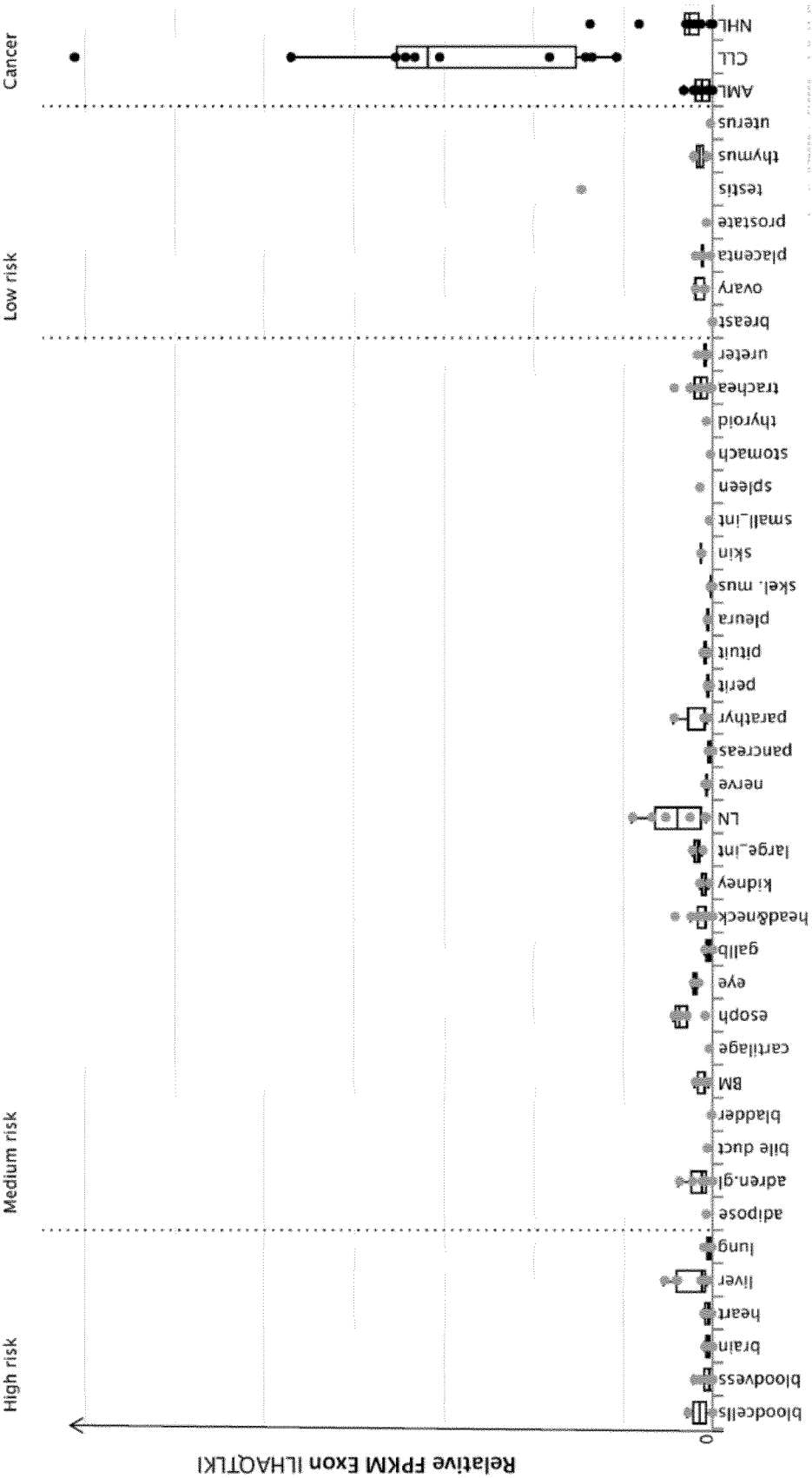


Figure 1E

Gene: FCRL2

Peptide: KTSNIVKIK

SEQ ID No: 32

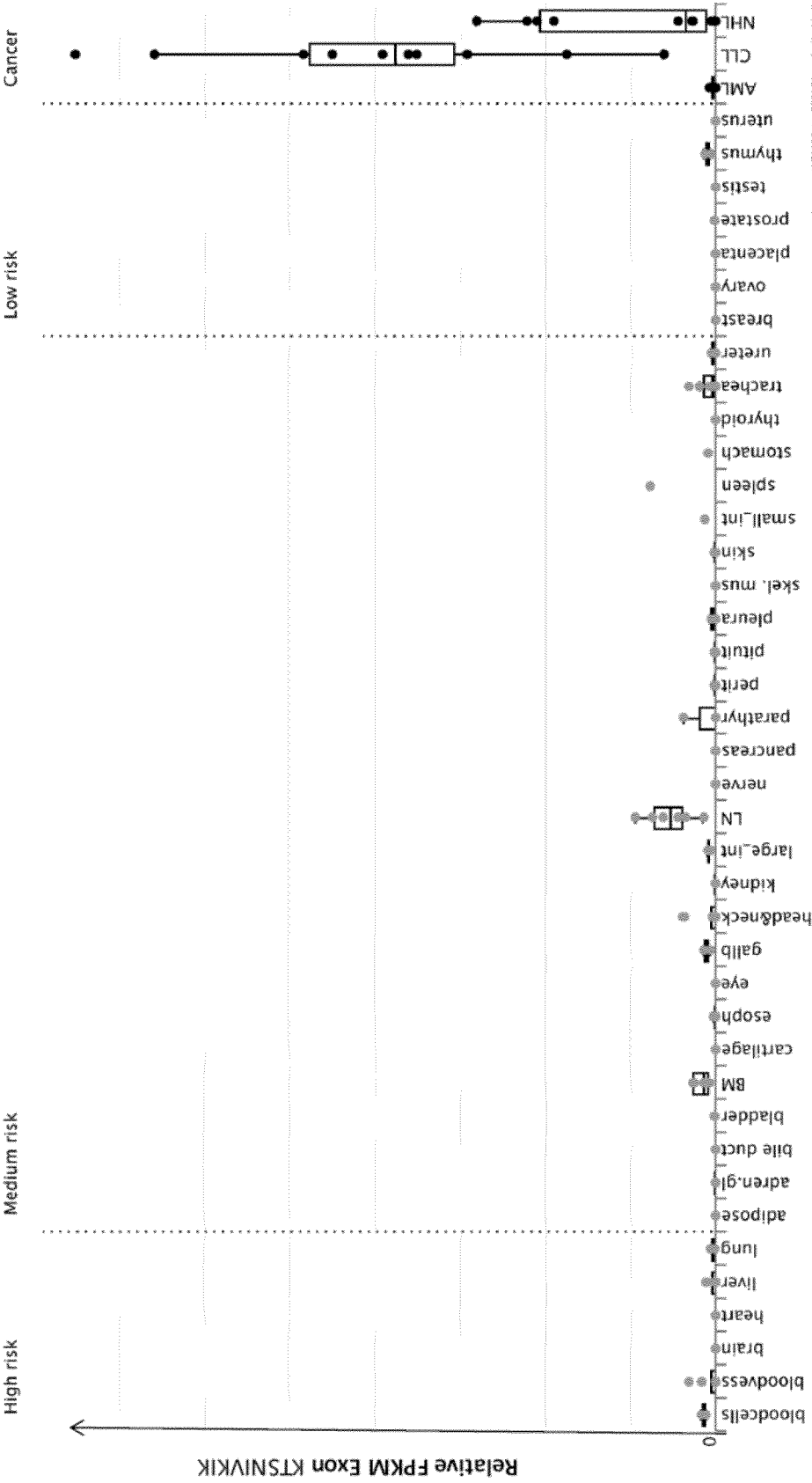


Figure 1F

Gene: KBTBD8

Peptide: RSKEYIRKK

SEQ ID No: 40

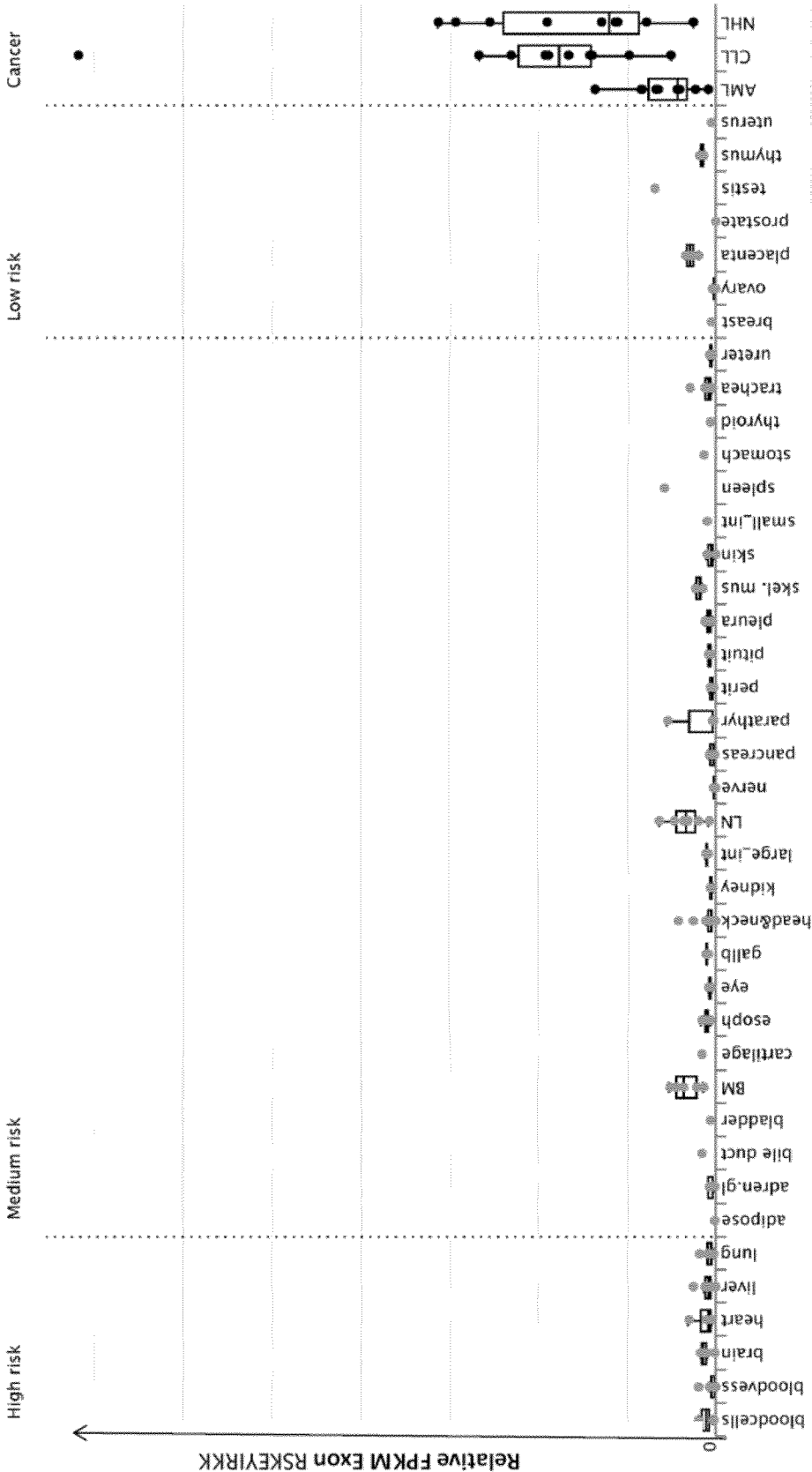


Figure 1G

Gene: ZNF92

Peptide: KAFNQSSLTK

SEQ ID No: 52

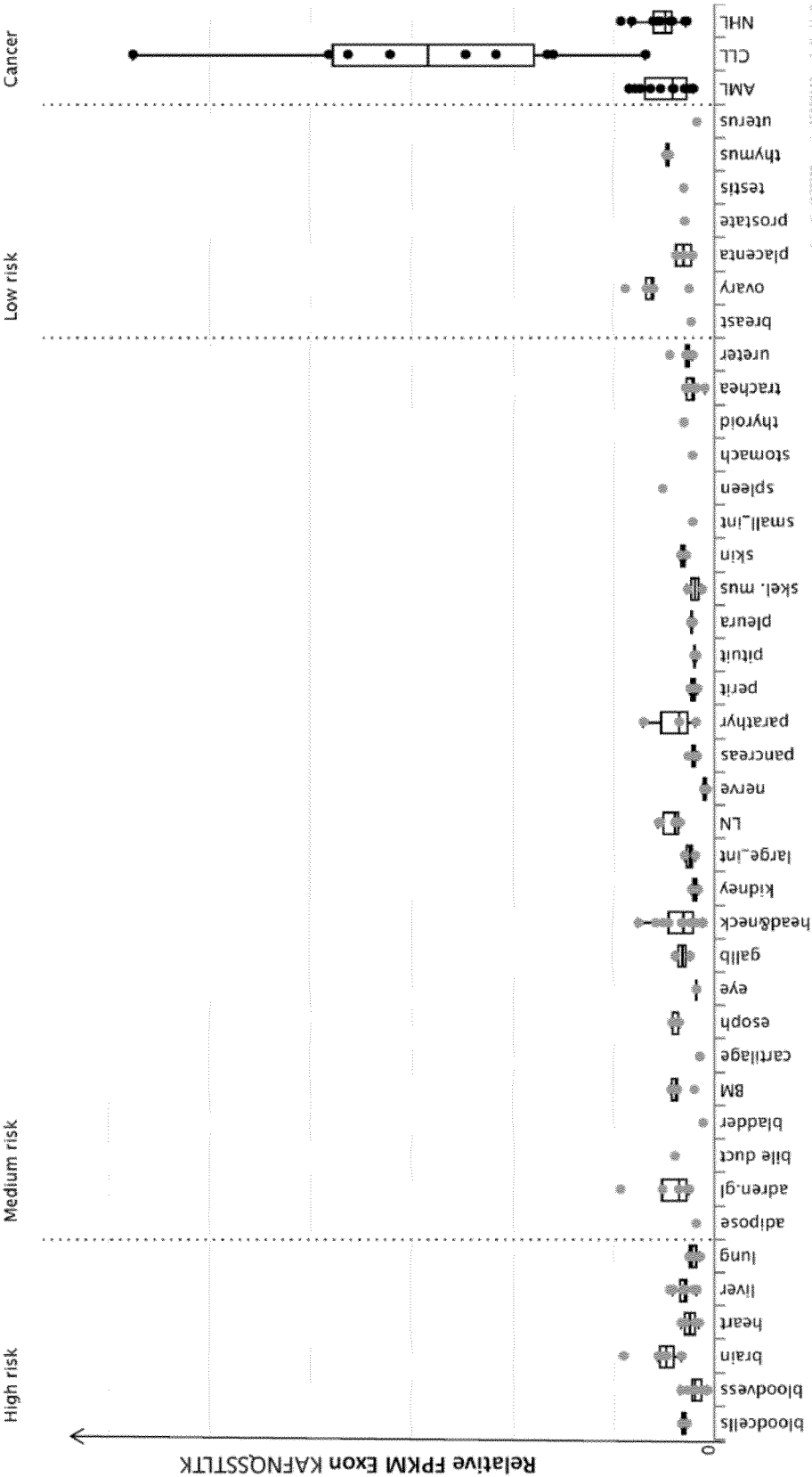


Figure 1H

Gene: ADAM28

Peptide: KYIEYLV

SEQ ID No: 53

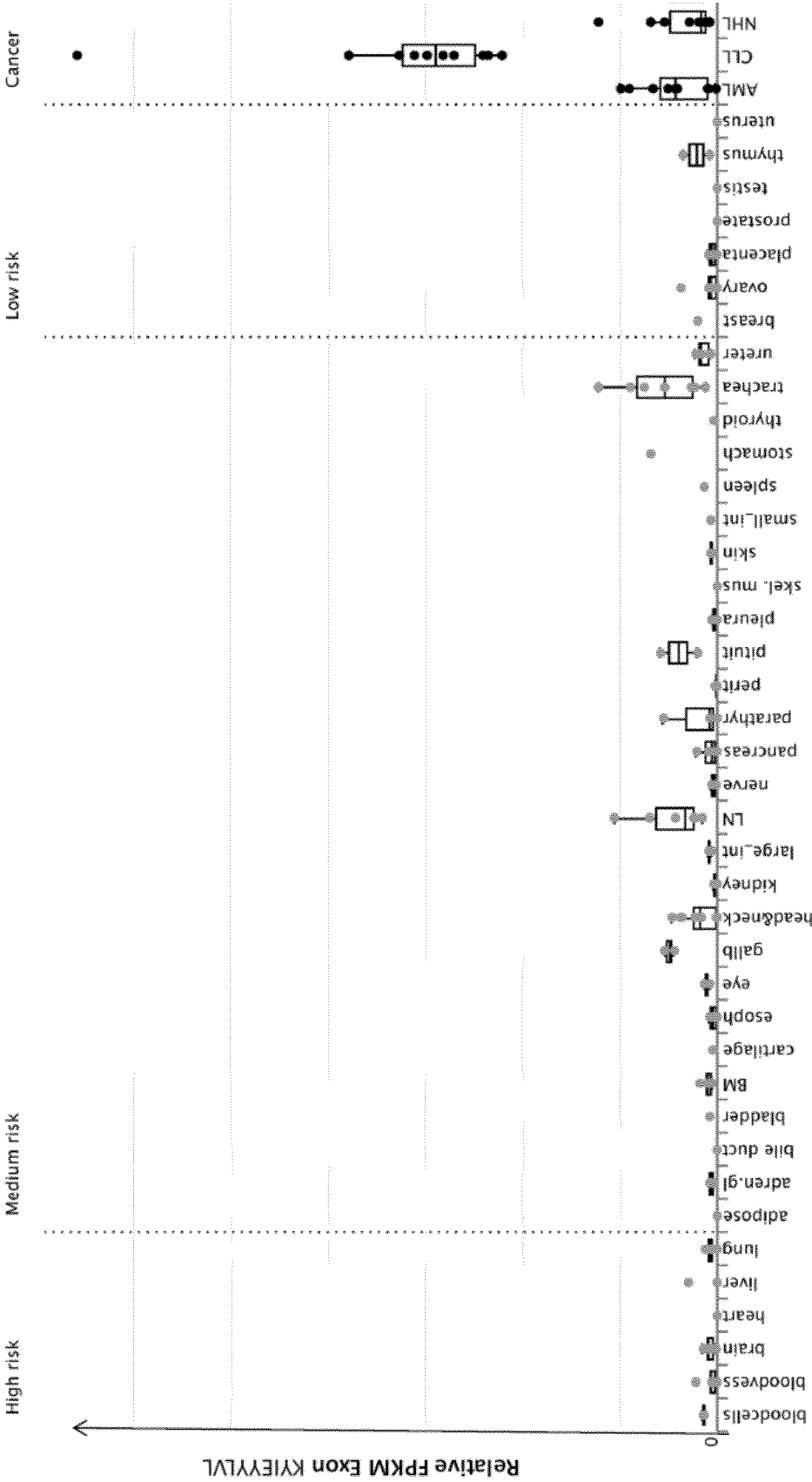


Figure 11

Gene: FLT3

Peptide: IFKEHNFSF

SEQ ID No: 61

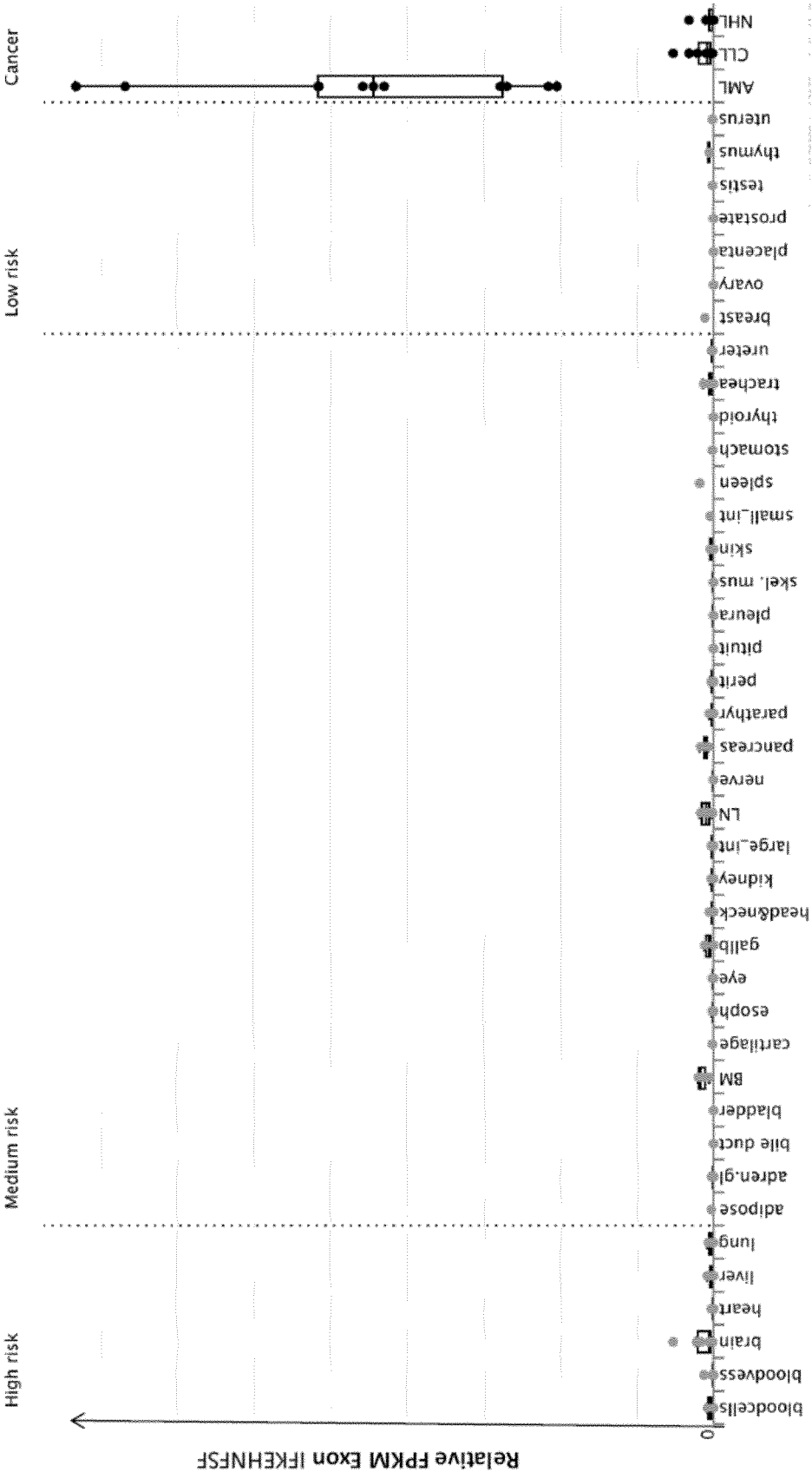


Figure 1J

Gene: ZNF92

Peptide: KAFSWSSAF

SEQ ID No: 76

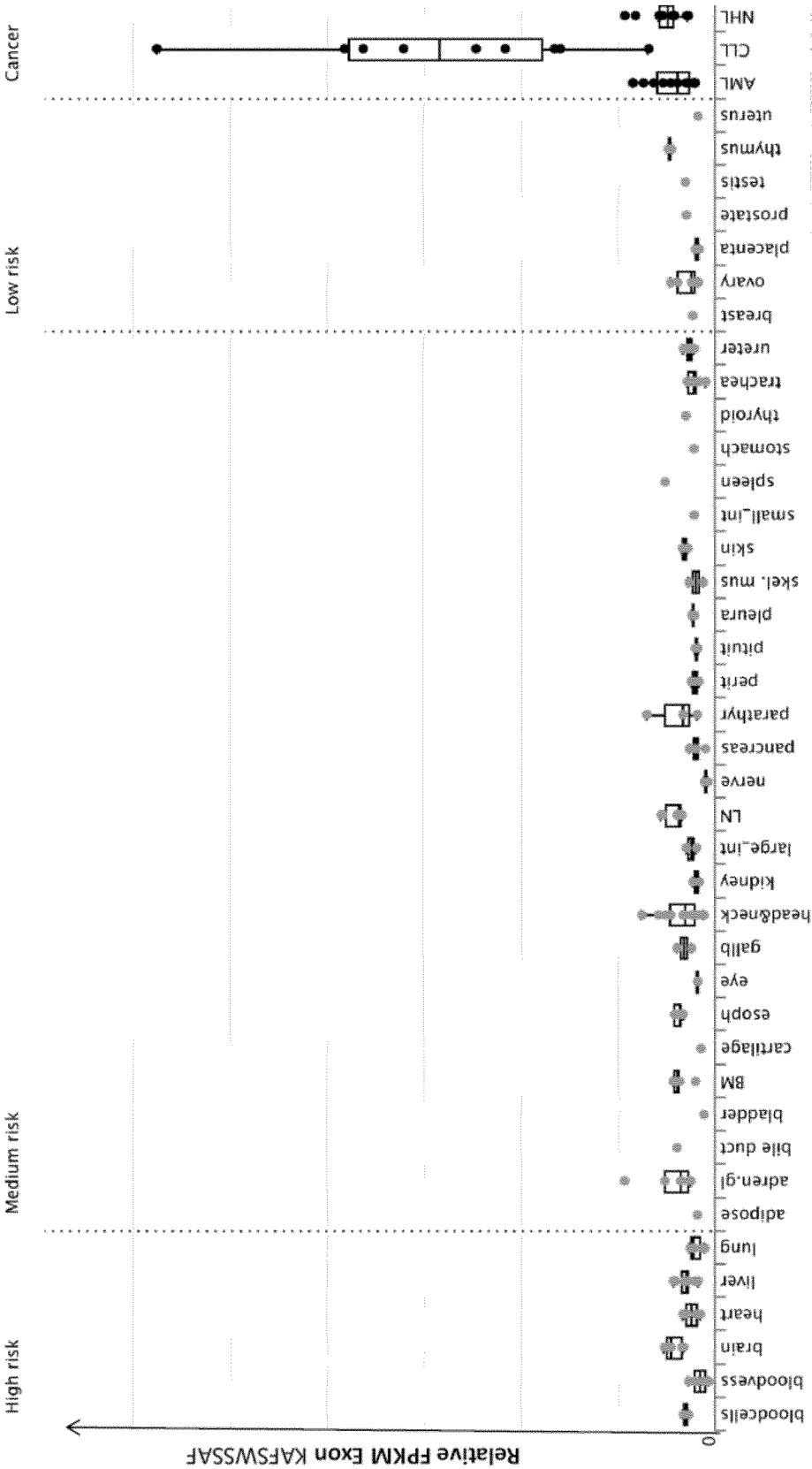


Figure 1K

Gene: FCRL3

Peptide: IPVSHPVL

SEQ ID No: 85

11/31

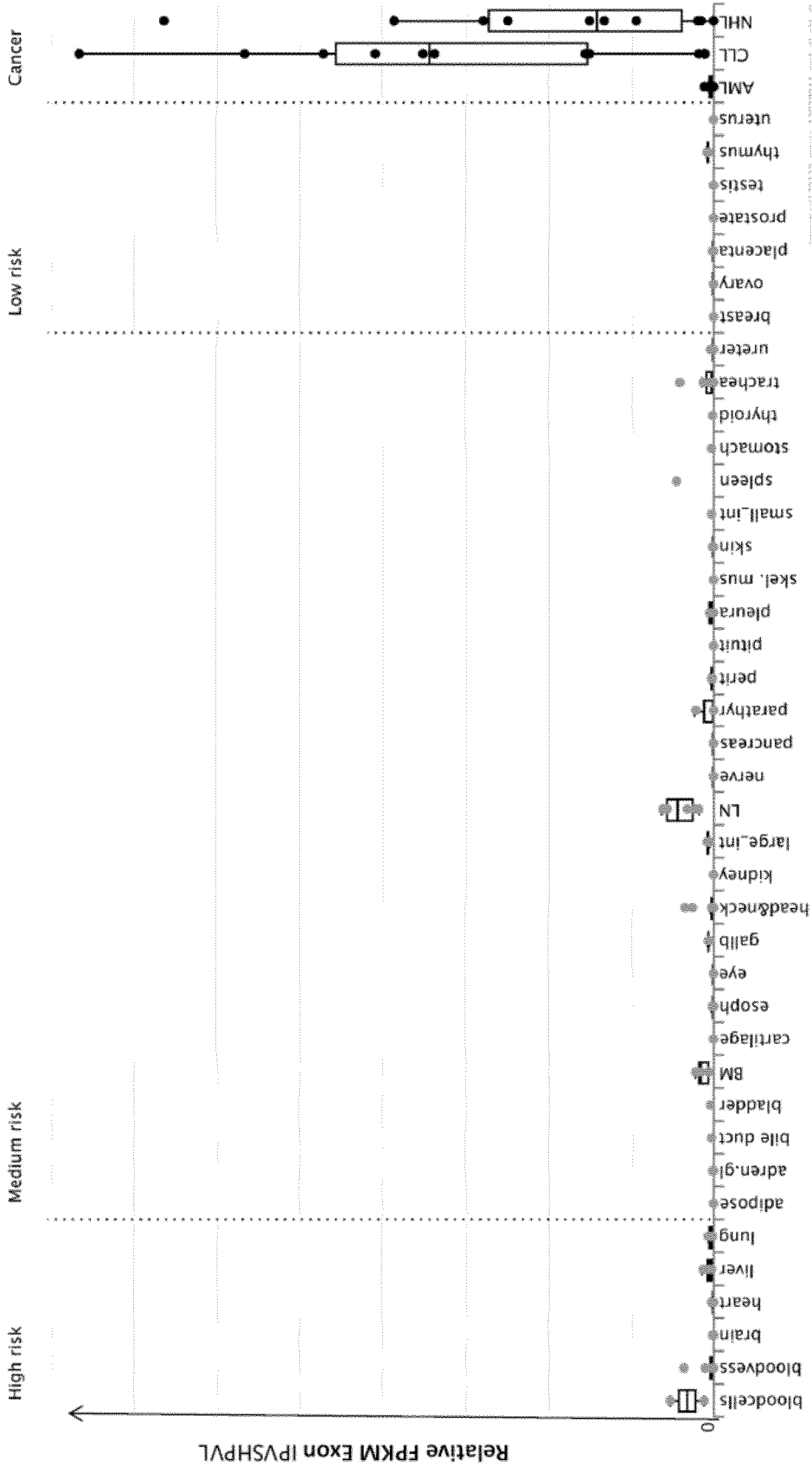


Figure 1L

Gene: CDK6

Peptide: FGLARIYSF

SEQ ID No: 110

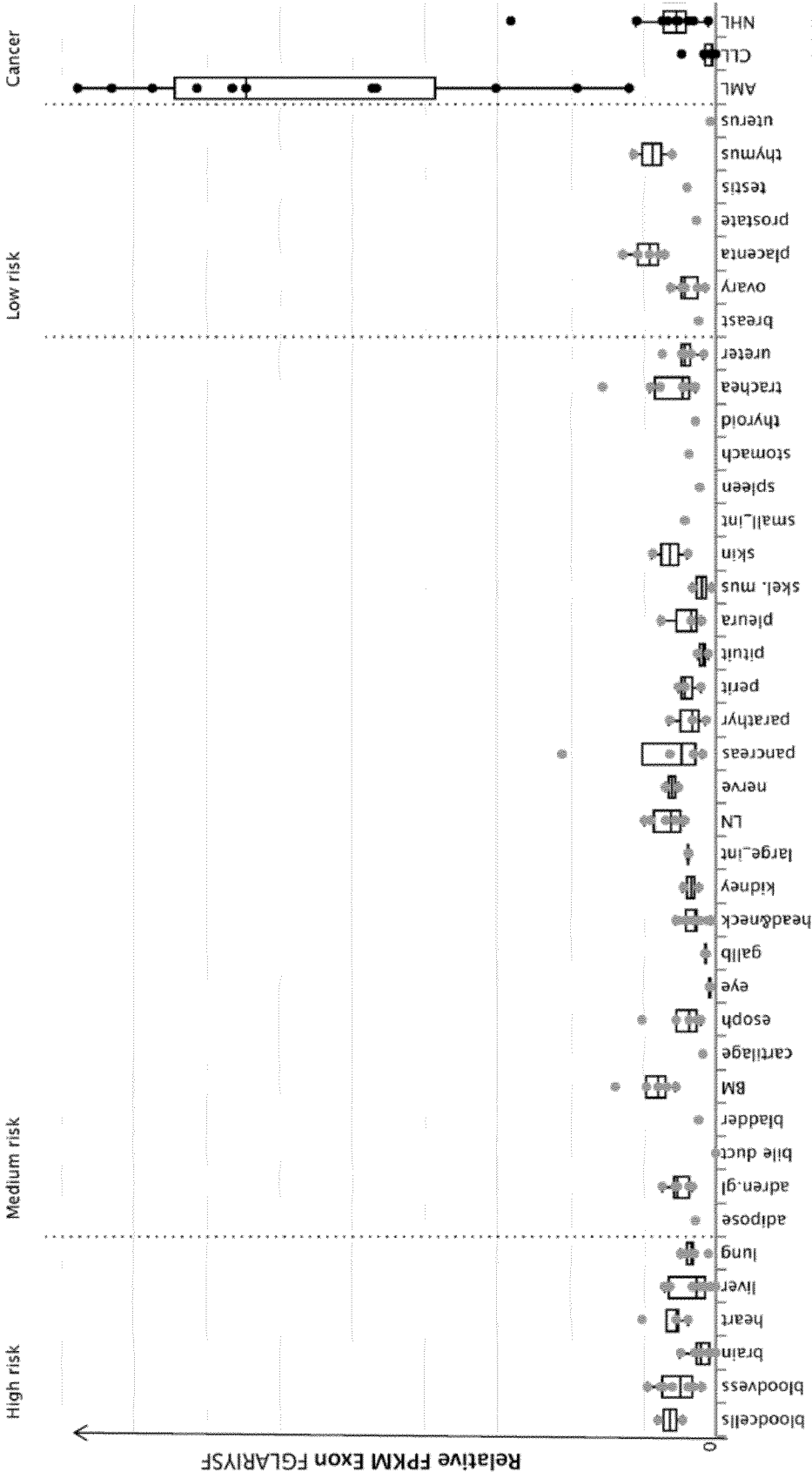


Figure 1M

Gene: CLEC17A

Peptide: VTLLIKYQEL

SEQ ID No: 111

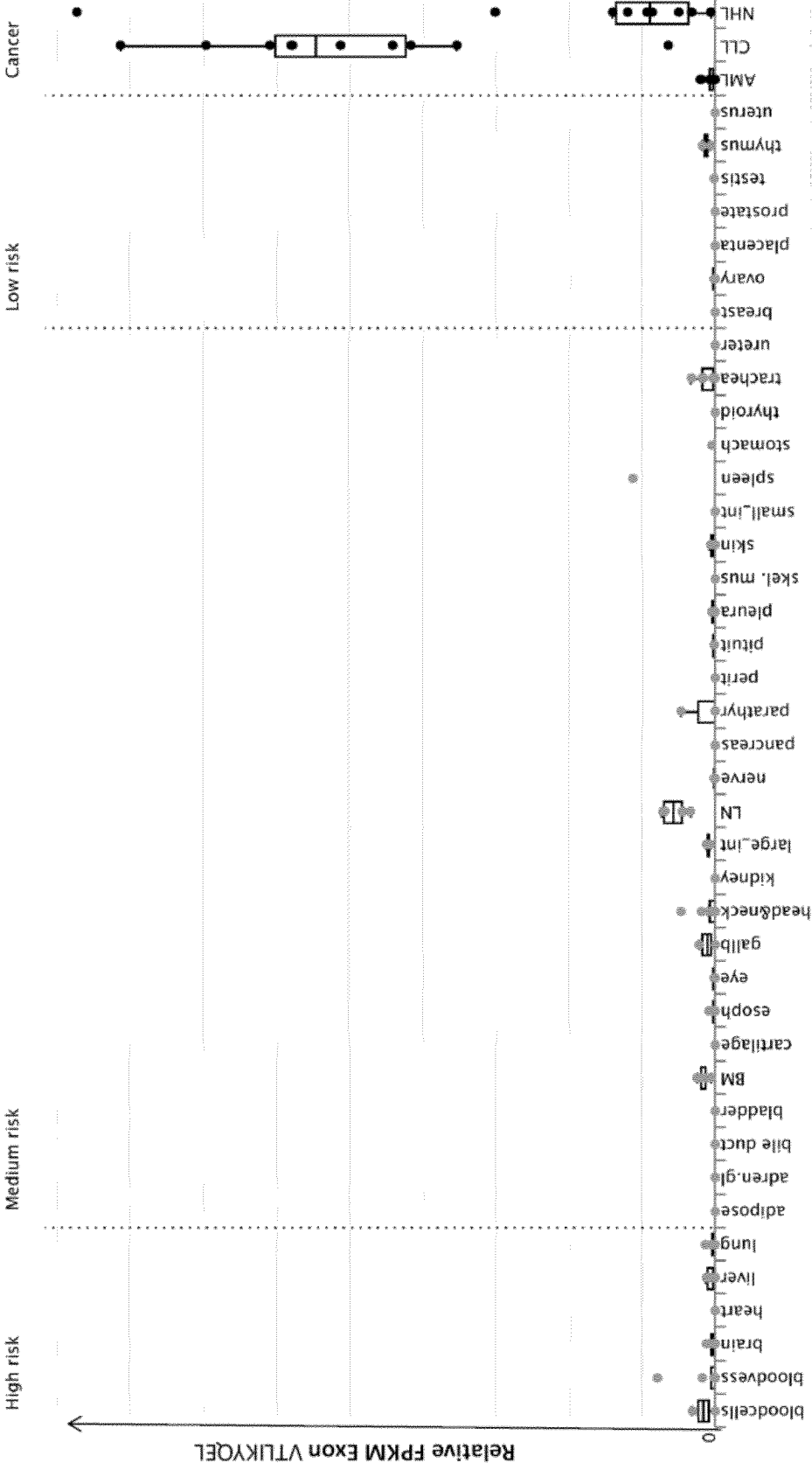


Figure 1N

Gene: RALGPS2

Peptide: YIKTAKKL

SEQ ID No: 117

14/31

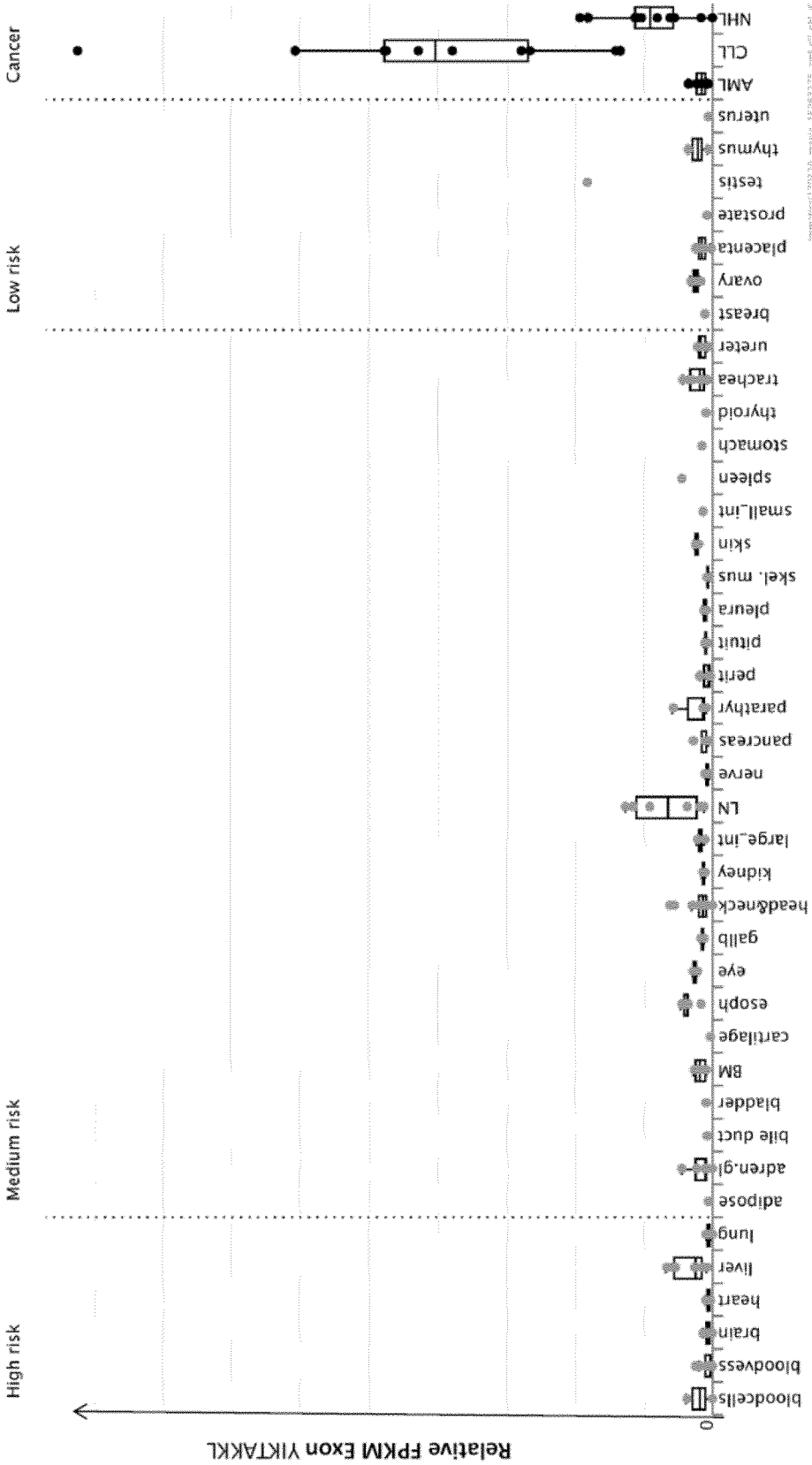


Figure 10

Gene: CDK6

Peptide: GEGAYGKVF

SEQ ID No: 129

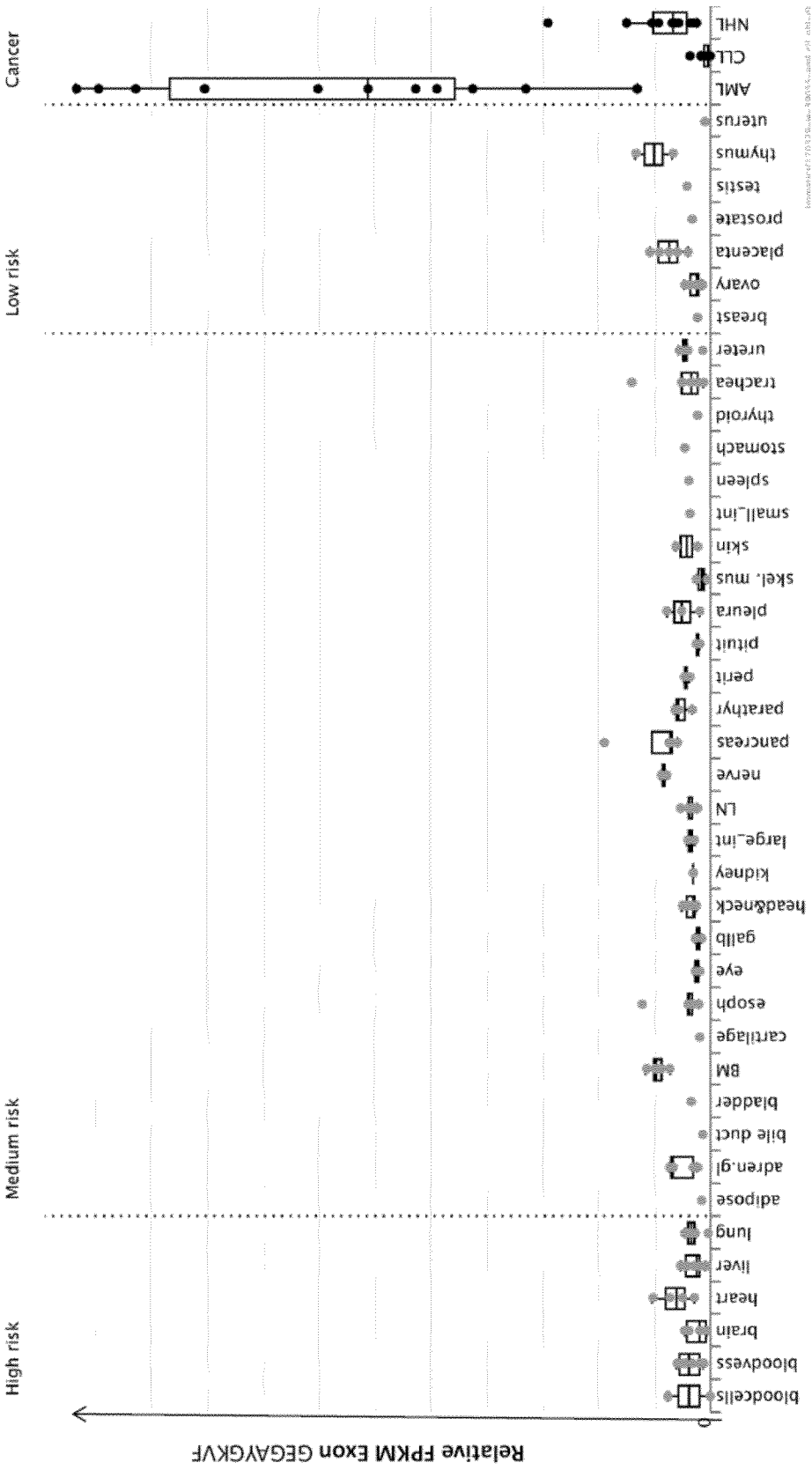


Figure 1P

Gene: FCRL2

Peptide: RENQVLGSGW

SEQ ID No: 139

16/31

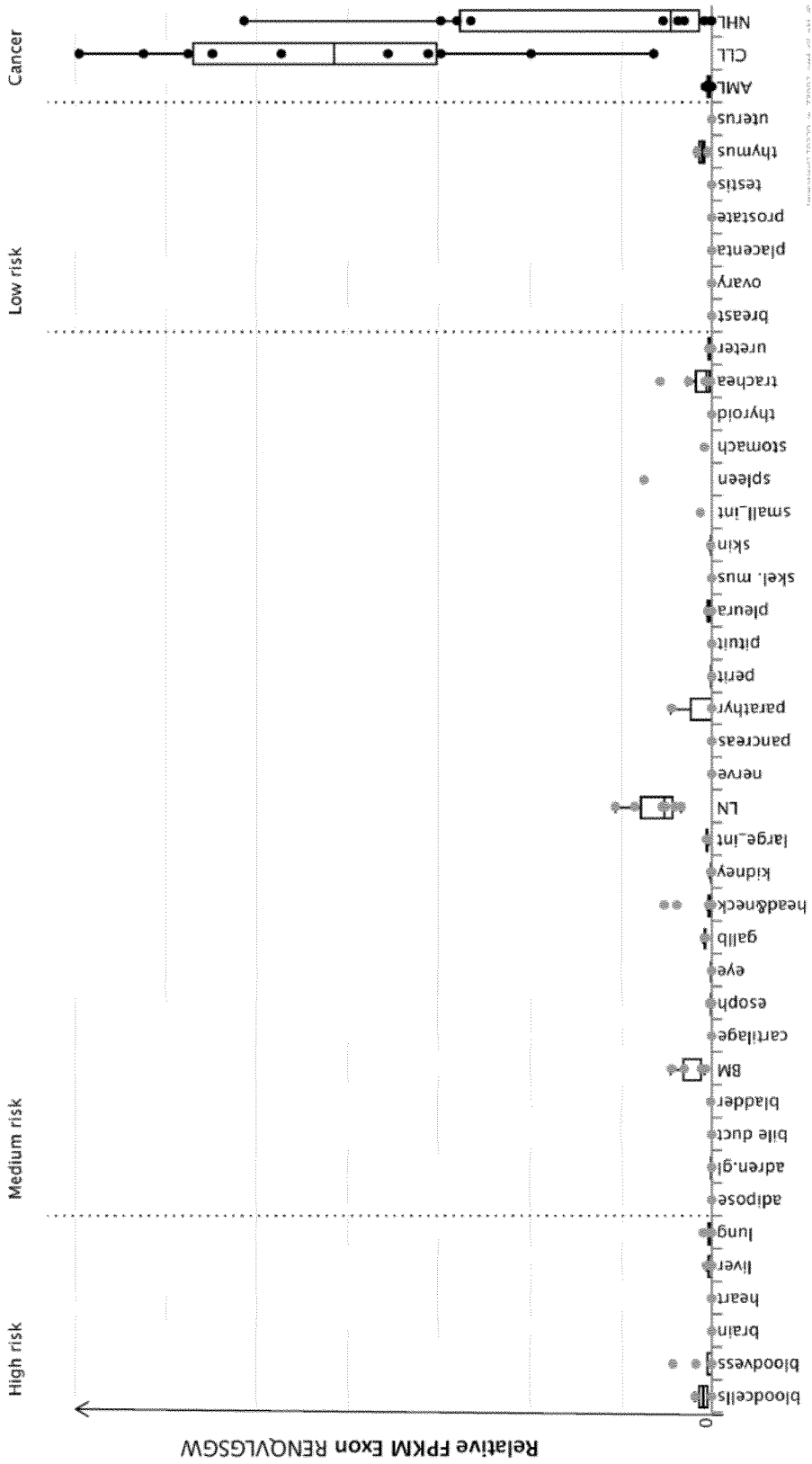


Figure 1Q

Gene: FLT3

Peptide: REYEDLKWEF

SEQ ID No: 141

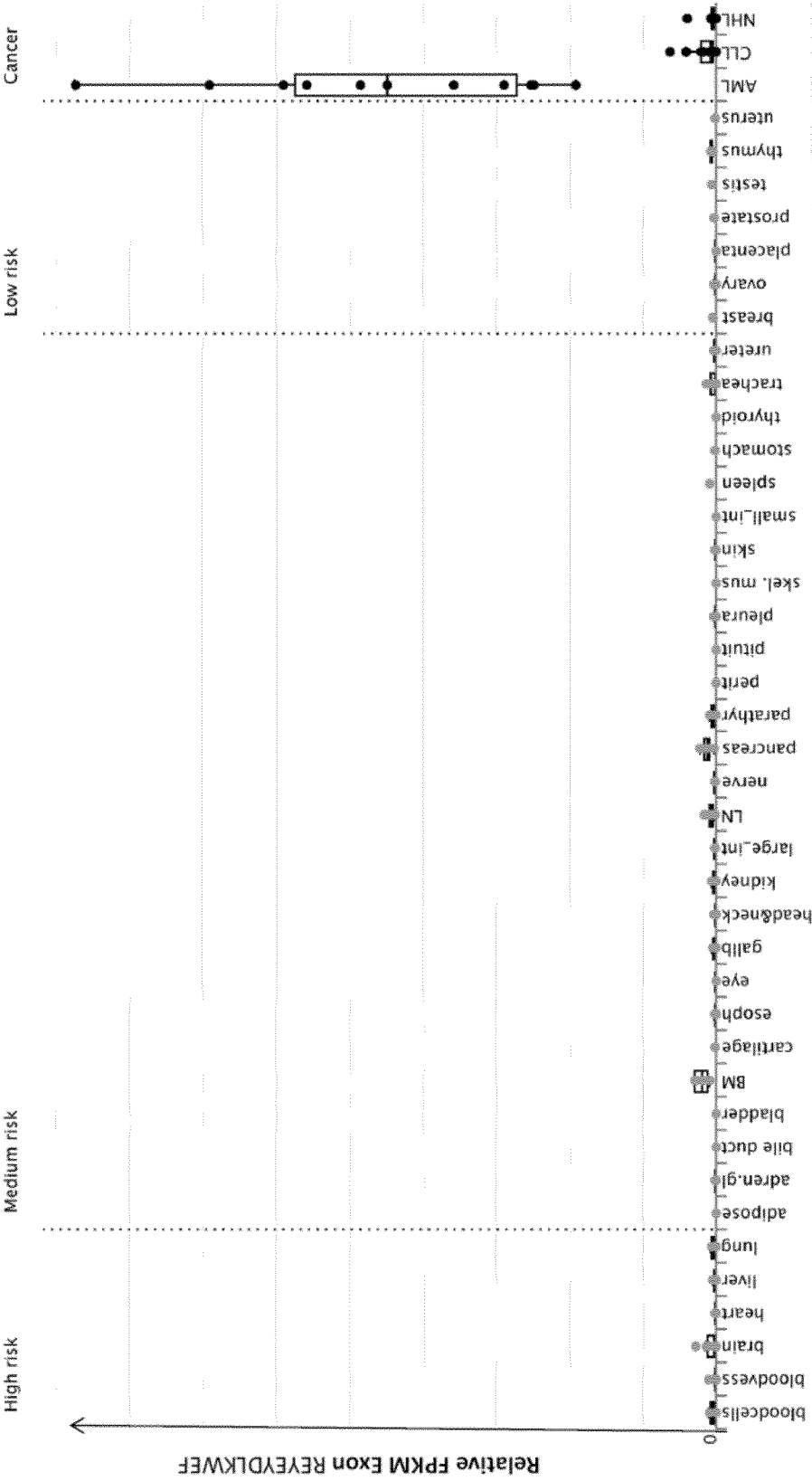


Figure 1R

Gene: BMF

Peptide: VTEEPQRLFY

SEQ ID No: 189

18/31

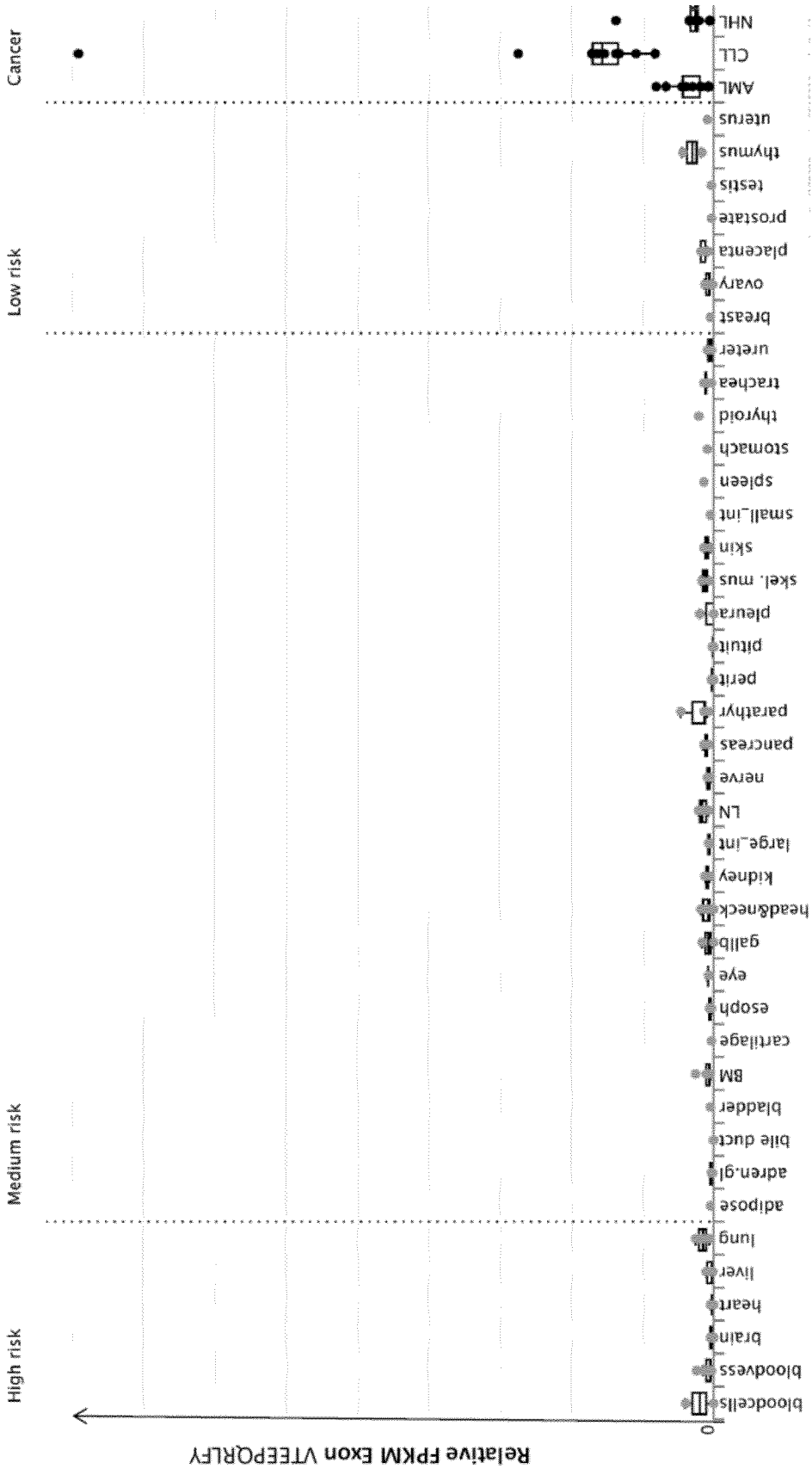


Figure 1S

Gene: FCER2
Peptide: LLWHWDTTQSLK
SEQ ID No: 212

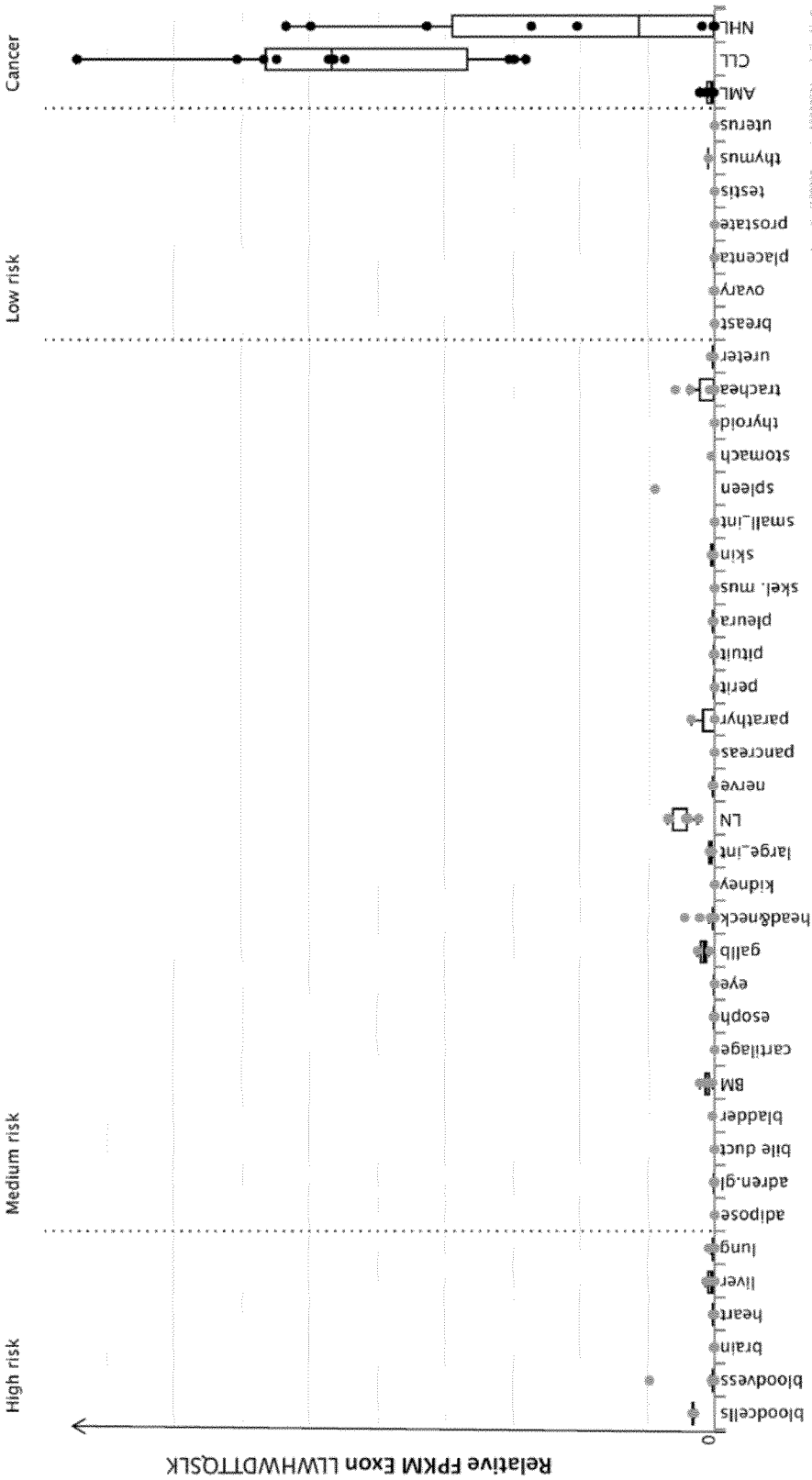


Figure 1T

Gene: CDK6

Peptide: MPLSTIREV

SEQ ID No: 231

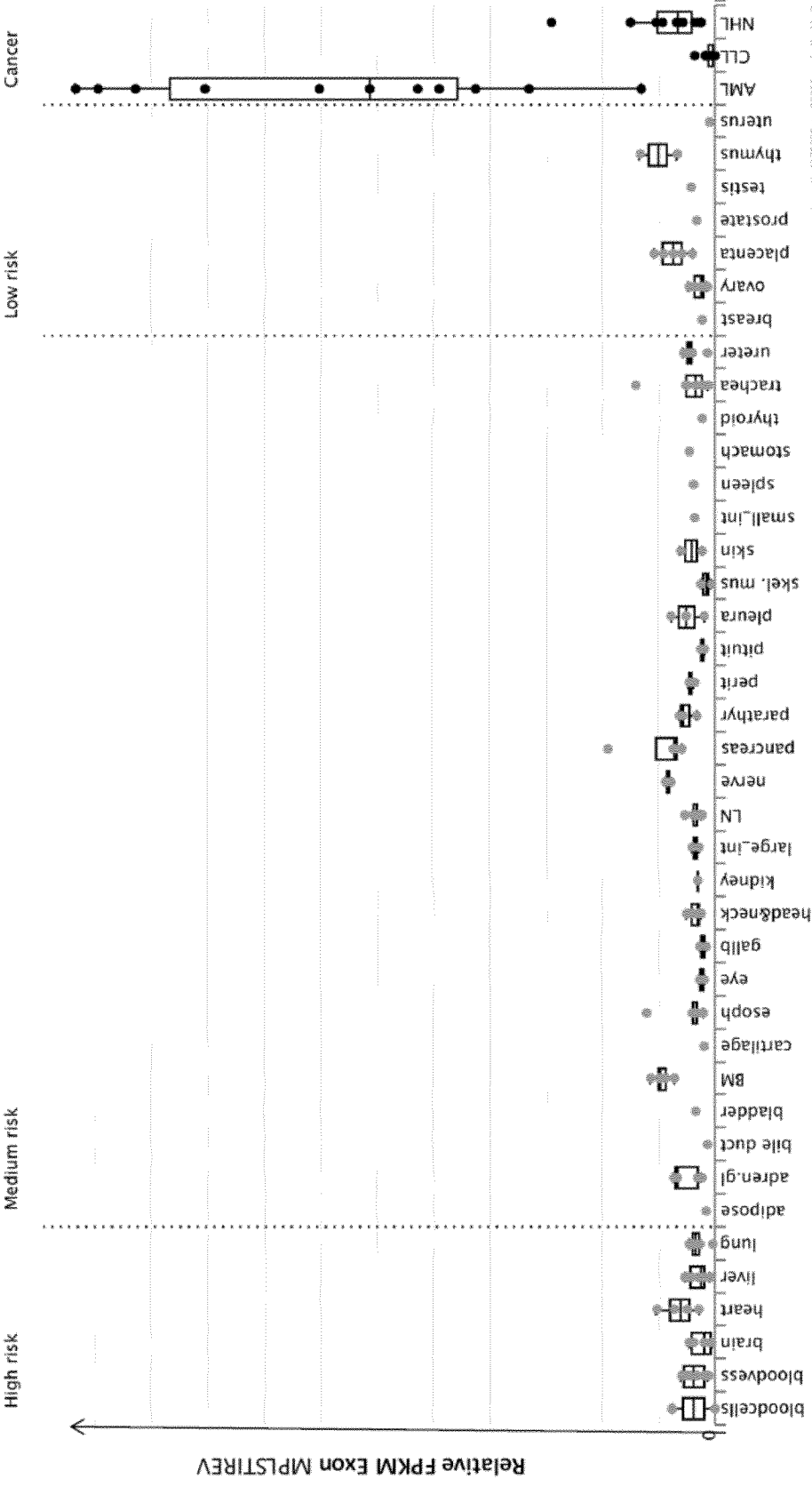


Figure 1U

Gene: CLEC17A

Peptide: SPRVYWLGL

SEQ ID No: 233

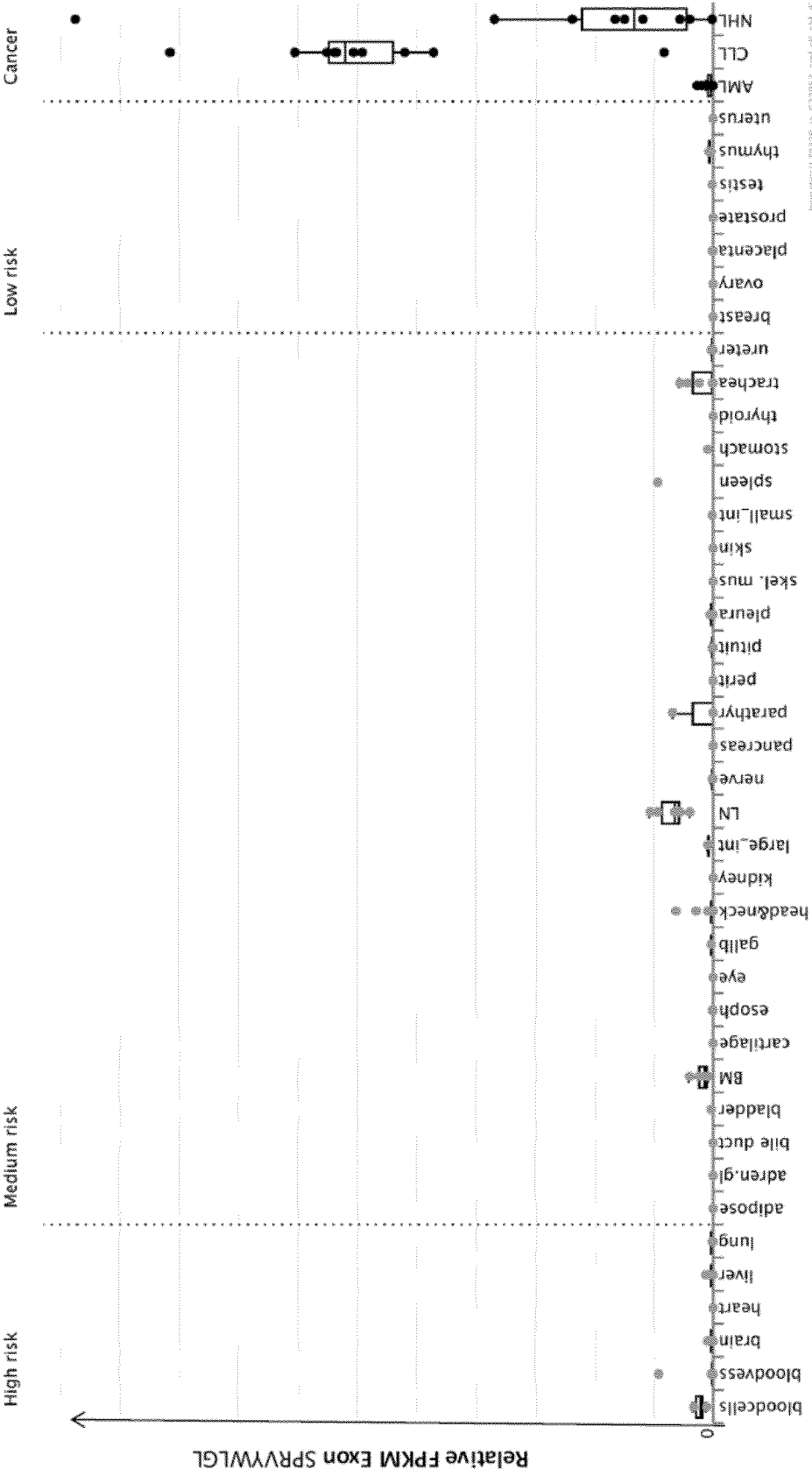


Figure 1V

Gene: PMAIP1

Peptide: QPSPARAPAEI

SEQ ID No: 247

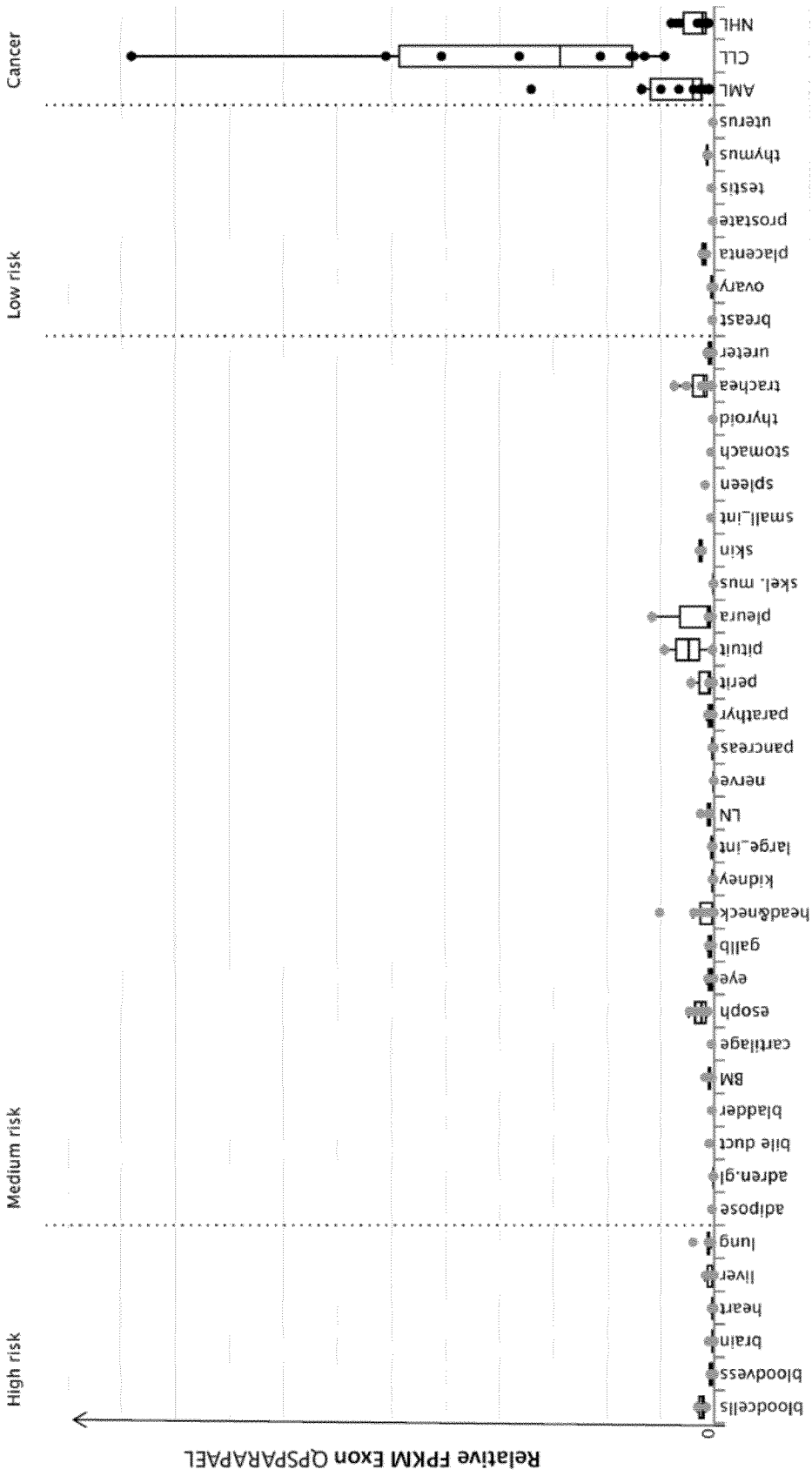


Figure 1W

Gene: CDK6

Peptide: AEIGEGAYGKVF

SEQ ID No: 260

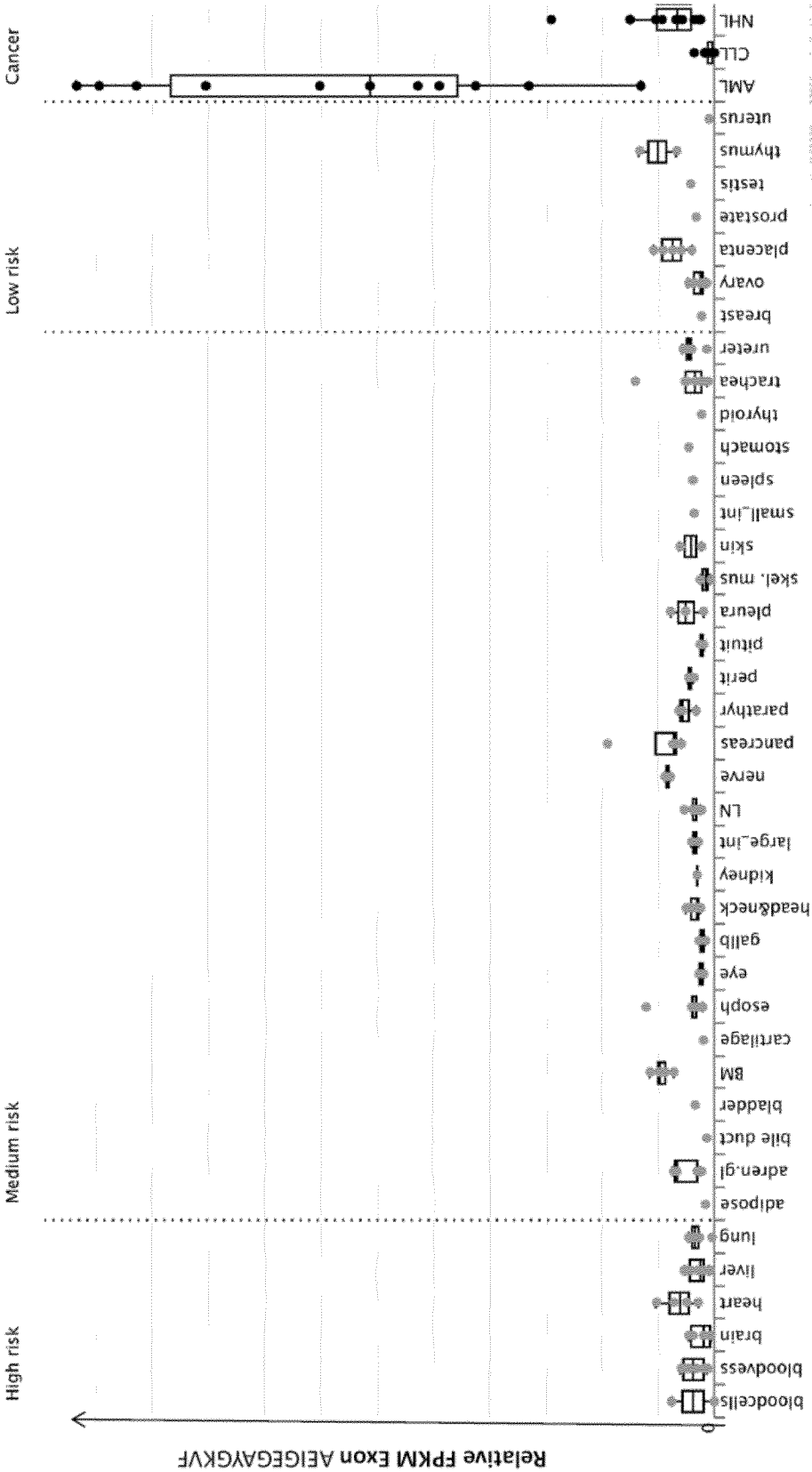


Figure 2

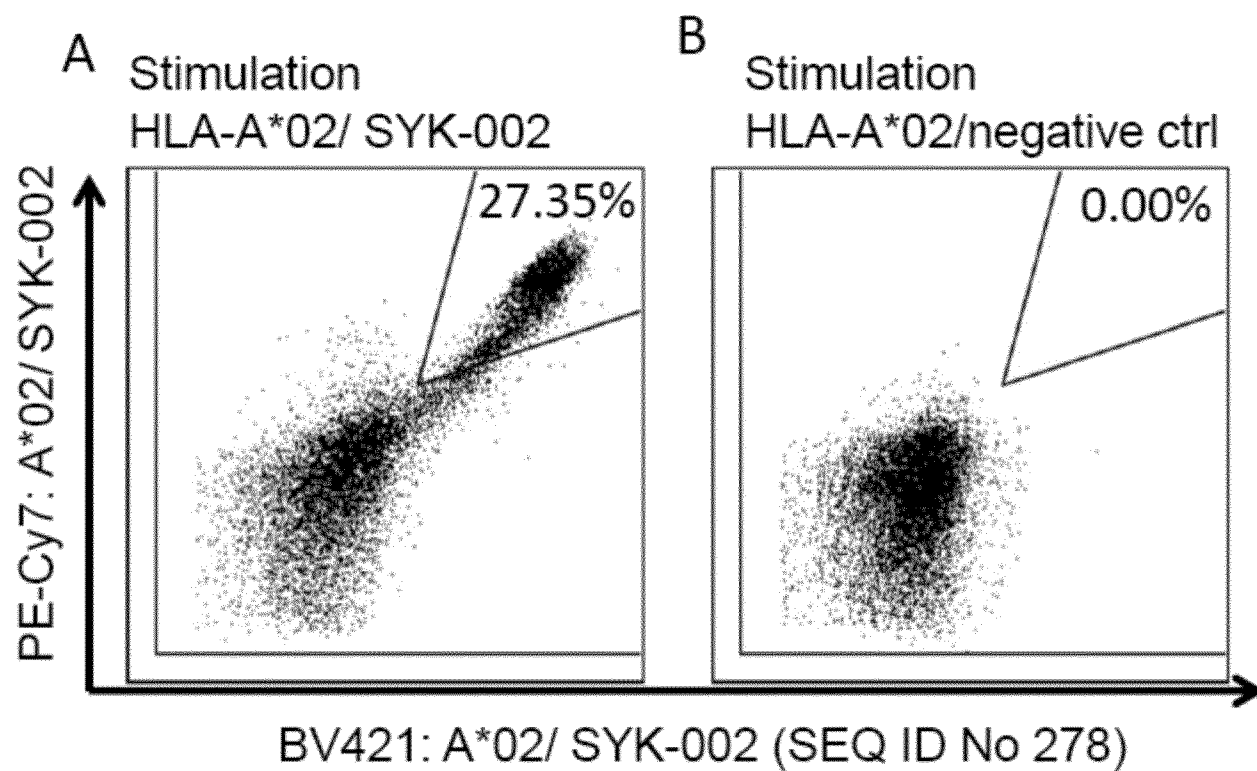


Figure 3

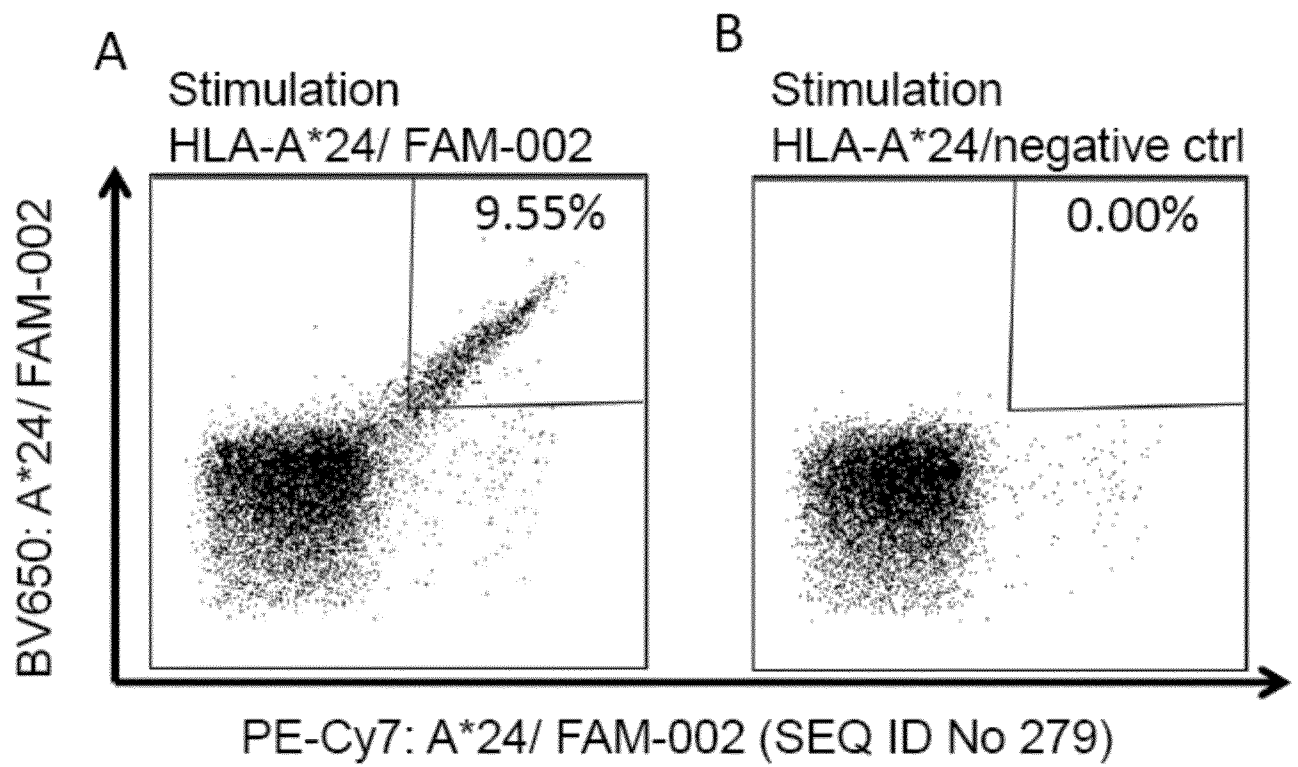


Figure 4

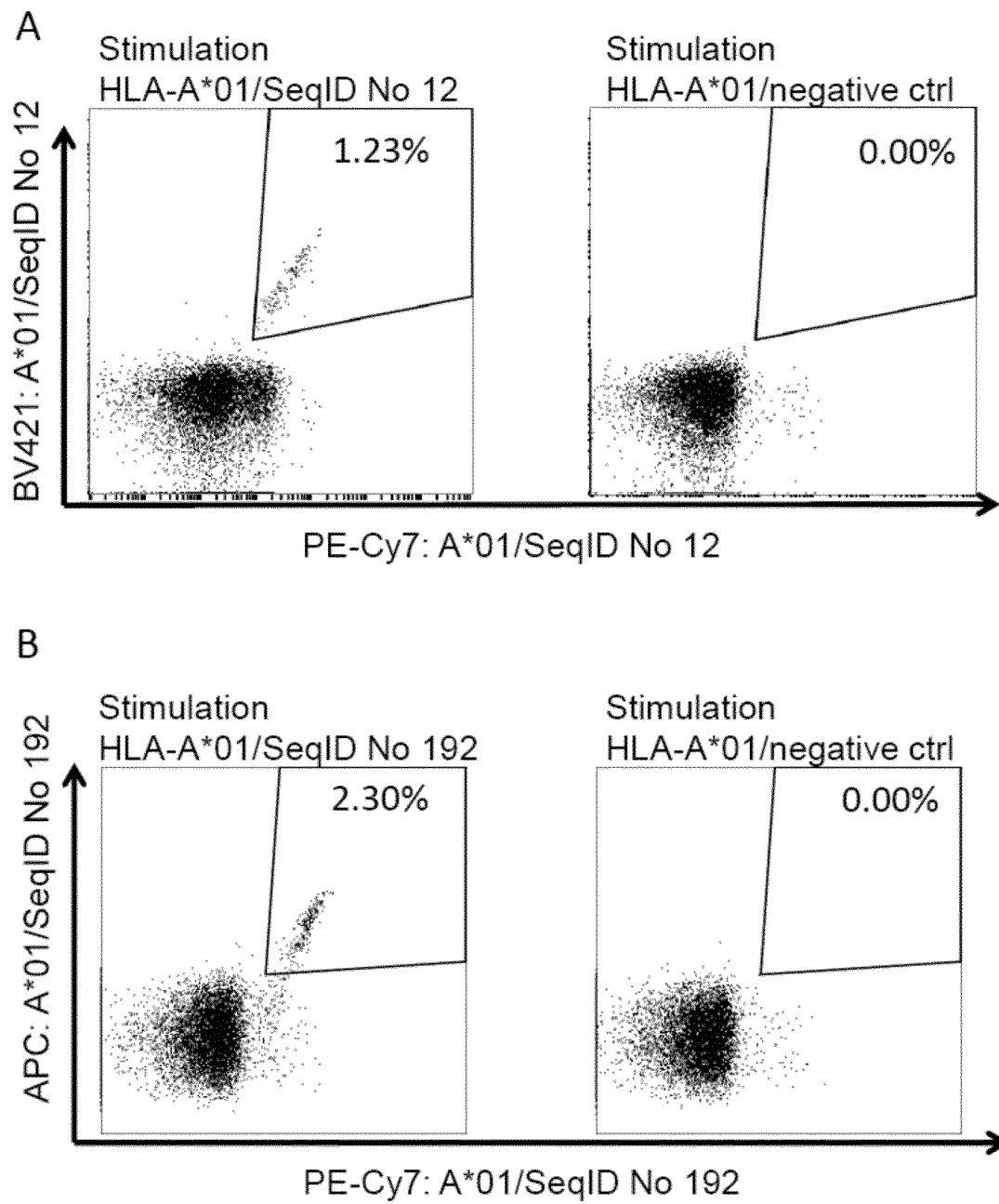


Figure 5

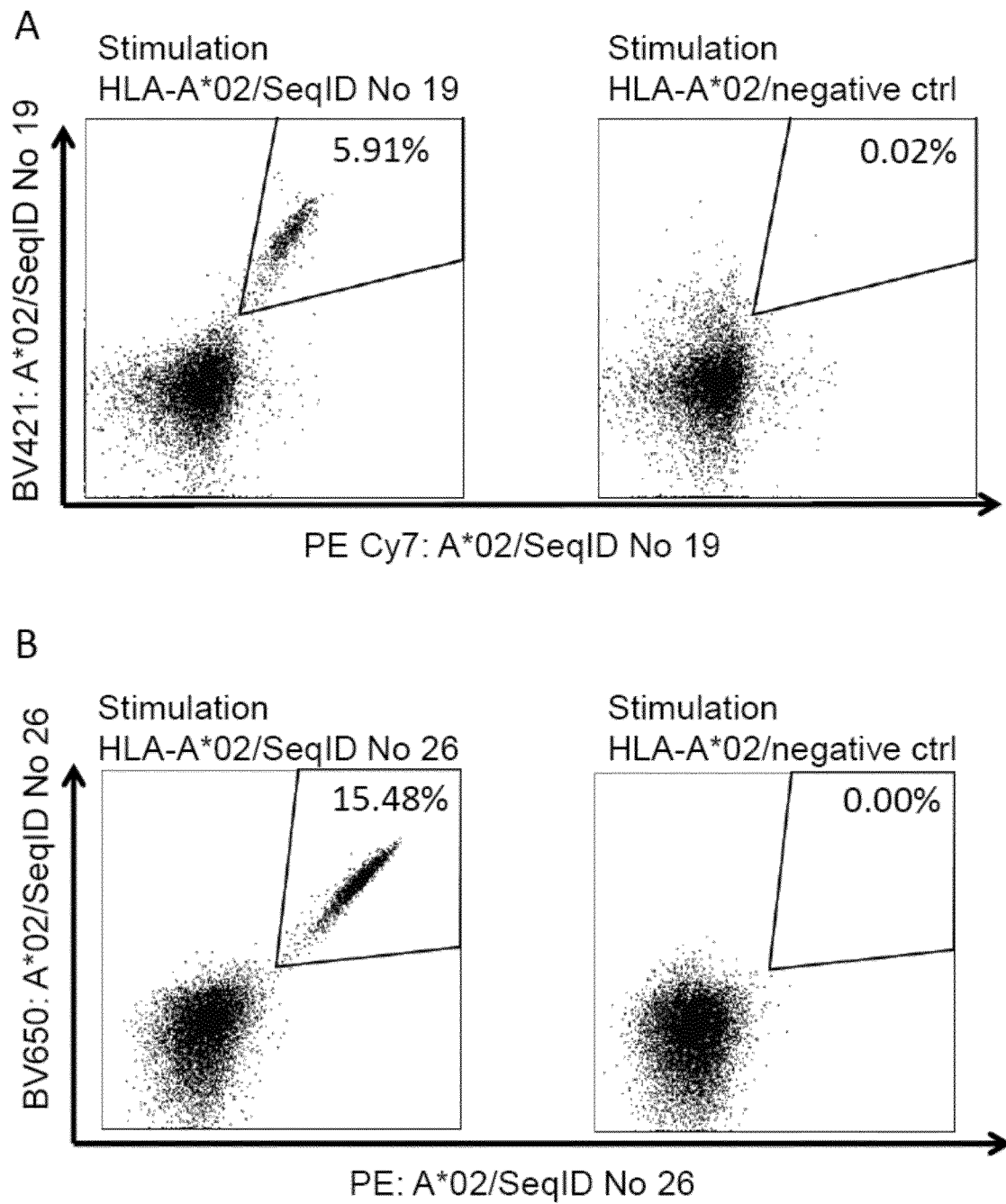


Figure 6

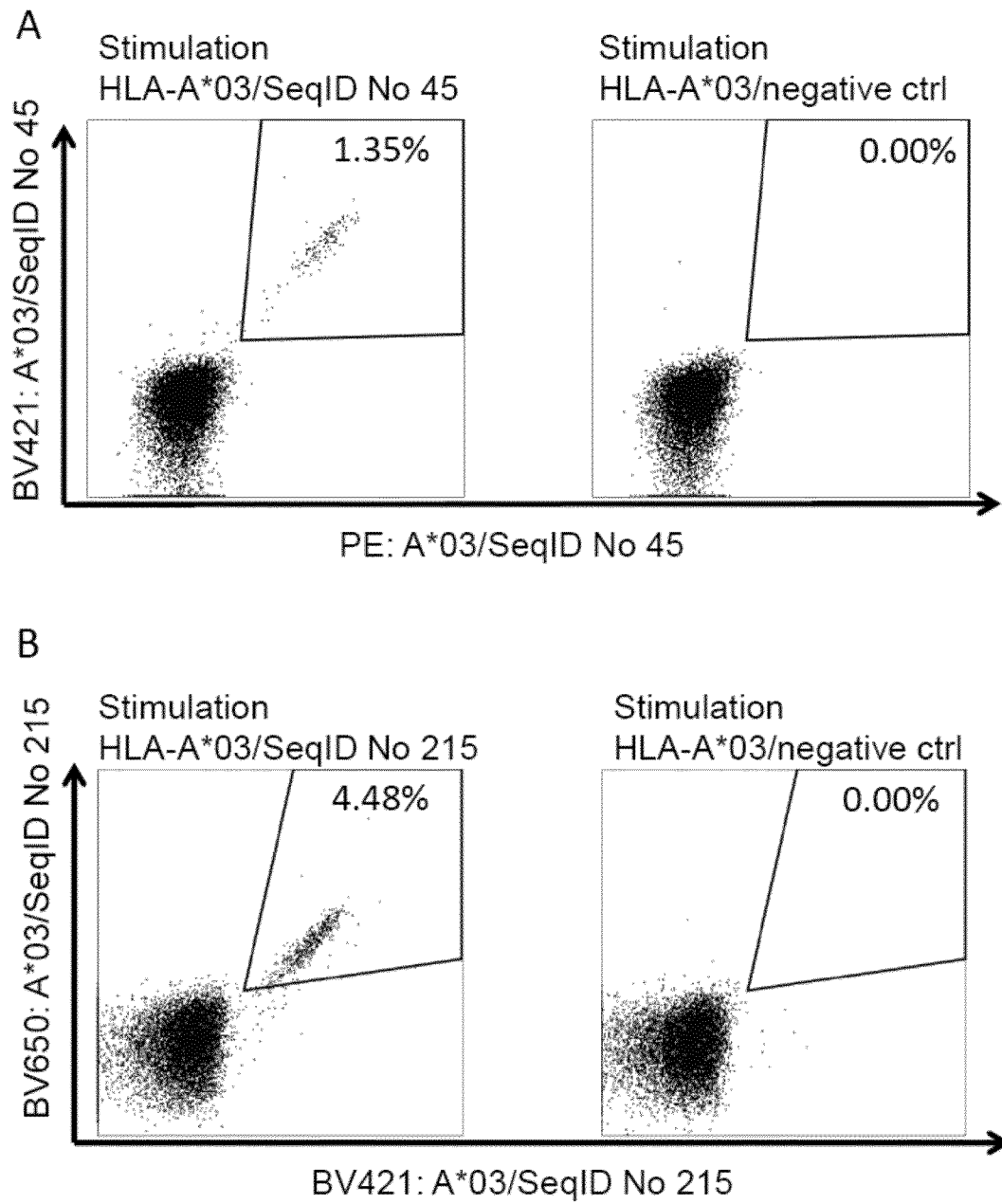


Figure 7

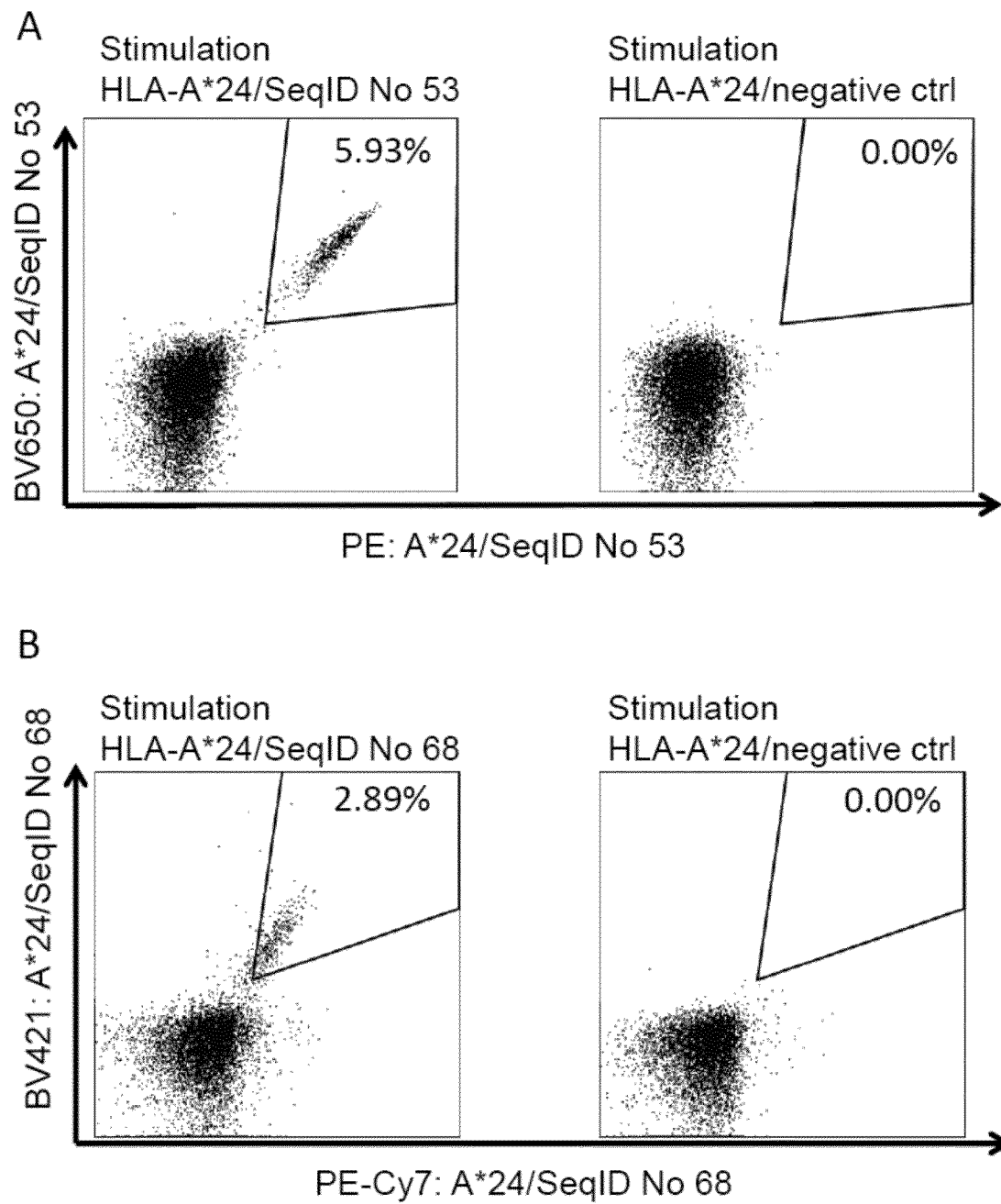


Figure 8

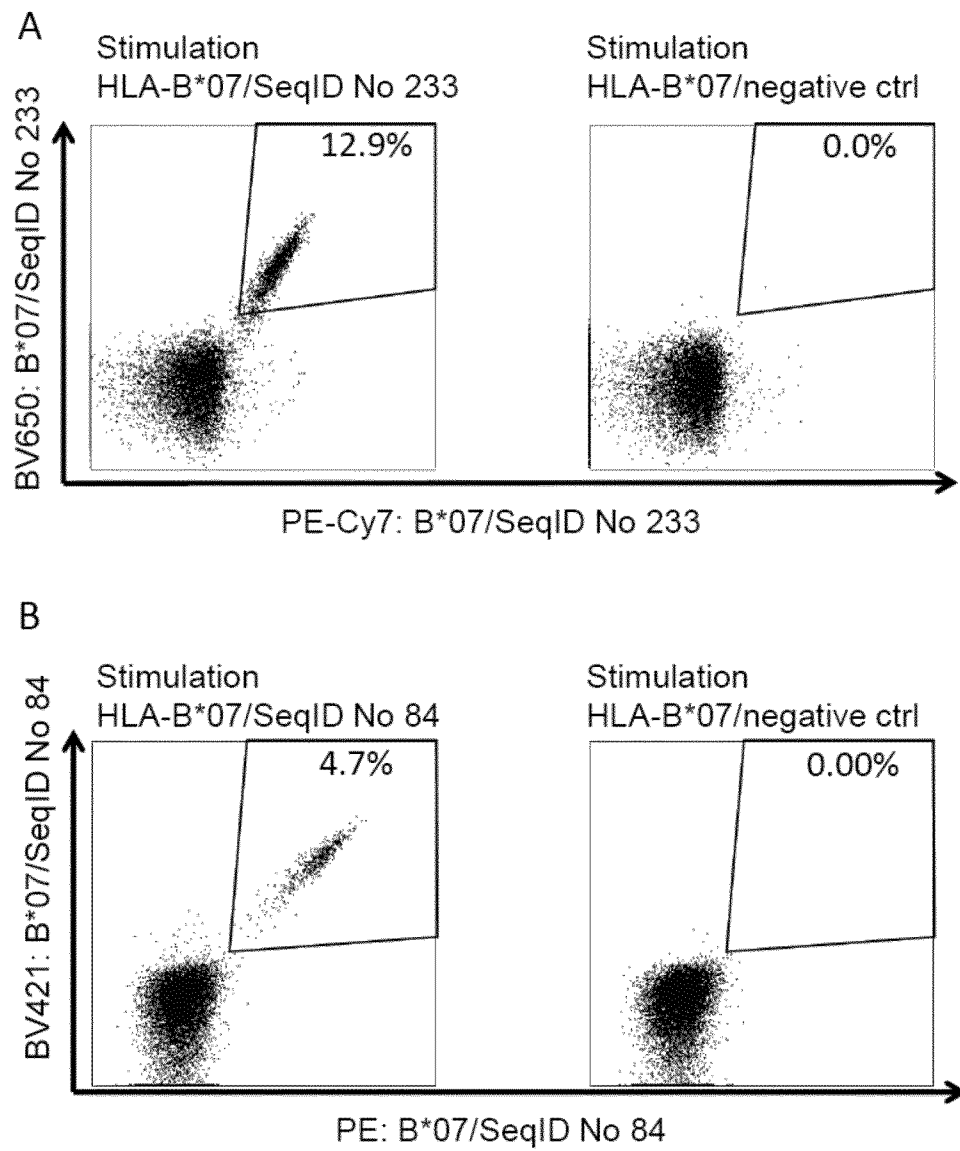


Figure 9

