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(54) Title: NOVEL PEPTIDES AND COMBINATION OF PEPTIDES FOR USE IN IMMUNOTHERAPY AGAINST EPITHELIAL OVARIAN CANCER 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.

Novel peptides and combination of peptides for use in immunotherapy against epithelial ovarian cancer 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 as well as HLA class II 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

Epithelial ovarian cancer (EOC) remains the leading cause of death from gynecologic malignancies and the fifth leading cause of cancer related death in the western world, causing an estimated 22,000 new diagnoses and 14,000 deaths in the US in 2014(1). The only available curative treatment option is complete surgical tumor removal at an early non metastatic stage. However, most patients (>70%) are diagnosed with stage III or IV disease caused by of a lack of specific early symptoms. Despite progress in chemotherapy regimens and the recent approval of bevacizumab for first line therapy, the majority of patients relapse within few months or years after initial treatment (2, 3).

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 ovarian cancer in particular. There is also a need to identify factors representing biomarkers for cancer in general and ovarian cancer 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.

Over the last two decades, EOC has been recognized as a highly immunogenic tumor, based on diverse clinical findings. Showing frequent immune cell infiltration EOC was among the first cancers, where a definitive association of T-cell infiltration and clinical prognosis could be established. Within these infiltrating T-cell population tumor reactive and antigen specific T-cells have been identified. Tumor resident regulatory T-cells (Tregs) in contrast are negatively correlated with clinical outcome. Further, immune stimulatory cytokines have been shown to induce compelling tumor responses in individual patients.

The effectiveness of immunotherapeutic approaches for cancer therapy has been illustrated by the recent development and approval of immune checkpoint inhibitors shown in melanoma treatment. Moreover, antigen specific peptide vaccination and adoptive T-cell transfer begin to show success in melanoma and other immunogenic tumors, e.g. renal cell carcinoma. Personalized immunotherapy even has curative potential and stunning results were presented for individual patients.

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 literature (Brossart and Bevan, 1997; Rock et al., 1990). MHC class II molecules can be found predominantly on professional antigen presenting cells (APCs), and primarily present peptides of exogenous or transmembrane proteins that are taken up by APCs e.g. during endocytosis, and are subsequently processed.

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

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

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

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

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

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

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

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

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

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

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

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

Therefore, TAAs are a starting point for the development of a T cell based therapy including but not limited to tumor vaccines. The methods for identifying and characterizing the TAAs are usually based on the use of T-cells that can be isolated from patients or healthy subjects, or they are based on the generation of differential transcription profiles or differential peptide expression patterns between tumors and normal tissues. However, the identification of genes over-expressed in tumor tissues or human tumor cell lines, or selectively expressed in such tissues or cell lines, does not provide precise information as to the use of the antigens being transcribed from these genes in an immune therapy. This is because only an individual subpopulation of

epitopes of these antigens are suitable for such an application since a T cell with a corresponding TCR has to be present and the immunological tolerance for this particular epitope needs to be absent or minimal. In a very preferred embodiment of the invention it is therefore important to select only those over- or selectively presented peptides against which a functional and/or a proliferating T cell can be found. Such a functional T cell is defined as a T cell, which upon stimulation with a specific antigen can be clonally expanded and is able to execute effector functions ("effector T cell").

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

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: 549 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: 549, 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: 549 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: 549, wherein said peptide or variant thereof has an overall length of between 8 and 100, preferably between 8 and 30, and most preferred of between 8 and 14 amino acids.

The following tables show the peptides according to the present invention, their respective SEQ ID NOs, and the prospective source (underlying) genes for these

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

Table 1: Peptides according to the present invention; X = S, R or G

SEQ ID No.	Sequence	Gene	HLA binding
1	QFITSTNTF	MUC16	A*24:02
2	STETSTVLY	MUC16	A*01
3	AHSKITTAM	MUC16	B*39:01
4	AVKTETSTSER	MUC16	A*31:01
5	AVTNVRTSI	MUC16	B*13
6	DALTPLVTI	MUC16	B*5101
7	DALVLKTV	MUC16	B*51
8	DPYKATSAV	MUC16	B*51
9	EPETTTSFITY	MUC16	B*35
10	ERSPVIQTL	MUC16	B*39:01
11	ETILTFHAF	MUC16	A*25
12	EVISSRGTSM	MUC16	A*25
13	EVITSSRTTI	MUC16	A*25
14	EVTSSGRTSI	MUC16	A*25
15	FPEKTTHSF	MUC16	B*35
16	FPHSEETTTM	MUC16	B*35
17	FPHSEITTL	MUC16	B*35
18	FQRQGQTAL	MUC16	B*15:01
19	GDVPRPSSL	MUC16	B*08:01
20	GHESHSPAL	MUC16	B*39:01
21	GHTTVSTSM	MUC16	B*39:01
22	GTHSPVTQR	MUC16	A*31:01
23	GTSGTPVSK	MUC16	A*11
24	HPDPQSPGL	MUC16	B*35
25	IPRVFTSSI	MUC16	B*51

26	ISDEVVTRL	MUC16	C*05
27	ISIGTIPRI	MUC16	B*15:17
28	ISKEDVTSI	MUC16	B*15:17
29	ITETSAVLY	MUC16	A*01
30	ITRLPTSSI	MUC16	B*15:17
31	KDTAHTEAM	MUC16	B*44:02
32	KEDSTALVM	MUC16	B*40/B*44
33	KEVTSSSSVL	MUC16	B*40/B*44/?
34	LPHSEITTL	MUC16	B*35
35	LTISTHKTI	MUC16	B*15:17
36	LTKSEERTI	MUC16	B*15:17
37	RDSLYVNGF	MUC16	B*44:02
38	RETSTSQKI	MUC16	B*18:01
39	RSSGVTFSR	MUC16	A*31:01
40	SAFESHSTV	MUC16	B*51
41	SATERSASL	MUC16	C*03/?
42	SENSETTAL	MUC16	B*40/B*44/?
43	SEQRTSPSL	MUC16	?
44	SESPSTIKL	MUC16	B*40/?
45	SPAGEAHSL	MUC16	B*07/B*56
46	SPAGEAHSLLA	MUC16	B*56:01
47	SPHPVSTTF	MUC16	B*07:02
48	SPHPVTALL	MUC16	B*07:02
49	SPLFQRSSL	MUC16	B*0702
50	SPQNLRNTL	MUC16	B*35/B*07:02
51	SPRLNTQGNTAL	MUC16	B*07:02
52	SPSEAITRL	MUC16	B*07:02
53	SPSKAFASL	MUC16	B*35/B*07:02
54	SPSSPTPKV	MUC16	B*07:02
55	SPSSQAPVL	MUC16	B*07:02
56	SQGFSHSQM	MUC16	B*15:01
57	SRTEVISSR	MUC16	B*27
58	SSAVSTTTI	MUC16	B*15:17
59	SSPLRVTSL	MUC16	n/a
60	STASSSLSK	MUC16	A*11
61	STQRVTTSM	MUC16	B*07?
62	STSQEIHSATK	MUC16	A*11
63	SVLADLVTTK	MUC16	A*03:01
64	SVPDILSTSW	MUC16	A*24:02
65	TAGPTTHQF	MUC16	C*03
66	TEISSSRTSI	MUC16	B*49:01

67	TENTGKEKL	MUC16	B*40/B*44
68	TETEAIHVF	MUC16	B*18
69	TEVSRTEVI	MUC16	B*49:01
70	TExVLQGLL	MUC16	B*40/B*44/?
71	TPGGTRQSL	MUC16	B*07:02/B*35
72	TPGNRAISL	MUC16	B*07:02/B*35
73	TPNSRGETSL	MUC16	B*07:02
74	TSGPVTEKY	MUC16	B*35
75	TSPAGEAHSL	MUC16	?
76	VHESHSSVL	MUC16	B*39:01
77	VPRSAATTL	MUC16	B*07:02/B*35
78	VTSAPGRSI	MUC16	B*15:17
79	VTSSSRTSI	MUC16	B*15:17
80	YPDPSKASSAM	MUC16	B*35
81	AAWLRSAAA	MMP11	B*55/B*56
82	APAAWLRSAA	MMP11	B*55/B*56
83	APAAWLRSAAA	MMP11	B*55/B*56
84	LPSPVDAAF	MMP11	B*35
85	RGVPSEIDAAF	MMP11	B*58
86	EAGPPAFYR	ESR1	A*66
87	STSSHSLQK	ESR1	A*03/A*11
88	APHLHLSA	KLK10	B*56:01
89	APHLHLSAA	KLK10	B*56:01
90	RALAKLLPL	KLK10	B*08/A*02
91	SAASGARAL	KLK10	C*03
92	VLVDQSWVL	KLK10	A*02
93	DYLKRFYLY	MMP7	A*24
94	SETKNANSL	MMP7	B*44/B*41/B*40
95	SSDPNAVMY	MMP7	A*01
96	YPFDGPGNTL	MMP7	B*35
97	YPFDGPGNTLAH	MMP7	B*35
98	NEIERVFVW	EYA2	B*44:02
99	NVGGLIGTPK	EYA2	A*03
100	RVKEMYNTY	EYA2	A*30/A*32
101	SAPLRVSQL	EYA2	?
102	DTDEYVLKY	EFHC1	A*01
103	KDSTKTAF	EFHC1	B*44
104	SKAPVLTY	EFHC1	B*15:03
105	AEYTDVLQKI	EPS8L1	B*49
106	EYTDVLQKI	EPS8L1	A*24
107	RPHLTSDA	EPS8L1	B*56

108	RPHLTSDAV	EPS8L1	B*56
109	RPHLTSDAVA	EPS8L1	B*56
110	SAKSIYEQR	EPS8L1	A*31
111	SPEEGARVY	EPS8L1	B*35
112	SQYPVNHLV	EPS8L1	B*15
113	YPVNHLVTF	EPS8L1	B*35
114	AAASAIKVI	IDO1	C*12
115	IHDHVNPKAFF	IDO1	B*38
116	NPKAFFSVL	IDO1	B*07
117	NPSVREFVL	IDO1	B*35
118	RSYHLQIVTK	IDO1	A*11/A*03
119	RYMPPAHRNF	IDO1	A*24
120	TEFEQYLHF	SOX17	B*18/B*44
121	VSDASSAVYY	SOX17	A*01
122	AEIEADRSY	LAMC2	B*44
123	AQKVDTRAK	LAMC2	A*03
124	HPSAHDVIL	LAMC2	B*35:03
125	RIKQKADSL	LAMC2	B*08
126	SEGASRSLGL	LAMC2	B*37
127	SVDEEGLVLL	LAMC2	A*02
128	SVHKITSTF	LAMC2	A*25
129	TREATQAEI	LAMC2	B*39
130	VYFVAPAKF	LAMC2	A*24
131	APQSAHAAF	SGPL1	B*07
132	ETIIIFHSL	EYA3	A*25
133	TELLVKAY	SGPL1	B*18
134	WQEGRASGTVY	SGPL1	B*15
135	IRSENFEEL	CRABP2	B*39
136	KIAVAAASK	CRABP2	A*03
137	NVMLRKIAV	CRABP2	B*08
138	RELTNDGELIL	CRABP2	B*40/B*44
139	VAAASKPAV	CRABP2	?
140	SPNAIFKAL	SOX9	B*07
141	SSKNKPHVKR	SOX9	A*31
142	TPASAGHVW	SOX9	B*07
143	YTDHQNSSSY	SOX9	A*01
144	AEVLLPRL	MSLN	B*40
145	AVLPLTVAEVQK	MSLN	A*03
146	LPTARPLL	MSLN	B*07
147	RVRELAVAL	MSLN	A*02
148	NLPIFLPRV	MLPH	A*02

149	RVHPEEQGW	MLPH	B*58
150	TVKPSGKPR	MLPH	A*31
151	YYEHVKARF	MLPH	A*24
152	AARPAGATL	ERBB2	B*07
153	MPNPEGRYTF	ERBB2	B*35
154	FYIKTSTTV	CRABP2	A*24
155	RTTEINFKV	CRABP2	A*02
156	YIKTSTTV	CRABP2	B*08
157	GQAAQGPTI	DDR1	B*15
158	HRFLAEDAL	DDR1	B*39:01
159	EEVARFYAA	FOLR1	B*45
160	NPNEEVARF	FOLR1	B*35
161	NPNEEVARFY	FOLR1	B*35
162	KSQTLLGK	ULK1	A*11/A*03
163	DELISKSF	YPEL1	B*18
164	HDELISKSF	YPEL1	B*35
165	GRAYLFNSV	YPEL1	B*27
166	YLFNSVVNV	YPEL1	A*02
167	APDNRPAL	MUC1	B*07/B*35
168	HHSDTPTTL	MUC1	B*38/B*39
169	HPMSEYPTY	MUC1	B*35
170	LQRDISEM	MUC1	B*51
171	LQRDISEMF	MUC1	B*51
172	AIAEIGNQL	MMP9	A*02
173	DVAQVTGALR	MMP9	A*68
174	SEDLPRAVI	MMP9	B*49/B*40
175	APDAKSFVL	LGALS1	B*35
176	EVAPDAKSF	LGALS1	A*25
177	FPFQPGSVAEV	LGALS1	B*35
178	GEVAPDAKSFVL	LGALS1	B*40
179	LPDGYEFKF	LGALS1	B*35

Table 2: Additional peptides according to the present invention, X = S, R or G

SEQ ID No.	**Sequence**	**MHC class**	**Gene**
180	DKAFTAATTEVSR	II	MUC16
181	ELGPYTLDRNSLYVN	II	MUC16
182	ELGPYTLDRNSLYVNG	II	MUC16
183	FDKAFTAATTEVSR	II	MUC16
184	GPYTLDRNSLYVN	II	MUC16

185	LGPYTLDRDSLYVN	II	MUC16
186	LGPYTLDRNSLYVN	II	MUC16
187	LGPYTLDRNSLYVNG	II	MUC16
188	STETITRLSTFPFVTG	II	MUC16
189	ELQWEQAQDYLKR	II	MMP7
190	ELQWEQAQDYLKRF	II	MMP7
191	GINFLYAATHELGHS	II	MMP7
192	LQWEQAQDYLKR	II	MMP7
193	LQWEQAQDYLKRF	II	MMP7
194	SELQWEQAQDYLKR	II	MMP7
195	SELQWEQAQDYLKRF	II	MMP7
196	VPYNILTPYPGPR	II	EPS8L1
197	YVPYNILTPYPGPR	II	EPS8L1
198	GNWKIIRSENFEEL	II	CRABP2
199	GNWKIIRSENFEELLK	II	CRABP2
200	NWKIIRSENFEEL	II	CRABP2
201	PNFSGNWKIIRSENF	II	CRABP2
202	VMLRKIAVAAASKPA	II	CRABP2
203	WKIIRSENFEEL	II	CRABP2
204	LQRYSSDPTGALT	II	EGFR
205	NPTTYQMDVNPEGK	II	EGFR
206	NPTTYQMDVNPEGKY	II	EGFR
207	DDGGQFVVTTNPVNNDG	II	CDH1
208	DKEGKVFYSITGQGADTPP	II	CDH1
209	DKEGKVFYSITGQGADTPPV	II	CDH1
210	DKNMFTINRNTGVI	II	CDH1
211	DKNMFTINRNTGVIS	II	CDH1
212	DPELPDKNMFTINRNTG	II	CDH1
213	DPELPDKNMFTINRNTGVI	II	CDH1
214	DPELPDKNMFTINRNTGVIS	II	CDH1
215	DPELPDKNMFTINRNTGVISV	II	CDH1
216	DPELPDKNMFTINRNTGVISV V	II	CDH1
217	DPELPDKNMFTINRNTGVISV VT	II	CDH1
218	DVNTYNAAIAYTILS	II	CDH1
219	DVNTYNAAIAYTILSQ	II	CDH1
220	EGKVFYSITGQGADT	II	CDH1
221	EGKVFYSITGQGADTPP	II	CDH1
222	EGKVFYSITGQGADTPPV	II	CDH1
223	ELPDKNMFTINRNTGVIS	II	CDH1
224	GGQFVVTTNPVNN	II	CDH1

225	GKVFYSITGQGADT	II	CDH1
226	GPFPKNLVQIKSNKDK	II	CDH1
227	GPFPKNLVQIKSNKDKE	II	CDH1
228	GPFPKNLVQIKSNKDKEGK	II	CDH1
229	KNMFTINRNTGVI	II	CDH1
230	KNMFTINRNTGVIS	II	CDH1
231	LPDKNMFTINRNTG	II	CDH1
232	LPDKNMFTINRNTGVI	II	CDH1
233	LPDKNMFTINRNTGVIS	II	CDH1
234	PELPDKNMFTINRNTGVI	II	CDH1
235	PELPDKNMFTINRNTGVIS	II	CDH1
236	QDPELPDKNMFTINRNTGVIS	II	CDH1
237	SQDPELPDKNMFTINRNTGVIS	II	CDH1
238	SQDPELPDKNMFTINRNTGVISVVT	II	CDH1
239	SVPRYLPRPANPDE	II	CDH1
240	TDGVITVKRPLRFHNPQ	II	CDH1
241	TRAELDREDFEHVK	II	CDH1
242	VPRYLPRPANPDE	II	CDH1
243	ALEFRALEPQGLL	II	AGRN
244	ALEFRALEPQGLLL	II	AGRN
245	DTRIFFVNPAPPY	II	AGRN
246	DTRIFFVNPAPPYL	II	AGRN
247	DTRIFFVNPAPPYLW	II	AGRN
248	DTRIFFVNPAPPYLWP	II	AGRN
249	DTRIFFVNPAPPYLWPA	II	AGRN
250	EFRALEPQGLLL	II	AGRN
251	GAPVPAFEGRSFLAFPTL	II	AGRN
252	GDTRIFFVNPAPPYLWP	II	AGRN
253	GDTRIFFVNPAPPYLWPA	II	AGRN
254	IVDVHFDPTTAFRAPD	II	AGRN
255	KVRVWRYLKGKDLVAR	II	AGRN
256	LALEFRALEPQGLLL	II	AGRN
257	LEFRALEPQGLLL	II	AGRN
258	SGPFLADFNGFSH	II	AGRN
259	TGDTRIFFVNPAPPYLWPA	II	AGRN
260	TRIFFVNPAPPYL	II	AGRN
261	VDVHFDPTTAFRAPD	II	AGRN
262	VDVHFDPTTAFRAPDV	II	AGRN
263	VRVWRYLKGKDLVAR	II	AGRN
264	APVPAFEGRSFLAFPT	II	AGRN

265	APVPAFEGRSFLAFPTL	II	AGRN
266	ALRGLLPVLGQPIIR	II	MSLN
267	DLPGRFVAESAEVLLP	II	MSLN
268	DLPGRFVAESAEVLLPR	II	MSLN
269	GQPIIRSIPQGIV	II	MSLN
270	GQPIIRSIPQGIVA	II	MSLN
271	LGQPIIRSIPQGIVA	II	MSLN
272	LPAALACWGVRGSL	II	MSLN
273	LPGRFVAESAEVLL	II	MSLN
274	LPGRFVAESAEVLLP	II	MSLN
275	LPGRFVAESAEVLLPR	II	MSLN
276	LRGLLPVLGQPIIR	II	MSLN
277	PGRFVAESAEVLLPR	II	MSLN
278	PGRFVAESAEVLLPRL	II	MSLN
279	QPIIRSIPQGIVA	II	MSLN
280	RGLLPVLGQPIIR	II	MSLN
281	SRTLAGETGQEAAPL	II	MSLN
282	STERVRELAVALAQK	II	MSLN
283	TDAVLPLTVAEVQ	II	MSLN
284	VAEVQKLLGPHVEG	II	MSLN
285	VAEVQKLLGPHVEGLK	II	MSLN
286	VLGQPIIRSIPQGIVA	II	MSLN
287	VRGSLLSEADVRALG	II	MSLN
288	VRGSLLSEADVRALGG	II	MSLN
289	LPAALACWGVRGSLL	II	MSLN
290	AIKVLRENTSPKANKE	II	ERBB2
291	DPSPLQRYSEDPTVPLPS	II	ERBB2
292	DPSPLQRYSEDPTVPLPSE	II	ERBB2
293	ELVSEFSRMARD	II	ERBB2
294	ELVSEFSRMARDPQ	II	ERBB2
295	IPVAIKVLRENTSPKANKE	II	ERBB2
296	RRLLQETELVEPLTPS	II	ERBB2
297	SPQPEYVNQPDVRPQPP	II	ERBB2
298	VKPDLSYMPIWKFPDE	II	ERBB2
299	ASGMRYLATLNFVHR	II	DDR1
300	IASGMRYLATLNFVHR	II	DDR1
301	KEVKIMSRLKDPN	II	DDR1
302	LNQFLSAHQLEDK	II	DDR1
303	NPAYRLLLATYARPP	II	DDR1
304	NPAYRLLLATYARPPR	II	DDR1
305	SNPAYRLLLATYARPP	II	DDR1

306	SNPAYRLLLATYARPPR	II	DDR1
307	DPSTDYYQELQRDISE	II	MUC1
308	VETQFNQYKTEAASR	II	MUC1
309	GRQVWVYTGASVLGPR	II	MMP9
310	NQLYLFKDGKYWRFSEG	II	MMP9
311	RQVWVYTGASVLGPR	II	MMP9
312	SGRQVWVYTGASVLG	II	MMP9
313	SGRQVWVYTGASVLGP	II	MMP9
314	SGRQVWVYTGASVLGPR	II	MMP9
315	VDPRSASEVDRMFPG	II	MMP9
316	GEVAPDAKSFVLN	II	LGALS1
317	LTVKLPDGYEFKFPNRLNL	II	LGALS1
318	VRGEVAPDAKSFVLN	II	LGALS1
319	VRGEVAPDAKSFVLNLG	II	LGALS1

Table 3: Additional peptides useful for cancer therapies, X = S, R or G

SEQ ID No.	**Sequence**	**MHC class**	**Gene**
320	ATSKIPLAL	I	MUC16
321	ITSSRTTI	I	MUC16
322	LNFTITNLQ	I	MUC16
323	TATSPMVPAS	I	MUC16
324	TTLPESRPS	I	MUC16
325	VELRVLALP	I	LRFN4
326	AEDNLIHKF	I	NLRP2
327	REDLERLGV	I	NLRP7
328	DTKDPAVTEW	I	TLR7
329	ILISKLLGA	I	TLR7
330	SESLRTLEF	I	TLR7
331	VLAELVAKL	I	TLR7
332	INTSILLIF	I	TLR3
333	ALQPLLHTV	I	IL17RD
334	RLMDNLPQL	I	IL17RD
335	LIISPTREL	I	DDX10
336	ADSKVLLF	I	WDR35
337	DSLLEQANNAI	I	WDR35
338	DYQGIKFVKR	I	WDR35
339	EVVGYFGRF	I	WDR35
340	KYVKGLISI	I	WDR35
341	SIGTPLDPK	I	WDR35

342	TASDKILIV	I	WDR35
343	GVIKVISGF	I	NOC3L
344	KVKLENKLK	I	NOC3L
345	SSSEPVHAK	I	NOC3L
346	SSSEPVHAKK	I	NOC3L
347	LSDQLAQAI	I	DNASE1
348	LSDIVIEKY	I	WDR27
349	SLDDHVVAV	I	WDR27
350	SQIDQQNSV	I	LRIF1
351	STIDPSGTRSK	I	LRIF1
352	VFRDQEPKI	I	LRIF1
353	VLREKEAAL	I	LRIF1
354	TRLQQAQAL	I	POLR2J3
355	VAAPEHISY	I	POLR2J3
356	NSKKKVAL	I	DDX52
357	QNSKKKVAL	I	DDX52
358	RDNTVHSF	I	DDX52
359	KQVSEFMTW	I	RASGEF1B
360	KTKPQSIQR	I	RASGEF1B
361	THIELERL	I	RASGEF1B
362	IAPKILQL	I	RASGEF1B
363	DIASVSGRW	I	BICC1
364	KPKQPSKSV	I	BICC1
365	MPAETIKEL	I	BICC1
366	SAVKEGTAM	I	BICC1
367	EEEKLQAAF	I	COMMD10
368	DEFNLQKM	I	EMC1
369	DEYKVTAF	I	EMC1
370	ETNIGGLNW	I	EMC1
371	FPQTALVSF	I	EMC1
372	GEFGKKADGLL	I	EMC1
373	GSMGSFSEK	I	EMC1
374	IFLIDGVTGRI	I	EMC1
375	IPPEVQRI	I	EMC1
376	IPYSPDVQI	I	EMC1
377	QVAPPVLKR	I	EMC1
378	TEKNVIAAL	I	EMC1
379	VGKVKFASL	I	EMC1
380	VPFSHVNI	I	EMC1
381	VVYQYWNTK	I	EMC1
382	YPSKQFDVL	I	EMC1

383	AADDSADKV	I	ZNF217
384	HHKEKQTDV	I	ZNF217
385	KQTDVAAEV	I	ZNF217
386	KSAFPAQSK	I	ZNF217
387	NEVVQVHAA	I	ZNF217
388	SEDLNKHVL	I	ZNF217
389	GETIHIPTM	I	BCAT1
390	GPKLASRIL	I	BCAT1
391	GVKKPTKAL	I	BCAT1
392	KEKPDPNNL	I	BCAT1
393	KVSERYLTM	I	BCAT1
394	LPVFDKEEL	I	BCAT1
395	LSKLTDIQY	I	BCAT1
396	DLSNIINKL	I	WDR12
397	RVWDVESGSLK	I	WDR12
398	SPTTSHVGA	I	WDR12
399	VEIEYVEKY	I	WDR12
400	VERNKVKAL	I	WDR12
401	REAVSKEDL	I	PANK2
402	IMGGNSILHSA	I	STXBP6
403	KQFEGSTSF	I	STXBP6
404	EEFLRQEHF	I	OASL
405	ETIPSEIQVF	I	OASL
406	EVGEALKTVL	I	DMD
407	KLEDLEEQL	I	DMD
408	LKIQSIAL	I	DMD
409	MNVLTEWLAAT	I	DMD
410	AIQDKLFQV	I	CHCHD6
411	FPNFDKQEL	I	SMARCAD1
412	GQTKEVLVI	I	SMARCAD1
413	KLIESTSTM	I	SMARCAD1
414	KPYQKVGL	I	SMARCAD1
415	KQESIVLKL	I	SMARCAD1
416	NANNRLLL	I	SMARCAD1
417	SEVPNGKEV	I	SMARCAD1
418	TNNIGSIAR	I	PANK2
419	DAKGRTVSL	I	GPX8
420	IIKKKEDL	I	GPX8
421	DVIDVVQAL	I	C20orf194
422	EEFKITSF	I	C20orf194
423	SDFEKTGF	I	C20orf194

424	DEDRLLVVF	I	USP34
425	HHSNIPMSL	I	USP34
426	LFPSLIKNL	I	USP34
427	NTNIPIGNK	I	USP34
428	SDQVADLR	I	USP34
429	THFSFPLRL	I	USP34
430	TYDSVTDKF	I	USP34
431	AESLYEIRF	I	TM9SF1
432	DEFLGLTHTY	I	TM9SF1
547	IITEVITRL	I	MUC16
548	KMISAIPTL	I	MUC16
549	TYSEKTTLF	I	MUC16

Table 4: Additional peptides useful for cancer therapies, X = S, R or G

SEQ ID No.	**Sequence**	**MHC class**	**Gene**
433	ALDFFGNGPPVNY	II	IFI30
434	ALDFFGNGPPVNYKT	II	IFI30
435	DFFGNGPPVNYK	II	IFI30
436	DFFGNGPPVNYKT	II	IFI30
437	DFFGNGPPVNYKTGN	II	IFI30
438	DFFGNGPPVNYKTGNL	II	IFI30
439	DFFGNGPPVNYKTGNLY	II	IFI30
440	LQALDFFGNGPPVNYKTGN	II	IFI30
441	QALDFFGNGPPVNYK	II	IFI30
442	QPPHEYVPWVTVNGKP	II	IFI30
443	SPLQALDFFGNGPPVNYKTG	II	IFI30
444	SPLQALDFFGNGPPVNYKTGN	II	IFI30
445	SPLQALDFFGNGPPVNYKTGNLY	II	IFI30
446	GPPFSSSQSIPVVPR	II	GPR64
447	LPSSLMNNLPAHDM	II	GPR64
448	LPSSLMNNLPAHDME	II	GPR64
449	LPSSLMNNLPAHDMEL	II	GPR64
450	SPIGEIQPLSPQPSAPI	II	GPR64
451	DEVTQPFVIDEKTAEIR	II	PCDHB5
452	KYPELVLDKALDREER	II	PCDHB5
453	KYPELVLDKALDREERPE	II	PCDHB5
454	VTQPFVIDEKTAEIR	II	PCDHB5
455	DGRTIVDLEGTPVVSPD	II	FNDC1
456	DGRTIVDLEGTPVVSPDG	II	FNDC1

457	DKPILSLGGKPLVG	II	FNDC1
458	GDGRTIVDLEGTPVVSPD	II	FNDC1
459	GDGRTIVDLEGTPVVSPDG	II	FNDC1
460	GGDGRTIVDLEGTPVVSPD	II	FNDC1
461	GGDGRTIVDLEGTPVVSPDG	II	FNDC1
462	GRTIVDLEGTPVVSPD	II	FNDC1
463	KVKEYILSYAPALKPF	II	FNDC1
464	KVKEYILSYAPALKPFG	II	FNDC1
465	LGGDGRTIVDLEGTPVVSPDG	II	FNDC1
466	RTHEIKKLASESVYV	II	FNDC1
467	VKEYILSYAPALKPF	II	FNDC1
468	YSKTQYNQVPSEDFERTPQ	II	CXADR
469	AAPNLSRMGAIPVMIP	II	CXADR
470	AAPNLSRMGAIPVMIPA	II	CXADR
471	APNLSRMGAIPVMIP	II	CXADR
472	APNLSRMGAIPVMIPA	II	CXADR
473	GYSKTQYNQVPSEDFERTPQ	II	CXADR
474	SKTQYNQVPSEDFER	II	CXADR
475	SKTQYNQVPSEDFERTP	II	CXADR
476	SKTQYNQVPSEDFERTPQ	II	CXADR
477	VAAPNLSRMGAIPVMIPA	II	CXADR
478	VIILYSGDKIYD	II	CXADR
479	YSKTQYNQVPSEDFER	II	CXADR
480	GHLFALRSLDYE	II	PCDHB3
481	AAEPGYLVTKVVAVDG	II	PCDHB3
482	AAEPGYLVTKVVAVDGD	II	PCDHB3
483	AAEPGYLVTKVVAVDGDS	II	PCDHB3
484	AAEPGYLVTKVVAVDGDSG	II	PCDHB3
485	AEPGYLVTKVVAVDG	II	PCDHB3
486	AEPGYLVTKVVAVDGD	II	PCDHB3
487	AEPGYLVTKVVAVDGDS	II	PCDHB3
488	EPGYLVTKVVAVDG	II	PCDHB3
489	EPGYLVTKVVAVDGD	II	PCDHB3
490	EPGYLVTKVVAVDGDS	II	PCDHB3
491	AEPGYLVTKVVAVD	II	PCDHB3
492	ADSTEFRPNAPVPLVI	II	CTPS2
493	ADSTEFRPNAPVPLVID	II	CTPS2
494	DADSTEFRPNAPVPLVI	II	CTPS2
495	DADSTEFRPNAPVPLVID	II	CTPS2
496	DADSTEFRPNAPVPLVIDM	II	CTPS2
497	DADSTEFRPNAPVPLVIDMP	II	CTPS2

498	DADSTEFRPNAPVPLVIDMPE	II	CTPS2
499	DSTEFRPNAPVPL	II	CTPS2
500	DSTEFRPNAPVPLV	II	CTPS2
501	DSTEFRPNAPVPLVI	II	CTPS2
502	DSTEFRPNAPVPLVID	II	CTPS2
503	DSTEFRPNAPVPLVIDMP	II	CTPS2
504	DSTEFRPNAPVPLVIDMPE	II	CTPS2
505	KDADSTEFRPNAPVPLVID	II	CTPS2
506	STEFRPNAPVPL	II	CTPS2
507	STEFRPNAPVPLVI	II	CTPS2
508	STEFRPNAPVPLVID	II	CTPS2
509	STEFRPNAPVPLVIDMP	II	CTPS2
510	AGDYTIANARKLIDE	II	RP2
511	ETLERLQEL		DMD
512	ADITYAIEADSESVK	II	FAT1
513	DITYAIEADSESVK	II	FAT1
514	KRDNYQIKVVASDHGE	II	FAT1
515	KRDNYQIKVVASDHGEK	II	FAT1
516	RDESFVIDRQSGRLK	II	FAT1
517	RDNYQIKVVASDHGE	II	FAT1
518	SPSELDRDPAYAIVT	II	FAT1
519	TPPQFSSVKVIHVTSPQ	II	FAT1
520	VPLPDIQEFPNY	II	FAT1
521	GPQLFHMDPSGTFVQ	II	PSMA5
522	DKNYFEGTGYARVPTQP	II	LAMA3
523	DKNYFEGTGYARVPTQPH	II	LAMA3
524	DSKPLYTPSSSFGVS	II	LAMA3
525	IQRQVKEINSLQSDFT	II	LAMA3
526	KNYFEGTGYARVPT	II	LAMA3
527	KNYFEGTGYARVPTQP	II	LAMA3
528	KNYFEGTGYARVPTQPH	II	LAMA3
529	SPRVVPNESIPIIPIP	II	PTPRG
530	SPRVVPNESIPIIPIPD	II	PTPRG
531	SSPRVVPNESIPIIP	II	PTPRG
532	SSPRVVPNESIPIIPIP	II	PTPRG
533	SSPRVVPNESIPIIPIPD	II	PTPRG
534	DDKGYTLMHPSLTRPY	II	CACHD1
535	DVGGAGYVVTISHTIHS	II	CACHD1
536	GAGYVVTISHTIH	II	CACHD1
537	GAGYVVTISHTIHS	II	CACHD1
538	GGAGYVVTISHTIH	II	CACHD1

539	GGAGYVVTISHTIHS	II	CACHD1
540	VGGAGYVVTISHTIHS	II	CACHD1
541	MTRTFHDLEGNAVKRDSG	II	ERMP1
542	RTFHDLEGNAVKR	II	ERMP1
543	RTFHDLEGNAVKRDSG	II	ERMP1
544	SGTFFPYSSNPANPK	II	ERMP1
545	SGTFFPYSSNPANPKP	II	ERMP1
546	TRTFHDLEGNAVKR	II	ERMP1

The present invention furthermore generally relates to the peptides according to the present invention for use in the treatment of proliferative diseases, such as, for example, ovarian cancer, non-small cell lung cancer, small cell lung cancer, kidney cancer, brain cancer, colon or rectum cancer, stomach cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, esophageal cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, bile duct cancer and other tumors that show an overexpression of a protein from which a peptide SEQ ID No. 1 to SEQ ID No. 319 is derived from.

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: 549. More preferred are the peptides – alone or in combination - selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 319 (see Table 1 and 2), and their uses in the immunotherapy of ovarian cancer, non-small cell lung cancer, small cell lung cancer, kidney cancer, brain cancer, colon or rectum cancer, stomach cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, esophageal cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, and bile duct cancer, and preferably ovarian cancer.

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 ovarian cancer, non-small cell lung cancer, small cell lung cancer, kidney cancer, brain cancer, colon or rectum cancer, stomach cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast

cancer, Merkel cell carcinoma, melanoma, esophageal cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, and bile duct cancer.

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: 549.

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.: 549, preferably containing SEQ ID No. 1 to SEQ ID No. 319, or a variant amino acid sequence.

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

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

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

Preferably, said medicament is for 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 ovarian cancer, non-small cell lung cancer, small cell lung cancer, kidney cancer, brain cancer, colon or rectum cancer, stomach cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, esophageal cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, and bile duct cancer, and preferably ovarian cancer 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 ovarian cancer 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.

Both therapeutic and diagnostic uses against additional cancerous diseases are disclosed in the following more detailed description of the underlying expression products (polypeptides) of the peptides according to the invention.

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 present invention further relates to a peptide according to the present invention, wherein said peptide is modified and/or includes non-peptide bonds as described herein below.

The present invention further relates to a peptide 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, i.e. binds to dendritic cells.

The present invention further relates to a nucleic acid, encoding for a peptide 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, expressing, and/or presenting 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.

The present invention further relates to antibodies as described further below, and methods of making them. Preferred are antibodies that are specific for the peptides of

the present invention, and/or for the peptides of the present invention when bound to their MHC. Preferred antibodies can be monoclonal.

The present invention further relates to T-cell receptors (TCR), in particular soluble TCR (sTCRs) targeting the peptides according to the invention and/or the peptide –MHC complexes thereof, and methods of making them.

The present invention further relates to antibodies or other binding molecules targeting the peptides according to the invention and/or the peptide–MHC complexes thereof, and methods of making them.

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. The present invention further relates to the host cell according to the present invention, wherein the antigen presenting cell is 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 the host cell and/or its culture medium.

The present invention further relates to an *in vitro* method for producing activated T-cells, 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 for a period of time sufficient to activate said T cells in an antigen specific manner, wherein said antigen is at least one peptide according to the present invention.

The present invention further relates to a method, wherein 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 present invention, wherein the antigen-presenting cell comprises an expression vector capable of expressing said peptide containing SEQ ID NO: 1 to SEQ ID NO: 549, or a variant amino acid sequence.

The present invention further relates to activated T cells, produced by the method according to the present invention, which selectively recognize 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 T-cell 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 said medicament is a vaccine, a cell, a cell population, such as, for example, a cell line, sTCRs and monoclonal antibodies.

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 present invention, wherein said cancer cells are cells of ovarian cancer.

The present invention further relates to particular marker proteins and biomarkers based on the peptides according to the present invention that can be used in the diagnosis and/or prognosis of ovarian cancer.

Furthermore, the present invention relates to the use of these novel targets for cancer treatment.

Further, the present invention relates to a method for producing a personalized anti-cancer vaccine for an individual patient using a database (herein designated also as “warehouse”) of pre-screened tumor associated peptides.

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).

Tremendous progress in the field of cancer immunotherapy during the last years has led to its wide appreciation as a potentially curative addition or alternative to standard chemotherapeutic approaches. Several papers demonstrate the importance of HLA

presented mutated and wild type tumor associated antigens as valuable tumor rejection antigens. Therefore, large scale identification of HLA presented cancer specific tumor antigens adds another important piece to the puzzle of our understanding how the immune system identifies and recognizes tumor cells.

In the present invention the inventors focused on epithelial ovarian cancer (EOC) with the goal to comprehensively characterize the immunopeptidome of EOC and evaluate the HLA presented antigens for their usefulness in clinical applications. So far, only few HLA presented antigens have been identified for EOC and most clinical studies have relied on predicted or established cancer testis antigens not necessarily also frequently presented by EOC, a fact that could be confirmed by our analysis.

The inventors demonstrate a consistent and high expression of HLA class I molecules on ovarian tumor cells in line with previously published data. Furthermore, the inventors show on a single cell level that EOC also display a strong expression of HLA-DR molecules. This strong expression was further underlined by our identification of large amounts of MHC class II ligands emanating from ovarian tumors as well as from highly enriched tumor cell fractions.

Profiling of the immunopeptidome of 34 ovarian tumors in comparison to more than 85 benign sources of different origin, revealed several hundred EOC associated antigens. Among the TOP100 HLA class I EOC antigens not presented on any of the tissues in our benign dataset MUC16 was clearly most exceptional. Concerning both the number of HLA ligands identified (> 80) and the frequency of presentation in the patient cohort (~80%) this is unprecedented for any other tumor antigen and tumor entity the inventors have investigated so far. Moreover, the inventors could establish that more than 70% of HLA ligands derived from MUC16 are immunogenic and able to prime T cells in healthy individuals rendering mucin 16 an unparalleled first-class antigen for EOC immunotherapy. Immunopeptidome profiling further provides a showcase for apparent mechanistic insights into EOC, which are reflected in the HLA ligandome of both HLA class I and class II ligands. HLA ligands from important kinases and phosphatases

(DDR1, EYA2), transcription factors (SOX9, SOX17), proteins associated with immunosuppression (IDO1, Galectin 1) as well as established and suspected molecular markers for EOC (MUC1, KLK10, FOLR1) are only a few to mention. Notably for HLA class II, mesothelin an established ligand of MUC16 has been identified as the TOP1 tumor associated antigen. Several studies have demonstrated the pivotal role of the MUC16/MSLN axis for cell invasion and metastasis in EOC as well as in other tumors such as pancreatic cancer or mesothelioma, suggesting that T-cell epitopes of these antigens should be further tested in other malignancies. The inventors could show that MSLN staining is directly correlated with MUC16 staining and high MSLN expression forms a negative prognostic factor in EOC.

For the first time several different benign tissues and cell types (PBMCs, bone marrow, liver, kidney, colon, ovary) have been used for this kind of selective immunopeptidome profiling. Due to restrictions in the number of different tissues available for investigation the inventors cannot completely exclude that individual antigens might also be presented by HLA molecules in other organs. The established functional relevance of those antigens for EOC and particularly the immunogenicity of the respective peptides in healthy individuals however, make a presentation of these antigens in other tissues unlikely.

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

and in case of MHC class II peptides (elongated variants of the peptides of the invention) they can be as long as 15, 16, 17, 18, 19 or 20 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 “the peptides of the present invention” shall also include the peptides consisting of or comprising a peptide as defined above according to SEQ ID NO: 1 to SEQ ID NO: 549.

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

Allele	Population	Calculated phenotype from allele frequency
A*02	Caucasian (North America)	49.1%

Allele	Population	Calculated phenotype from allele frequency
A*02	African American (North America)	34.1%
A*02	Asian American (North America)	43.2%
A*02	Latin American (North American)	48.3%
DR1	Caucasian (North America)	19.4%
DR2	Caucasian (North America)	28.2%
DR3	Caucasian (North America)	20.6%
DR4	Caucasian (North America)	30.7%
DR5	Caucasian (North America)	23.3%
DR6	Caucasian (North America)	26.7%
DR7	Caucasian (North America)	24.8%
DR8	Caucasian (North America)	5.7%
DR9	Caucasian (North America)	2.1%
DR1	African (North) American	13.20%
DR2	African (North) American	29.80%
DR3	African (North) American	24.80%
DR4	African (North) American	11.10%
DR5	African (North) American	31.10%
DR6	African (North) American	33.70%
DR7	African (North) American	19.20%
DR8	African (North) American	12.10%
DR9	African (North) American	5.80%
DR1	Asian (North) American	6.80%
DR2	Asian (North) American	33.80%
DR3	Asian (North) American	9.20%
DR4	Asian (North) American	28.60%
DR5	Asian (North) American	30.00%
DR6	Asian (North) American	25.10%
DR7	Asian (North) American	13.40%
DR8	Asian (North) American	12.70%
DR9	Asian (North) American	18.60%
DR1	Latin (North) American	15.30%
DR2	Latin (North) American	21.20%
DR3	Latin (North) American	15.20%
DR4	Latin (North) American	36.80%
DR5	Latin (North) American	20.00%
DR6	Latin (North) American	31.10%
DR7	Latin (North) American	20.20%
DR8	Latin (North) American	18.60%

Allele	Population	Calculated phenotype from allele frequency
DR9	Latin (North) American	2.10%
A*24	Philippines	65%
A*24	Russia Nenets	61%
A*24:02	Japan	59%
A*24	Malaysia	58%
A*24:02	Philippines	54%
A*24	India	47%
A*24	South Korea	40%
A*24	Sri Lanka	37%
A*24	China	32%
A*24:02	India	29%
A*24	Australia West	22%
A*24	USA	22%
A*24	Russia Samara	20%
A*24	South America	20%
A*24	Europe	18%

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

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 disclosed.

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.

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

In accordance with the present invention, the term “percent identity” or “percent identical”, when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the “Compared Sequence”) with the described or claimed sequence (the “Reference Sequence”). The percent identity is then determined according to the following formula:

percent identity = 100 [1 -(C/R)]
wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence, wherein
(i) each base or amino acid in the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and
(ii) each gap in the Reference Sequence and
(iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, constitutes a difference and
(iiii) the alignment has to start at position 1 of the aligned sequences;
and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

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

As mentioned above, the present invention thus provides a peptide comprising a sequence that is selected from the group of consisting of SEQ ID NO: 1 to SEQ ID NO: 549 or a variant thereof which is 88% homologous to SEQ ID NO: 1 to SEQ ID NO: 549, 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: 549. 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 549, by maintaining the known anchor

residues, and would be able to determine whether such variants maintain the ability to bind MHC class I or II molecules. The variants of the present invention retain the ability to bind to the TCR of activated T cells, which can subsequently cross-react with and kill cells that express a polypeptide containing the natural amino acid sequence of the cognate peptide as defined in the aspects of the invention.

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

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

Less conservative substitutions might involve the replacement of one amino acid by another that has similar characteristics but is somewhat different in size, such as replacement of an alanine by an isoleucine residue. Highly non-conservative replacements might involve substituting an acidic amino acid for one that is polar, or

even for one that is basic in character. Such “radical” substitutions cannot, however, be dismissed as potentially ineffective since chemical effects are not totally predictable and radical substitutions might well give rise to serendipitous effects not otherwise predictable from simple chemical principles.

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

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

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

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

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 6.

Table 6: Combinations of the elongations of peptides of the invention

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

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

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

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

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

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

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

recognized by T cells from more than one individual, at least two, and more preferably three individuals.

In a particularly preferred embodiment of the invention the peptide consists or consists essentially of an amino acid sequence according to SEQ ID NO: 1 to SEQ ID NO: 549.

“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 549 or a variant thereof contains additional N- and/or C-terminally located stretches of amino acids that are not necessarily forming part of the peptide that functions as an epitope for MHC molecules epitope.

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

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

In a reverse peptide bond amino acid residues are not joined by peptide (-CO-NH-) linkages but the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere et al (1997) (Meziere et al., 1997), incorporated herein by reference. This approach

involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Meziere et al. (Meziere et al., 1997) show that for MHC binding and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

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

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

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

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

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

Selective reduction of disulfide bonds in proteins is also common. Disulfide bonds can be formed and oxidized during the heat treatment of biopharmaceuticals. Woodward's Reagent K may be used to modify specific glutamic acid residues. N-(3-(dimethylamino)propyl)-N'-ethylcarbodiimide can be used to form intra-molecular crosslinks between a lysine residue and a glutamic acid residue. For example, diethylpyrocarbonate is a reagent for the modification of histidyl residues in proteins.

Histidine can also be modified using 4-hydroxy-2-nonenal. The reaction of lysine residues and other α-amino groups is, for example, useful in binding of peptides to surfaces or the cross-linking of proteins/peptides. Lysine is the site of attachment of poly(ethylene)glycol and the major site of modification in the glycosylation of proteins. Methionine residues in proteins can be modified with e.g. iodoacetamide, bromoethylamine, and chloramine T.

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

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

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

A peptide or variant, wherein the peptide is modified or includes non-peptide bonds is a preferred embodiment of the invention. Generally, peptides and variants (at least those containing peptide linkages between amino acid residues) may be synthesized by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lukas et al. (Lukas et al., 1981) and by references as cited therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is done using 20% piperidine in N, N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl

derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalizing agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N, N-dicyclohexyl-carbodiimide/1hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50 % scavenger mix. Scavengers commonly used include ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesized. Also a combination of solid phase and solution phase methodologies for the synthesis of peptides is possible (see, for example, (Bruckdorfer et al., 2004), and the references as cited therein).

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

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

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

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

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 TUMAPs recorded from ovarian cancer 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 ovarian cancer patients.

The discovery pipeline XPRESIDENT® v2.1 (see, for example, US 2013-0096016, which is hereby incorporated by reference in its entirety) allows the identification and selection of relevant over-presented peptide vaccine candidates based on direct relative quantitation of HLA-restricted peptide levels on cancer tissues in comparison to several different non-cancerous tissues and organs. This was achieved by the development of

label-free differential quantitation using the acquired LC-MS data processed by a proprietary data analysis pipeline, combining algorithms for sequence identification, spectral clustering, ion counting, retention time alignment, charge state deconvolution and normalization.

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

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

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

The present invention provides peptides that are useful in treating cancers/tumors, preferably ovarian cancer 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 ovarian cancer 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 ovarian tissue cells or other normal tissue cells, demonstrating a high degree of tumor association of the source genes. Moreover, the peptides themselves are strongly over-presented on tumor tissue – “tumor tissue” in relation to this invention shall mean a sample from a patient suffering from ovarian cancer, but not on normal tissues.

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

The present description further relates to T-cell receptors (TCRs) comprising an alpha chain and a beta chain (“alpha/beta TCRs”). Also provided are HAVCR1-001 peptides capable of binding to TCRs and antibodies when presented by an MHC molecule. The

present description also relates to nucleic acids, vectors and host cells for expressing TCRs and peptides of the present description; and methods of using the same.

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

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

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

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

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

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

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

As used herein in connect with TCRs of the present description, “specific binding” and grammatical variants thereof are used to mean a TCR having a binding affinity (KD) for an HAVCR1-001 peptide-HLA molecule complex of 100 µM or less.

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

With or without the introduced inter-chain bond mentioned above, alpha/beta hetero-dimeric 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, an inventive 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 an inventive peptide 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/ inventive 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 TCRαβ gene loci (1.1 and 0.7 Mb), whose T-cells express a diverse human TCR repertoire that compensates for mouse TCR deficiency, immunizing the mouse with an inventive peptide, incubating PBMCs obtained from the transgenic mice with tetramer-phycoerythrin (PE), and isolating the high avidity T-cells by fluorescence activated cell sorting (FACS)–Calibur analysis.

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

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

To increase the expression, nucleic acids encoding TCRs of the present description may be operably linked to strong promoters, such as retroviral long terminal repeats (LTRs), cytomegalovirus (CMV), murine stem cell virus (MSCV) U3, phosphoglycerate

kinase (PGK), β-actin, ubiquitin, and a simian virus 40 (SV40)/CD43 composite promoter, elongation factor (EF)-1a and the spleen focus-forming virus (SFFV) promoter. In a preferred embodiment, the promoter is heterologous to the nucleic acid being expressed.

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

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

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

Nucleic acids encoding TCRs of the present description may be codon optimized to increase expression from a host cell. Redundancy in the genetic code allows some amino acids to be encoded by more than one codon, but certain codons are less "op-timal" than others because of the relative availability of matching tRNAs as well as other factors (Gustafsson et al., 2004). Modifying the TCR-alpha and TCR-beta gene

sequences such that each amino acid is encoded by the optimal codon for mammalian gene expression, as well as eliminating mRNA instability motifs or cryptic splice sites, has been shown to significantly enhance TCR-alpha and TCR-beta gene expression (Scholten et al., 2006).

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

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

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

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

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

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

Preferably, the medicament of the present invention is an immunotherapeutics 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. 549, and at least one additional peptide, preferably two to 50, more preferably two to 25, even more preferably two to 20 and most preferably two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen or eighteen peptides. The peptide(s) may be derived from one or more specific TAAs and may bind to MHC class I molecules.

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

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

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

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

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

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend

upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

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

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

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

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

A typical mammalian cell vector plasmid for constitutive expression comprises the CMV or SV40 promoter with a suitable poly A tail and a resistance marker, such as neomycin. One example is pSVL available from Pharmacia, Piscataway, NJ, USA. An example of

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

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

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

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

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

and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

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

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

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

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

In another embodiment the peptide, the nucleic acid or the expression vector of the invention are used in medicine. For example, the peptide or its variant may be prepared for intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, intramuscular (i.m.) injection. Preferred methods of peptide injection include s.c., i.d., i.p., i.m., and i.v. Preferred methods of DNA injection include i.d., i.m., s.c., i.p. and i.v. Doses of e.g. between 50 µg and 1.5 mg, preferably

125 µg to 500 µg, of peptide or DNA may be given and will depend on the respective peptide or DNA. Dosages of this range were successfully used in previous trials (Walter et al., 2012).

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

The medicament of the invention may also include one or more adjuvants. Adjuvants are substances that non-specifically enhance or potentiate the immune response (e.g., immune responses mediated by CD8-positive T cells and helper-T (TH) cells to an antigen, and would thus be considered useful in the medicament of the present invention. Suitable adjuvants include, but are not limited to, 1018 ISS, aluminum salts, AMPLIVAX®, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, flagellin or TLR5 ligands derived from flagellin, FLT3 ligand, GM-CSF, IC30, IC31, Imiquimod (ALDARA®), resiquimod, ImuFact IMP321, Interleukins as IL-2, IL-13, IL-21, Interferon-alpha or -beta, or pegylated derivatives thereof, IS Patch, ISS, ISCOMATRIX, ISCOMs, JuvImmune®, LipoVac, MALP2, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, water-in-oil and oil-in-water emulsions, OK-432, OM-174, OM-197-MP-EC, ONTAK, OspA, PepTel® vector system, poly(lactid co-glycolid) [PLG]-based and dextran microparticles, talactoferrin SRL172,

Virosomes and other Virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox, Quil, or Superfos. Adjuvants such as Freund's or GM-CSF are preferred. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described previously (Allison and Krummel, 1995). Also cytokines may be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-), accelerating the maturation of dendritic cells into efficient antigen-presenting cells for T-lymphocytes (e.g., GM-CSF, IL-1 and IL-4) (U.S. Pat. No. 5,849,589, specifically incorporated herein by reference in its entirety) and acting as immunoadjuvants (e.g., IL-12, IL-15, IL-23, IL-7, IFN-alpha. IFN-beta) (Gabrilovich et al., 1996).

CpG immunostimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a vaccine setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non-adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen-specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses, dendritic cell vaccines, autologous cellular vaccines and polysaccharide conjugates in both prophylactic and therapeutic vaccines. More importantly it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of TH1 cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T cell help. The TH1 bias induced by TLR9 stimulation is maintained even in the presence of vaccine adjuvants such as alum or incomplete Freund’s adjuvant (IFA) that normally promote a TH2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nanoparticles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enable the antigen doses to be reduced by approximately two orders of magnitude, with comparable antibody responses to the full-dose vaccine without CpG in some

experiments (Krieg, 2006). US 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen to induce an antigen-specific immune response. A CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen (Berlin, Germany) which is a preferred component of the pharmaceutical composition of the present invention. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 may also be used.

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

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

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

In a preferred embodiment, the pharmaceutical composition according to the invention the adjuvant is selected from the group consisting of colony-stimulating factors, such as

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

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

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

biomarker assessments for antigen expression, but it is still ensured that several targets are simultaneously attacked by the induced immune response, which is important for efficacy (Banchereau et al., 2001; Walter et al., 2012).

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

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

Each scaffold can comprise a labeling 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 labeled with a fluorescent dye or any other applicable cellular marker molecule. Such marker molecules are well known in the art. For example a fluorescence-labeling, for example provided by a fluorescence dye, can provide a visualization of the bound aptamer by fluorescence or laser scanning microscopy or flow cytometry.

Each scaffold can be conjugated with a second active molecule such as for example IL-21, anti-CD3, anti-CD28.

For further information on polypeptide scaffolds see for example the background section of WO 2014/071978A1 and the references cited therein.

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

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

DNA aptamers can be selected to reveal broad-spectrum recognition properties for various cancer cells, and particularly those derived from solid tumors, while non-tumorigenic and primary healthy cells are not recognized. If the identified aptamers

recognize not only a specific tumor sub-type but rather interact with a series of tumors, this renders the aptamers applicable as so-called broad-spectrum diagnostics and therapeutics.

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

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

Aptamers can be selected against complex targets such as cells and tissues and complexes of the peptides comprising, preferably consisting of, a sequence according to any of SEQ ID NO 1 to SEQ ID NO 549, according to the invention at hand with the MHC molecule, using the cell-SELEX (Systematic Evolution of Ligands by Exponential enrichment) technique.

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

Therefore, it is a further aspect of the invention to provide a method for producing a recombinant antibody specifically binding to a human major histocompatibility complex (MHC) class I or II being complexed with a HLA-restricted antigen, the method comprising: immunizing a genetically engineered non-human mammal comprising cells expressing said human major histocompatibility complex (MHC) class I or II with a soluble form of a MHC class I or II molecule being complexed with said HLA-restricted

antigen; isolating mRNA molecules from antibody producing cells of said non-human mammal; producing a phage display library displaying protein molecules encoded by said mRNA molecules; and isolating at least one phage from said phage display library, said at least one phage displaying said antibody specifically binding to said human major histocompatibility complex (MHC) class I or II being complexed with said HLA-restricted antigen.

It is a further aspect of the invention to provide an antibody that specifically binds to a human major histocompatibility complex (MHC) class I or II being complexed with a HLA-restricted antigen, wherein the antibody preferably is a polyclonal antibody, monoclonal antibody, bi-specific antibody and/or a chimeric antibody.

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

Preferably, the antibody is binding with a binding affinity of below 20 nanomolar, preferably of below 10 nanomolar, to the complex, which is 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: 549, or a variant thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 549 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: 549 or a variant

thereof which is at least 88% homologous (preferably identical) to SEQ ID NO: 1 to SEQ ID NO: 549, 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: 549.

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.

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: 48 and has been synthetically produced (e.g. synthesized) as a pharmaceutically acceptable salt. Methods to synthetically produce peptides are well known in the art. The salts of the peptides according to the present invention differ substantially from the peptides in their state(s) *in vivo*, as the peptides as generated *in vivo* are no salts. The non-natural salt form of the peptide mediates the solubility of the peptide, in particular in the context of pharmaceutical compositions comprising the peptides, e.g. the peptide vaccines as disclosed herein. A sufficient and at least substantial solubility of the peptide(s) is required in order to efficiently provide

the peptides to the subject to be treated. Preferably, the salts are pharmaceutically acceptable salts of the peptides. These salts according to the invention include alkaline and earth alkaline salts such as salts of the Hofmeister series comprising as anions PO_4^{3-}, SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3^-, ClO_4^-, I^-, SCN^- and as cations NH_4^+, Rb^+, K^+, Na^+, Cs^+, Li^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+}. Particularly salts are selected from $(NH_4)_3PO_4$, $(NH_4)_2HPO_4$, $(NH_4)H_2PO_4$, $(NH_4)_2SO_4$, NH_4CH_3COO, NH_4Cl, NH_4Br, NH_4NO_3, NH_4ClO_4, NH_4I, NH_4SCN, Rb_3PO_4, Rb_2HPO_4, RbH_2PO_4, Rb_2SO_4, Rb_4CH_3COO, Rb_4Cl, Rb_4Br, Rb_4NO_3, Rb_4ClO_4, Rb_4I, Rb_4SCN, K_3PO_4, K_2HPO_4, KH_2PO_4, K_2SO_4, KCH_3COO, KCl, KBr, KNO_3, $KClO_4$, KI, KSCN, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, Na_2SO_4, $NaCH_3COO$, NaCl, NaBr, $NaNO_3$, $NaClO_4$, NaI, NaSCN, $ZnCl_2$ Cs_3PO_4, Cs_2HPO_4, CsH_2PO_4, Cs_2SO_4, $CsCH_3COO$, CsCl, CsBr, $CsNO_3$, $CsClO_4$, CsI, CsSCN, Li_3PO_4, Li_2HPO_4, LiH_2PO_4, Li_2SO_4, $LiCH_3COO$, LiCl, LiBr, $LiNO_3$, $LiClO_4$, LiI, LiSCN, Cu_2SO_4, $Mg_3(PO_4)_2$, Mg_2HPO_4, $Mg(H_2PO_4)_2$, Mg_2SO_4, $Mg(CH_3COO)_2$, $MgCl_2$, $MgBr_2$, $Mg(NO_3)_2$, $Mg(ClO_4)_2$, MgI_2, $Mg(SCN)_2$, $MnCl_2$, $Ca_3(PO_4)$,, Ca_2HPO_4, $Ca(H_2PO_4)_2$, $CaSO_4$, $Ca(CH_3COO)_2$, $CaCl_2$, $CaBr_2$, $Ca(NO_3)_2$, $Ca(ClO_4)_2$, CaI_2, $Ca(SCN)_2$, $Ba_3(PO_4)_2$, Ba_2HPO_4, $Ba(H_2PO_4)_2$, $BaSO_4$, $Ba(CH_3COO)_2$, $BaCl_2$, $BaBr_2$, $Ba(NO_3)_2$, $Ba(ClO_4)_2$, BaI_2, and $Ba(SCN)_2$. Particularly preferred are NH acetate, $MgCl_2$, KH_2PO_4, Na_2SO_4, KCl, NaCl, and $CaCl_2$, such as, for example, the chloride or acetate (trifluoroacetate) salts.

Generally, peptides and variants (at least those containing peptide linkages between amino acid residues) may be synthesized by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lukas et al. (Lukas et al., 1981) and by references as cited therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is done using 20% piperidine in N, N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made

of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalizing agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N, N-dicyclohexyl-carbodiimide/1hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrine, 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. acetonitril/water gradient separation.

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 ovarian cancer.

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: 549 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 ovarian cancer cells or other solid or hematological tumor cells such as pancreatic cancer, brain cancer, kidney cancer, colon or rectal cancer, leukemia.

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 ovarian cancer. 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 ovarian cancer marker polypeptide, delivery of a toxin to a ovarian cancer cell expressing a cancer marker gene at an increased level, and/or inhibiting the activity of a ovarian cancer 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 ovarian cancer 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: 549, 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 ovarian cancer marker polypeptide used to generate the antibody according to the invention.

One of skill in the art will realize that the generation of two or more different sets of monoclonal or polyclonal antibodies maximizes the likelihood of obtaining an antibody with the specificity and affinity required for its intended use (e.g., ELISA, immunohistochemistry, *in vivo* imaging, immunotoxin therapy). The antibodies are tested for their desired activity by known methods, in accordance with the purpose for which the antibodies are to be used (e.g., ELISA, immunohistochemistry,

immunotherapy, etc.; for further guidance on the generation and testing of antibodies, see, e.g., Greenfield, 2014 (Greenfield, 2014)). For example, the antibodies may be tested in ELISA assays or, Western blots, immunohistochemical staining of formalin-fixed lung cancers or frozen tissue sections. After their initial *in vitro* characterization, antibodies intended for therapeutic or *in vivo* diagnostic use are tested according to known clinical testing methods.

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

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

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

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

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

The antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab' or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired

specificity, affinity and capacity. In some instances, Fv framework (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues, which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

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

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

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

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

Effective dosages and schedules for administering the antibodies may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of antibodies that must be administered will vary depending on, for example, the subject that will receive the antibody, the route of administration, the particular type of antibody used and other drugs being administered. A typical daily dosage of the antibody used alone might range from about 1 (µg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. Following administration of an antibody, preferably for treating ovarian cancer, 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 lung cancer in a subject receiving treatment may be monitored using standard tumor imaging techniques. A therapeutically-administered antibody that arrests tumor growth, results in

tumor shrinkage, and/or prevents the development of new tumors, compared to the disease course that would occurs in the absence of antibody administration, is an efficacious antibody for treatment of lung cancer.

It is a further aspect of the invention to provide a method for producing a soluble T-cell receptor (sTCR) recognizing a specific peptide-MHC complex. Such soluble T-cell receptors can be generated from specific T-cell clones, and their affinity can be increased by mutagenesis targeting the complementarity-determining regions. For the purpose of T-cell receptor selection, phage display can be used (US 2010/0113300, (Liddy et al., 2012)). For the purpose of stabilization of T-cell receptors during phage display and in case of practical use as drug, alpha and beta chain can be linked e.g. by non-native disulfide bonds, other covalent bonds (single-chain T-cell receptor), or by dimerization domains (Boulter et al., 2003; Card et al., 2004; Willcox et al., 1999). The T-cell receptor can be linked to toxins, drugs, cytokines (see, for example, US 2013/0115191), domains recruiting effector cells such as an anti-CD3 domain, etc., in order to execute particular functions on target cells. Moreover, it could be expressed in T cells used for adoptive transfer. Further information can be found in WO 2004/033685A1 and WO 2004/074322A1. A combination of sTCRs is described in WO 2012/056407A1. Further methods for the production are disclosed in WO 2013/057586A1.

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

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

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

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

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

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

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

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

If an antigen-presenting cell is transfected to express such an epitope, preferably the cell comprises an expression vector capable of expressing a peptide containing SEQ ID NO: 1 to SEQ ID NO: 549, or a variant amino acid sequence thereof.

A number of other methods may be used for generating T cells in vitro. For example, autologous tumor-infiltrating lymphocytes can be used in the generation of CTL. Plebanski et al. (Plebanski et al., 1995) made use of autologous peripheral blood lymphocytes (PLBs) in the preparation of T cells. Furthermore, the production of autologous T cells by pulsing dendritic cells with peptide or polypeptide, or via infection with recombinant virus is possible. Also, B cells can be used in the production of autologous T cells. In addition, macrophages pulsed with peptide or polypeptide, or infected with recombinant virus, may be used in the preparation of autologous T cells. S. Walter et al. (Walter et al., 2003) describe the in vitro priming of T cells by using artificial antigen presenting cells (aAPCs), which is also a suitable way for generating T cells against the peptide of choice. In the present invention, aAPCs were generated by the coupling of preformed MHC:peptide complexes to the surface of polystyrene

particles (microbeads) by biotin:streptavidin biochemistry. This system permits the exact control of the MHC density on aAPCs, which allows to selectively elicit high- or low-avidity antigen-specific T cell responses with high efficiency from blood samples. Apart from MHC:peptide complexes, aAPCs should carry other proteins with co-stimulatory activity like anti-CD28 antibodies coupled to their surface. Furthermore such aAPC-based systems often require the addition of appropriate soluble factors, e. g. cytokines, like interleukin-12.

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

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

Activated T cells, which are produced by the above method, will selectively recognize a cell that aberrantly expresses a polypeptide that comprises an amino acid sequence of SEQ ID NO: 1 to SEQ ID NO 549.

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 normal levels of expression 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).

Because the underlying polypeptides of the peptides of the invention as mentioned in the Tables above are highly expressed in ovarian cancer, and are expressed at rather to extremely low levels in normal cells, targeting peptides derived from the protein products of the following genes may preferably be integrated into a therapeutic strategy:

The present invention further provides a medicament that is useful in treating cancer, in particular ovarian cancer and other malignancies.

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 ovarian cancer, the medicament of the invention is preferably used to treat ovarian cancer.

The present invention further includes 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 ovarian cancer 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 ovarian cancer tissues, the warehouse may contain HLA-A*02 and HLA-A*24 as well as HLAs with smaller abundance 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 immuno-competence 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, ovarian cancer 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 (ovarian cancer) compared with a range of normal organs and tissues
3. Identified HLA ligands were compared to gene expression data. Peptides over-presented or selectively presented on tumor tissue, preferably encoded by selectively expressed or over-expressed genes as detected in step 2 were considered suitable TUMAP candidates for a multi-peptide vaccine.
4. Literature research was performed in order to identify additional evidence supporting the relevance of the identified peptides as TUMAPs
5. The relevance of over-expression at the mRNA level was confirmed by redetection of selected TUMAPs from step 3 on tumor tissue and lack of (or infrequent) detection on healthy tissues.
6. In order to assess, whether an induction of *in vivo* T-cell responses by the selected peptides may be feasible, in vitro immunogenicity assays were performed using human T cells from healthy donors as well as from ovarian cancer 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 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 ovarian cancer 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 ovarian cancer. Presence of groups of peptides can enable classification or sub-classification of diseased tissues.

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

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

The present invention will now be described in the following examples, which describe preferred embodiments thereof, nevertheless, without being limited thereto. For the

purposes of the present invention, all references as cited herein are incorporated by reference in their entireties.

FIGURES

Figure 1 shows the HLA-A,B,C (a) and HLA-DR (b) expression of different cell subsets within ovarian cancer and benign ovarian tissue. For Figure 1 the two-tailed unpaired Student's t-test with Welch's correction was used owing to unequal variance between the two comparison groups. HLA class I (A) and HLA-DR (B) expression on different cell types within EOC and benign ovarian tissue after enzymatic dissociation characterized by distinct cell surface markers (leukocyte compartments: CD45+, tumor cells/epithelial cell compartments: CD45-EpCam+, endothelial cell compartments: CD45-CD31+). Each data point represents the mean of triplicate experiments performed for each sample. Two sided t-tests were used to test for significance (*$p<0.05$; ** $p<0.01$).

Figures 2A to D show the comparative profiling of the immunopeptidome of EOC vs. benign tissues. (A) Comparative profiling of HLA class I ligand source proteins represented in EOC (n=34) and benign tissues. The frequency of HLA restricted presentation of source proteins is indicated on the y-axis separately for EOC (above x-axis) and benign sources (below x-axis). The source proteins were ranked (from left to right) according to their frequency of EOC specific presentation. The box on the left side highlights the TOP100 HLA ligand source proteins exclusively presented by EOC. (B) Word cloud of the TOP 100 EOC specific HLA class I ligand source proteins (uniprot recommended gene name). Font size (5-26) correlates with absolute number of cancer patients presenting HLA ligands of respective source proteins. (C) Comparative profiling of HLA class II ligand source proteins represented in EOC (n=22) and benign tissues. (D) Word cloud of the TOP 100 EOC specific HLA class II ligand source proteins (uniprot recommended gene name). Font size (3-11) correlates with absolute number of cancer patients presenting HLA ligands of respective source proteins.

Figure 3 shows the cellular origin of the TOP100 EOC associated HLA class I ligands. Volcano plots of the relative abundance of HLA ligands in the class I immunopeptidome

of enriched cell populations of OvCa 84 analyzed by label free quantitation. Panels show on the left side (A) tumor infiltrating leukocytes (CD45+) vs. tumor cells (CD45-Epcam+) and on the right side (B) stroma cells (CD45-EpCam-) vs. tumor cells. The horizontal dashed line indicates significance threshold ($p < 0.05$). TOP100 EOC exclusive ligands (MUC16 (red), DDR1, EYA2, SOX9, TLR7, OASL) as well as ligands derived from leukocyte associated antigens (CD132, CD8, LSP1) and stroma (endothelial cell) associated antigens (vWF) are highlighted.

Figure 4 shows the immunohistochemical staining and serum levels as surrogate markers for ligand presentation. Immunohistochemical staining of high-grade serous ovarian carcinomas for MUC16 (CA-125) with low (IRS4), intermediate (IRS6) and high (IRS12) immunoreactivity score (A). Immunohistochemical staining for Mesothelin (right, IRS8) and IDO1 (left, IRS 12; all at 200 x magnification) (B). Correlation of HLA ligand presentation and source protein expression of selected TOP100 EOC associated antigens. Expression of MUC16 (n=23), IDO1 (n=23) and MSLN (n=16) was analyzed by immunohistochemical staining (C) or serum marker analysis of CA-125 (n=30) at the day of surgery (D). For MSLN only the cases for which HLA class II immunopeptidome data were available were included. Non parametric Mann-Whitney test was employed to test for statistical significance ($p<0.05$ was considered significant).

Figure 5 shows the prognostic relevance of MUC16 and MSLN. Immunohistochemical stainings were performed on TMAs with 71 high-grade serous EOC samples from patients with documented optimal tumor debulking. (A) Kaplan Meier plot depicting the influence of MUC16 expression (left panel, low expression score < 7, n=41; high expression score ≥ 7, n=30) and MSLN expression (right panel, low expression < 6, n=15; high expression ≥ 6 , n=52) on overall survival. (B) Impact of CD3 T-cell infiltration into the intraepithelial compartment (left panel CD3E, low infiltration < 7 cell/HPF, n=13; high infiltration ≥ 7, n=57) or the fibrovascular stroma (right panel, CD3S, low infiltration < 7 cell/HPF, n=40; high infiltration ≥ 7, n=30) on overall survival of patients. (C) Subgroup analysis of combined CD3 and MLN staining (all scoring cutoffs as above) for intraepithelial CD3 T-cells (top panel, low MSLN/high CD3E, n=11;

low MSLN/low CD3E, n=40; high MSLN/low CD3E, n=14; high MSLN/high CD3E, n=1) or fibrovascular CD3 T-cells (bottom panel, low MSLN/high CD3S, n=30; high MSLN/low CD3S, n=7; low MSLN/low CD3S, n=21; high MSLN/high CD3S, n=8).

Figure 6 shows the flow cytometric analysis of EOC and benign ovarian tissue. Exemplary presentation of the gating strategy for OvCa 48 showing the selection of CD45+ leukocytes, CD45-CD31+ endothelial cells and CD45-EpCam+ tumor or epithelial cells.

Figure 7 shows the saturation analysis of HLA ligand source protein identifications for EOC. Saturation analysis for identifications of source proteins is depicted separately for HLA class I (A) and HLA class II (B) ligand proteins. The mean number of unique source proteins has been calculated for each source count by 1000 random samplings from the 34 EOC sources. Exponential regression was used to determine the calculated maximal attainable coverage of source protein accession (dotted lines) for EOC.

Figure 8 shows the frequency and number of HLA ligand presentation among EOC samples. HLA presentation of selected EOC associated antigens as well as the number of different HLA presented peptides (color coding) is visualized for each individual EOC (patient number on top of each column) both for class I (top) and class II (bottom) antigens.

EXAMPLES

Materials and methods

Tissue samples

All tissue samples were collected at the University Hospital of Tübingen after obtaining patient informed consent in accordance with the principles of the Declaration of Helsinki. All study protocols were approved by the local institutional review board. If not stated otherwise samples were stored at -80oC until further usage. Two-digit HLA typing was

performed by sequence specific primer (SSP) PCR using the HLA-Ready Gene System (Innotrain, Kronberg,Germany) and evaluated by SCORE Software (Olerup, Stockholm, Sweden) at the Department of Transfusion Medicine of the University Hospital of Tübingen. High resolution four-digit HLA typing was performed by next generation sequencing on a GS Junior Sequencer using the GS GType HLA Primer Sets (both Roche, Basel, Switzerland). Normal tissues were obtained from Bio-Options Inc, CA, USA; BioServe, Beltsville, MD, USA; Capital BioScience Inc, Rockville, MD, USA; Geneticist Inc., Glendale, CA, USA; University Hospital of Geneva; University Hospital of Heidelberg; University Hospital Munich; ProteoGenex Inc., Culver City, CA, USA; University Hospital of Tübingen. Written informed consents of all patients had been given before surgery or autopsy. Tissues were shock-frozen immediately after excision and stored until isolation of TUMAPs at -70°C or below.

Tissue dissociation

EOC as well as benign ovary and fallopian tube tissues were freshly collected from patients undergoing tumor resection/debulking or salpingoophorectomy. Tissues were minced into small pieces < 2 mm^3 and transferred into an enzymatic dissociation solution containing 400 U/ml Collagenase Type IV, 5 U/ml Dispase (both life technologies, Carlsbad, CA) and 0.1mg/ml DNAse (Roche, Basel, Switzerland) in DMEM (life technologies) with 10% fetal calf serum (Lonza, Basel, Switzerland). Dissociation was performed on a rotating shaker (Infors HT, Basel, Switzerland) for 3 hours at 37°C. Remaining tissue fragments (typically < 1% of initial weight) were removed using a 100 µm cell strainer (BD, Franklin Lakes, NJ). Single cell suspensions were washed twice with PBS and erythrocytes were lysed using ammonium chloride lysis buffer.

HLA surface molecule quantification

HLA surface expression was determined using QIFIKIT quantification flow cytometric assay (Dako, Glostrup, Denmark) according to manufacturer's instructions. Cells were stained with either pan-HLA class I specific monoclonal antibody W6/32, HLA-DR specific L243 or respective isotype control. Discrimination of cell types was based on

surface marker staining with fluorescently labeled antibodies directed against CD45 (AmCyan clone 2D1, BD), CD31 (PeCy7, clone WM59, Biolegend, San Diego, CA), EpCam (APC, clone HEA125, Miltenyi, Bergisch-Gladbach, Germany) and CD34 (APCCy7,clone 581, Biolegend). 7-AAD (BioLegend) was added as viability marker immediately before analysis on a LSR SORP Fortessa instrument (BD). Triplicates were recorded for each sample with median fluorescence intensities used for calculation of surface molecule expression.

Cell separation:

Cell separation was performed using two consecutive magnetic activated cell separation (MACS) protocols according to manufacturer's instructions (Miltenyi). Separations were performed using XS columns and a superMACS separator (both Miltenyi). The first separation aimed at positive selection of $CD45^+$ leukocytes. The negative fraction was subsequently enriched for $EpCam^+$ tumor cells. The remaining $CD45^-$ $EpCam^-$ fraction was assumed to represent the stroma cell fraction.

HLA ligand isolation

HLA class I and II molecules were isolated by standard immunoaffinity purification as described previously [42]. Pan-HLA class I specific mAb W6/32 was employed for HLA class I isolation and pan-HLA class II mAb Tü39 as well as HLA-DR specific mAb L243 were used for HLA class II isolation.

Immunopeptidome analysis by LC-MS/MS

Immunopeptidome analysis was performed on an LTQ OrbitrapXL mass spectrometer (Thermo Fisher, Waltham, MA) equipped with a nanoelectron spray ion source and coupled to an Ultimate 3000 RSLC Nano UHPLC System (Dionex, Sunnyvale, CA). Peptide samples were loaded with 3% of solvent B (20% H_2O, 80% acetonitrile and 0.04% formic acid) on a 2 cm PepMap 100 C18 Nanotrap column (Dionex) at a flow rate of 4 µL/min for 10 min. Separation was performed on a 50 cm PepMap C18 column with a particle size of 2 µm (Dionex) mounted in a column oven running at 50°C. The applied gradient ranged from 3 to 30% solvent B within 140 min at a flow rate of 175 nil/min.

(Solvent A: 99% H_2O, 1% ACN and 0.1% formic acid; Solvent B: 20% H2O, 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). Survey scans were recorded in the Orbitrap at a resolution of 60,000. 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 possible charge states 2+ and 3+ selected for fragmentation. For HLA class II mass range was set to 300-1500 m/z allowing for fragmentation with positive charge states ≥ 2.

HLA class I samples were analyzed in 5 technical replicates while for HLA class II samples 3 technical replicates were typically acquired. Initial runs were performed without dynamic exclusion, whereas for consecutive runs a dynamic exclusion of 5s was enabled.

Mass spectrometry data processing and analysis

MS data analysis was carried out using Proteome discoverer 1.3 (ThermoFisher). Peak lists were searched against the human proteome as comprised in the Swiss-Prot database (www.uniprot.org, released September 27th 2013; including 20,279 reviewed protein sequences) using Mascot search engine (Mascot 2.2.04, Matrix Science, Boston, MA). Mass tolerance for processing was 5 ppm for precursor ions and 0.5 Da for fragment ions. No cleavage specificity was selected and the only dynamic modification allowed was oxidized methionine. Peptide confidence was determined using percolator algorithm with a target value of q≤0.05 (5% FDR). Additional post processing filters were a Mascot Ionscore ≥ 20, search engine rank = 1 and peptide length of 8-12 amino acids for HLA class I ligands and 12-25 amino acids for HLA class II ligands. Protein grouping was disabled to ensure multiple annotations of peptides, if sequences map into multiple proteins due to conservation. HLA annotation was performed using HLA prediction algorithms hosted at SYFPEITHI (www.syfpeithi.de) and NETMHC 3.4 (http://www.cbs.dtu.dk/services/NetMHC/). In case of ambiguous

results multiple alleles are mentioned. For comparative profiling “one hit wonders” i.e. peptides only presented on one source with a PSM count ≤ 5 were removed from both of the datasets.

Label free quantitation of peptides on tumor vs. $CD45^+$ and tumor vs. stroma cells was performed using Sieve 2.1 (Thermo Fisher). At least 3 replicates of MS raw files for each cell enriched fraction as well as results from whole tissue MHC precipitations were aligned altogether with a maximum retention time (RT) shift of 2.5 mins. Frames were generated based on MS^2 scan events with a maximum RT width of 3.5 mins and 5 ppm mass tolerance. Identifications were imported from Proteome discoverer using Mascot search results (see above). Total ion current chromatogram normalization was used to accommodate for differences in sample intensities.

Immunogenicity analysis of HLA class I ligands

Priming of peptide specific cytotoxic lymphocytes (CTLs) was conducted using an established protocol involving artificial antigen presenting cells (aAPCs) (30). aAPCs consisted of streptavidin-coated polystyrene beads (5.6 µm in diameter; Bangs Laboratories, Fishers, IN). Beads were resuspended at $2x10^6$ particles per ml and incubated with 10 nM biotinylated peptide-MHC complexes and 10 nM stimulating anti-CD28 antibody (clone 9.3 derived from ATCC, Manassas, VA) each for 30 min at ambient temperature. T cells were isolated from whole blood of healthy donors using a CD8 magnetic cell isolation kit (Miltenyi). One million T-cells per well were cultured in 96 well plates (Corning, Corning, NY, USA) and stimulated with the same number of loaded aAPCs in the presence of 5 ng/ml IL-12 (PromoCell, Heidelberg, Germany). T cells were stimulated 3 times in total with weekly stimulation interval. 40 U/ml IL-2 was added 2 days subsequent to each stimulation. T-cell priming was assessed by MHC-multimer staining one week after the last stimulation round.

Construction of tissue microarrays (TMA)

Consecutive paraffin embedded tumor samples of patients with high-grade serous carcinoma of the ovary or fallopian tube (EOC) with at least FIGO stage II-III and

operated at the University Women's Hospital in Tübingen between 1999 and 2008 were retrieved from the archives of the Institute of Pathology. After confirmation of histological subtype and grading according to published criteria (43). 154 cases were initially included in the study. A tissue microarray (TMA) was constructed as described previously (44). We used six cores of 0.6 mm diameter of each patient (maximum three cores each from two different sites of the primary tumors – at least two separate cores). In addition we constructed a TMA using paraffin embedded tissue from the primary tumors of the prospectively collected cases for ligandome analysis. 3 µm thick sections were cut, rehydrated and subjected to specific pretreatment for immunohistochemistry. In total 23 cases were evaluable for immunoscoring and correlation with immunopeptidome data.

Immunohistochemistry

The following primary antibodies and dilutions were used for immunohistochemistry: CD3 (1:100, rat monoclonal SP7, DCS, Hamburg, Germany), CD8 (1:200, mouse monoclonal C8/144B, DAKO), MUC16 (1:450, mouse monoclonal M11, DAKO, Glostrup, Denmark), IDO1 (1:25, mouse monoclonal, ABCAM, Cambridge, UK) and MSLN (1:100, mouse monoclonal SPM143, GeneTex, Irvine, CA, USA). The tissue sections were pre-treated with EDTA-buffer solution (pH 8.6) at 95°C for 36 min. Immunohistochemical staining was performed on an automated immunostainer according to the manufacturer's instructions using the iView DAB detection kit (both Ventana, Tucson, AZ, USA).

Immunoscoring

Quantification of TILs was carried out by first assessing the average number of immunostained cells per high power field (HPF=400x) by counting at least 2 HPF for each core. In a second step, the average number of lymphocytes per HPF for the left and right triple core set was calculated, and for all cores together. This bilateral average count was used for further calculations. The fibro vascular tumor stroma (CD3S and CD8S), and the intraepithelial compartment of the tumor (CD3E and CD8E) were evaluated separately.

For expression of CA 125, IDO1 and MSLN staining intensity was graded from 0-3, multiplied by a score from 1-4 for the percentage of tumor cells (1: 0-10%; 2: 10-50%; 3: 50-80%; 4: 80-100%). For all parameters the cases were separated in quartiles and the best separation between two quartiles defined as cut-off value between high and low expression. Of the 154 cases on the TMA 71 patients had undergone documented optimal tumor debulking (< 1cm residual tumor mass) and could be successfully evaluated for TILs and expression of proteins. Immunoscoring and clinical data analysis were performed by independent investigators.

Statistical analysis / Visualization

If not mentioned otherwise all figures and statistical analyses were generated using Graphpad Prism 6.0 (Graphpad software, La Jolla, CA, USA) or Microsoft Office 2010 (Microsoft). Word clouds were created using an online applet (www.wordle.net). Kaplan-Meier analysis was performed using SPSS statistical software (Version 21, IBM Corp., Armonk, NY, USA). Two-tailed unpaired student's t-test was performed unless otherwise specified. P values less than 0.05 were considered statistically significant. D'Agostino-Pearson omnibus test was used to verify normality and the F-Test was used to verify equal variance. For Figure 1 the two-tailed unpaired Student's t-test with Welch's correction was used owing to unequal variance between the two comparison groups. Non-parametric Mann-Whitney-test was used in Figure 4 because normal distribution could not be assessed in all cases due to small sample sizes. Spearman correlation was used to correlate IHC scores of MSLN and MUC16 as the datasets were not showing normal distribution. P values comparing two Kaplan-Meier survival curves in Figure 5 were calculated using the log-rank (Mantel-Cox) test in Graphpad Prism.

Example 1: HLA count on cell surface and HLA typing

A major prerequisite for the development of T-cell mediated immunotherapies is the expression of MHC molecules on the surface of tumor cells. Therefore, the inventors analyzed and quantified the number of HLA-A, B, C as well as HLA-DR molecules by flow cytometry on different cell subsets of ovarian tumors (n=11) as well as benign

tissues from ovary and fallopian tube (n=8) obtained by enzymatic dissociation. The analysis aimed at the separate quantification of cell type specific HLA expression for leukocytes ($CD45^+$), tumor/epithelial cells ($Epcam^+$), and endothelial cells ($CD31^+$; the latter only in a subset of 7 ovarian tumors). For the complete gating strategy see figure 6. The median number of HLA molecules per cell was heterogeneous both among different cell types and individual patients, ranging from ~ 5,000 to 150,000 HLA class I and ~500 to 330,000 HLA-DR molecules. The number of HLA-A, B, and C molecules was significantly higher ($p = 0.0205$) on leukocytes isolated from tumor vs. benign tissue indicating an ongoing inflammatory reaction within the tumor. Strong differences in HLA class I expression were also seen when comparing tumor cells with epithelial cells derived from benign tissues. HLA class I molecule expression was significantly ($p = 0.0021$) higher on tumor cells (~75,000 molecules/cell) but remained in the range of other stromal cells such as endothelial cells (~95,000 molecules/cell). Surprisingly the inventors evidenced a strong (~105,000 molecules/cell) to some extent extraordinarily high expression of HLA-DR on EOC cells (>300,000 molecules/cell), whereas benign epithelial cells were virtually negative for HLA-DR ($p=0.0108$). Altogether, the inventors could observe an increased MHC class I and class II expression within the tumors.

HLA ligandome analysis and comparative profiling reveal EOC specific antigen presentation. In order to map the HLA ligand repertoire of EOC the inventors isolated HLA molecules from bulk tumor tissue and performed mass spectrometry to characterize the HLA ligandome for a total of 34 EOCs (for patient characteristics and HLA typing see Table 7).

Table 7

OvCa ID	Age	Tumor Type	TNM Staging	HLA typing MHC class I	HLA typing MHC class II
OvCa 9	65	serous ovarian carcinoma	T3cNxM1G2R1	A*02:01, A*03:01, B*07:02, B*40:02, C*07:02, C*12:01	DQB1*03:01, DQA1*03:01, DQA1*05:01, DRB1*11:01, DRB1*04:01, DRB3*02:02, DRB4*01:01, DPB1'02:01, DPB1*13:01

OvCa 10	60	serous ovarian carcinoma	T3bN1M1G2R1	A*02:01, A*11:01, B*44:05, B*51:01, C*02:02, C*15:02	DQB1*02:02, DQB1*05:01, DQA1*01:01, DQA1*03:01, DRB1*01:01, DRB1*09:01, DRB4*01:01, DPB1*04:01, DPB1*05:01
OvCa 12	62	serous ovarian carcinoma	T3cN0G2R0	A*24:02, A*31:01, B*35:03, B*49:01, C*07:01, C*12:03	DQB1*03:01, DQB1*05:04, DQA1*01:02, DQA1*03:01, DRB1*01:01, DRB1*04:01, DRB4*01:01, DPB1*02:01, DPB1*05:01
OvCa 13	62	serous ovarian carcinoma	T1cN1G3R0	A*02, B*35, B*40, C*03, C*04	DQB1*04, DQB1*06, DRB1*08, DRB1*13
OvCa 15	75	serous ovarian carcinoma	T3cN0G3R0	A*11:01, A*24:02, B*07:02, B*55:01, C*03:03, C*07:02	DQB1*03:01, DQA1*05:01, DRB1*11:01, DRB1*03:17, DRB3*02:02, DPB1*03:01
OvCa 16	45	serous ovarian carcinoma	T3bN1G3R0	A*02, B*40, B*44, C*03, C*05	DQB1*06, DRB1*08, DRB1*13, DRB1*14, DRB3
OvCa 23	29	serous ovarian carcinoma	T3aN1G3R0	A*01, A*03, B*08, B*35, C*04, C*07	DQB1*02, DQB1*03, DRB1*03, DRB1*12, DRB3
OvCa 28	66	serous ovarian carcinoma	T2bN0G3R0	A*01:01, A*02:01, B*27:05, B*52:01, C*01:02, C*02:02	DQB1*05:01, DQB1*06:01, DQA1*01:01, DQA1*03:01 DRB1*01:03, DRB1*15:02, DRB5*01:02, DPB1*04:01
OvCa 39	45	serous ovarian carcinoma	T3cN1G3R1	A*25:01, A*31:01, B*07:02, B*18:01, C*12:03, C*07:02	DQB1*06:02, DQA1*01:02, DRB1*15:01, DRB1*16:09, DRB5*01:01, DRB5*01:11, DPB1*04:01, DPB1*04:02
OvCa	66	serous	T3cN0G3R1	A*02, A*24,	DQB1*03, DQ7, DRB1*11,

41		and endo metrial ovaria n carcin oma		B*18, B*51, C*02, C*12	DRB3
OvCa 43	61	serous ovaria n carcin oma	T3cN1G3R2	A*02, A*32, B*18, B*35, C*04, C*07	DQB1*03, DQB1*05, DQ9, DRB1*01, DRB1*07, DRB4
OvCa 45	63	mixed differe ntiated (mostl y endo metroi d) ovaria n carcin oma	T1cN0G3R0	A*01, A*23, B*08, B*44, C*04, C*07	DQB1*02, DRB1*03, DRB1*07, DRB3, DRB4
OvCa 48	71	serous ovaria n carcin oma	T3cN1G3R0	A*02:01, A*25:01, B*15:01, B*41:02, C*03:04, C*17:01	DQB1*03:02, DQB1*03:04, DQA1*03:01, DRB1*04:01, DRB1*13:03, DRB3*01:01, DRB4*01:01, DPB1*02:01
OvCa 53	48	serous ovaria n carcin oma	T3bN1G3R0	A*02, A*03, B*27, B*35, C*02, C*04	DQB1*02, DQB1*03, DQ7, DRB1*03, DRB1*11, DRB3
OvCa 54	66	serous ovaria n carcin oma	T3cN1M1G3 R2	A*02:01, A*11:01, B*35:01, B*35:03, C*04:01, C*12:03	DQB1*05:01, DQB1*05:03, DQA1*01:01, DRB1*01:03, DRB1*14:01, DRB3*02:02, DPB1*04:01, DPB1*02:01
OvCa 57	58	endo metrioi d ovaria n	T1cN0G1R0	A*25, A*32, B*15, B*18, C*03, C*12	DQB1*05, DQB1*06, DRB1*01, DRB1*15, DRB5

		carcinoma			
OvCa 58	74	serous ovarian carcinoma	T3cN1G3R1	A*02, A*03, B*35, C*03, C*04	DQB1*05, DRB1*01
OvCa 59	47	serous ovarian carcinoma	T3cN1G3R2	A*03, A*30, B*13, C*06	DQB1*02, DRB1*07, DRB4
OvCa 60	50	serous ovarian carcinoma	T3cN1G3R1	A*24:02, A*25:01, B*13:02, B*18:01, C*12:03, C*06:02	DRB1*08:01, DRB1*13:01, DQB1*04:02, DQB1*06:03, DQA1*04:01, DQA1*01:03, DPB1*02:01, DPB1*03:01
OvCa 64	56	serous ovarian carcinoma	T3cN1G3R1	A*01, A*25, B*08, C*07	DQB1*02, DRB1*03, DRB3
OvCa 65	55	serous ovarian carcinoma	T3cN1M1G3R1	A*01, A*24, B*15, B*35, C*04, C*14	DQB1*03, DQB1*05, DRB1*10, DRB1*11, DRB3
OvCa 66	73	serous ovarian carcinoma	T2bN0G3R0	A*11:01, A*29:02, B*18:01, B*44:03, C*05:01, C*16:01	DRB1*03, DRB*0701, DRB3*0202, DRB4*0101, DQB1*02:01, DQB1*02:02, DQA1*02:01, DQA1*05:01, DPB1*02:02, DPB1*03:01
OvCa 68	69	serous ovarian carcinoma	T3cN1G3R1	A*02:01, A*01:01, B*44:02, B*37:01, C*06:02, C*05:01	DRB1*10:01, DRB1*04:01, DRB4*04:01, DQB1*05:01, DQB1*03:01, DQA1*01:01, DPB1*04:01
OvCa 69	68	serous ovarian carcinoma	T3cN0G1R1	n/a	n/a
OvCa	48	serous	T3cN1M1G1	A*01, A*02,	DQB1*03, DQB1*05,

70		ovarian carcinoma	R1	B*07, C*07	DRB1*09, DRB1*14, DRB3, DRB4
OvCa 72	53	serous ovarian carcinoma	T3bN1G3R0	A*03:01, A*01:01, B*08:01, B*07:02, C*07:02, C*07:01	DRB1*01:01, DRB1*03:01, DRB3*01:01, DQB1*05:01, DQB1*02:01, DQA1*01:01, DPB1*04:01
OvCa 73	69	serous ovarian carcinoma	T3cN1G3R0	A*01:01, B*08:01, C*07:01	DRB1*03:01, DRB1*03:42, DRB3*01:01, DRB3*01:14, DQB1*02:01, DQA1*05:01, DPB1*04:01
OvCa 74	79	endometrioid ovarian carcinoma	T3bNxG1R1	A*02:01, B*18:01, B*51:01, C*07:02, C*15:02	DRB1*11:04, DRB1*07:01, DRB3*02:02, DRB4*01:01, DQB1*03:01, DQB1*02:02, DQA1*02:01, DQA1*05:01, DPB1*04:02, DPB1*02:01
OvCa 79	57	endometrioid ovarian carcinoma	T2bN0G2R0	A*01:01, A*31:01, B*08:01, B*51:01, C*07:01, C*15:02	DQB1*03:03, DQA1*02:01, DRB1*07:01, DRB1*09:01, DRB4*01:01, DPB1*13:01, DPB1*02:01
OvCa 80	93	serous ovarian carcinoma	T3cNxG3R2	A*25:01, A*32:01, B*18:01, B*39:01, C*12:03	DRB1*01:01, DRB1*12:01, DRB3*02:02, DQB1*03:01, DQB1*05:01, DQA1*01:01, DQA1*05:01, DPB1*04:01
OvCa 81	78	serous ovarian carcinoma	T3cNxG3R2	A*02:01, B*45:01, B*56:01, C*07:02, C*01:02	DRB1*04:02, DRBB1*11:01, DRB4*01:01, DRB3*02:02, DQB1*03:01, DQB1*03:02
OvCa 82	48	serous ovarian carcinoma	T3cN1G3R0	A*01:01, A*03:01, B*08:01, B*38:01, C*07:01, C*12:03	DRB1*04:02, DRB1*03:01, DRB4*01:01, DRB3*01:01, DQB1*02:01, DQB1*03:02, DQA1*03:01, DQA1*05:01, DPB1*04:01, DPB1*13:01
OvCa	50	serous	T1cN0G2R0	A*02, A*11,	DQB1*03, DQB1*05,

83		ovarian carcinoma		B*51, B*55, C*03, C*15	DRB1*09, DRB1*14, DRB3, DRB4
OvCa 84	70	serous ovarian carcinoma	T3cN1G3R1	A*02:01, B*07:02, B*44:02, C*07:02, C*05:01	DRB1*15:01, DRB5*01:01, DQB1*06:02, DQA1*01:02, DPB1*04:01, DPB1*04:02

For MHC class I the inventors could identify 22,920 unique peptides (mean 1,263/sample) emanating from 9,136 different source proteins (mean 1,239/sample) reaching >90% of the estimated maximal attainable coverage (see Figure 7a).

Example 2, Identification of top cancer associated HLA ligands

Aiming to extract the most specific HLA ligands for EOC from this vast catalogue of data the inventors compared the HLA ligand source proteins with an in-house database of benign sources ("HLA benign ligandome database") consisting of samples from PBMCs (n=30), bone marrow (n=10), liver (n=15), colon (n=12), ovary (n=4) and kidney (n=16). The HLA benign ligandome database contains 31,032 peptides representing 10,012 source proteins and was established using blood or bone marrow from healthy donors as well as histopathologically evaluated normal tissues, all analyzed with exactly the same pipeline as used for EOCs. For comparative profiling "one hit wonders" (i.e. peptides only presented on one source with low PSM count) were removed from both datasets to accommodate for false positive hits. Comparative analysis of the two respective datasets (see Figure 2A) revealed 379 MHC class I source proteins to be presented exclusively by EOC in at least three of the tested patients, highlighting an EOC specific HLA peptide repertoire. The TOP100 EOC specific source proteins ranked according to their frequency of presentation are visualized in Figure 2B. The most important EOC specific HLA ligand source protein yielded by this analysis was mucin 16 (MUC16) also known as cancer antigen 125 (CA-125). Overall more than 80 different MUC16 derived HLA ligands (see Table 8) were presented in nearly 80% of patients (26/34).

Table 8

Sequence	ID No.	Sources	HLA
AHSKITTAM	3	OvCa 80	B*39:01
AVKTETSTSER	4	OvCa 12, OvCa 79	A*31:01
AVTNVRTSI	5	Ovca 59, OvCa 60	B*13
DALTPLVTI	6	OvCa 74	B*51:01
DALVLKTV	7	OvCa 41, OvCa 74, OvCa 79, OvCa 83	B*51
DPYKATSAV	8	OvCa 10, OvCa 41, OvCa 69 OvCa 74, OvCa 79, OvCa 83	B*51
EPETTTSFITY	9	OvCa 65	B*35
ERSPVIQTL	10	OvCa 80	B*39:01
ETILTFHAF	11	OvCa 48, OvCa 64, OvCa 80	A*25
EVISSRGTSM	12	OvCa 48, OvCa 60, OvCa 64, OvCa 80	A*25
EVITSSRTTI	13	OvCa 60, Ovca 64	A*25
EVTSSGRTSI	14	OvCa 60, Ovca 64, OvCa 80	A*25
FPEKTTHSF	15	OvCa 65	B*35
FPHSEETTTM	16	OvCa 13, OvCa 65	B*35
FPHSEITTL	17	OvCa 12, OvCa 13, OvCa 53	B*35
FQRQGQTAL	18	OvCa 48	B*15:01
GDVPRPSSL	19	OvCa 72	B*08:01
GHESHSPAL	20	OvCa 80	B*39:01
GHTTVSTSM	21	OvCa 80	B*39:01
GTHSPVTQR	22	OvCa 39, OvCa 79	A*31:01
GTSGTPVSK	23	OvCa 83	A*11
HPDPQSPGL	24	OvCa 65	B*35
IITEVITRL	547	OvCa 83	A*02
IPRVFTSSI	25	OvCa 41, OvCa 74	B*51
ISDEVVTRL	26	OvCa 16	C*05
ISIGTIPRI	27	OvCa 65	B*15:17
ISKEDVTSI	28	OvCa 65	B*15:17
ITETSAVLY	29	OvCa 65	A*01
ITRLPTSSI	30	OvCa 65	B*15:17
KDTAHTEAM	31	OvCa 68	B*44:02
KEDSTALVM	32	OvCa 16	B*40/B*44
KEVTSSSSVL	33	OvCa 16, OvCa 70	B*40/B*44/?
KMISAIPTL	548	OvCa 81, OvCa 83	A*02
LPHSEITTL	34	OvCa 12, OvCa 13	B*35
LTISTHKTI	35	OvCa 65	B*15:17
LTKSEERTI	36	OvCa 65	B*15:17
QFITSTNTF	1	OvCa 60	A*24:02

RDSLYVNGF	37	OvCa 68	B*44:02
RETSTSQKI	38	OvCa 60	B*18:01
RSSGVTFSR	39	OvCa 79	A*31:01
SAFESHSTV	40	OvCa 41, OvCa 74, OvCa 79, OvCa 83	B*51
SATERSASL	41	OvCa 13, OvCa 16, OvCa 70	C*03/?
SENSETTAL	42	OvCa 16, OvCa 70	B*40/B*44/?
SEQRTSPSL	43	OvCa 70	n.a.
SESPSTIKL	44	OvCa 13, OvCa 70	B*40/?
SPAGEAHSL	45	OvCa 72, OvCa 81, OvCa 84	B*07/B*56
SPAGEAHSLLA	46	OvCa 81	B*56:01
SPHPVSTTF	47	OvCa 84	B*07:02
SPHPVTALL	48	OvCa 9, OvCa 72, OvCa 84	B*07:02
SPLFQRSSL	49	Ovca 72	B*0702
SPQNLRNTL	50	OvCa 23, OvCa 72, OvCa 84	B*35/B*07:02
SPRLNTQGNTAL	51	OvCa 72, Ovca 84	B*07:02
SPSEAITRL	52	Ovca 84	B*07:02
SPSKAFASL	53	OvCa 9, OvCa 23, OvCa 39, OvCa 69, OvCa 72, OvCa 84	B*35/B*07:02
SPSSPTPKV	54	OvCa 72	B*07:02
SPSSQAPVL	55	OvCa 84	B*07:02
SQGFSHSQM	56	OvCa 48	B*15:01
SRTEVISSR	57	OvCa 53	B*27
SSAVSTTTI	58	OvCa 65	B*15:17
SSPLRVTSL	59	OvCa 69	n.a.
STASSSLSK	60	OvCa 83	A*11
STETSTVLY	2	OvCa 64, OvCa 65, OvCa 68	A*01
STQRVTTSM	61	OvCa 72	n.a.
STSQEIHSATK	62	OvCa 83	A*11
SVLADLVTTK	63	OvCa 72	A*03:01
SVPDILSTSW	64	OvCa 60	A*24:02
TAGPTTHQF	65	OvCa 58	C*03
TEISSSRTSI	66	OvCa 12	B*49:01
TENTGKEKL	67	OvCa 16	B*40/B*44
TETEAIHVF	68	OvCa 41, OvCa 80	B*18
TEVSRTEVI	69	OvCa 12	B*49:01
TExVLQGLL	70	OvCa 16, OvCa 66, OvCa 70	B*40/B*44/?
TPGGTRQSL	71	OvCa 9, OvCa 23, OvCa 39, OvCa 72, OvCa 84	B*07:02/B*35
TPGNRAISL	72	OvCa 23, OvCa 72, OvCa 84	B*07:02/B*35

TPNSRGETSL	73	OvCa 72	B*07:02
TSGPVTEKY	74	OvCa 58	B*35
TSPAGEAHSL	75	OvCa 81	n.a.
TTLPESRPS	324	OvCa 70	n.a.
TYSEKTTLF	549	OvCa 12, OvCa 41, OvCa 60, OvCa 65	A*24
VHESHSSVL	76	OvCa 80	B*39:01
VPRSAATTL	77	OvCa 23, OvCa 72, OvCa 84	B*07:02/B*35
VTSAPGRSI	78	OvCa 65	B*15:17
VTSSSRTSI	79	OvCa 65	B*15:17
YPDPSKASSAM	80	OvCa 65	B*35

Those data highlight the frequent processing and presentation of MUC16 by a multitude of different HLA allotypes unparalleled by any other EOC specific antigen and mirrored only by frequently (>95%) presented house-keeping proteins such as beta actin (overall 149 different peptides identified). Among the TOP100 EOC specific source proteins other well established tumor associated antigens like MUC1 or KLK10 as well as antigens with well documented immune-evasive functions like Indoleamine-2,3-dioxygenase (IDO1) or Galectin 1 (LGALS1) were identified.

Owing to the power of CD4 T cells in supporting or driving an anti-tumor immune response the inventors used the same approach to further analyze MHC class II presented peptides in EOC (n=22) yielding 9,162 peptides (mean 598/sample) representing 2,330 source protein (mean 319/sample) reaching > 80% of attainable coverage (see Figure 7B) . The HLA benign ligand dataset for MHC class II contained 7,267 peptides representing 1,719 source proteins derived from bone marrow (n=5), PBMCs (n=13), colon (n=2), liver (n=7) and kidney (n=17). Analysis of the TOP100 MHC class II presented antigens revealed a more heterogeneous and complex picture (Figure 2C). Notably, MHC presented peptides of mesothelin (MSLN) an established ligand of MUC16, could be identified in nearly 50% of patients (10/22; Figure 2D). MUC16 itself was not among the TOP100 class II antigens but respective ligands could nevertheless be detected in four patients.

Besides the TOP100 EOC specific HLA ligand source proteins, the inventors further looked for established cancer-testis and tumor associated antigens that have been previously employed for clinical application to verify their abundance (Her2neu, WT1, NY-ESO-1, hTert and p53). Although the inventors could identify HLA presented peptides for all antigens except for NY-ESO-1, none of them were exclusively presented on EOC (Table 9). The only ligands showing EOC specific presentation, albeit with low frequency (3/34), were HLA class I ligands (but not HLA class II) from Her2neu.

Table 9

SEQ ID	Her2neu	HLA restriction	Sources of presentation
	ERBB2 (Receptor tyrosine-protein kinase erbB-2)		
554	TYLPTNASLSF	A*23/A*24	2x OvCa
153	MPNPEGRYTF	B*35	1x OvCa
152	AARPAGATL	B*07	1x OvCa
291	AIKVLRENTSPKANKE	HLA class II	1x OvCa
292	DPSPLQRYSEDPTVPLPS	HLA class II	2x OvCa
293	DPSPLQRYSEDPTVPLPSE	HLA class II	1x OvCa
294	ELVSEFSRMARD	HLA class II	2x PBMCs
295	ELVSEFSRMARDPQ	HLA class II	2x PBMCs, 1x Kidney
296	IPVAIKVLRENTSPKANKE	HLA class II	1x OvCa
297	RRLLQETELVEPLTPS	HLA class II	2x Liver
298	SPQPEYVNQPDVRPQPP	HLA class II	1x OvCa
291	VKPDLSYMPIWKFPDE	HLA class II	1x OvCa
	WT-1		
	Wilms tumor protein		
558	RMFPNAPYL	A*02	8x PBMCs, 1x Liver
557	QRNMTKLQL	B*13	2x OvCa, 1x Liver, 1x PBMCs
555	GVFRGIQDV	B*13	2x OvCa
550	ALLPAVPSL	A*02	1x OvCa
	hTert		
	Telomerase reverse transcriptase		
556	LMSVYVVEL	A*02	2x PBMCs
	p53		
	Cellular tumor antigen p53		

552	RPILTIITL	B*07	4x PBMCs, 2x Liver, 2x Kidney, 3x OvCa
553	TYSPALNKMF	A*24	1x PBMCs, 1x Liver, 2x OvCa
551	GRNSFEVRV	B*27	1x PBMC, 1x Liver, 1x Kidney, 1x OvCa

Example 3: Cellular origin of EOC associated HLA presented peptides

Since EOCs embody not only cancer cells but rather represent a heterogeneous mixture of different cell types the inventors asked, whether the MHC class I TOP100 antigens were indeed originally presented by cancer cells. For this purpose the inventors digested EOCs and separated $CD45^+$ leukocytes, $EpCam^+$ tumor cells as well as stroma cells negative for the two markers (for enrichment efficiencies see Table 10) and subsequently the inventors performed HLA ligandomics individually for each of the subsets.

Table 10: Cell enrichment efficiencies:

Percentage of cells are given in each fraction before (PreSort) and after MACSorting

	PreSort			**$CD45^+$ fraction**			**$EpCam^+$ fraction**			**$EpCam^-$ fraction**		
Ovca	**$CD45^+$**	**$EpCam^+$**	**Viability**	**$CD45^+$**	**$EpCam^+$**	**Viability**	**$CD45^+$**	**$EpCam^+$**	**Viability**	**$CD45^+$**	**$EpCam^+$**	**Viability**
84	74.7	18.3	80.2	93.5	6.2	71.6	10.7	85.7	88.2	4.5	22.1	64.0
73	23.1	12.3	81.2	95.7	1.7	77.2	3.4	73.3	87.6	1.7	3.2	87.4
70	76.2	8.83	78.9	96	1.3	82.7	3.4	94	66.4	3.1	4.5	65.4
60	77.4	5.2	92.3	94.8	1.7	90.2	5.2	79.7	88.7	3.8	10.7	89.5
57	31.9	50.5	94.1	93.6	5.0	90.6	1.4	95.3	96.7	0.8	7.2	95.3

The inventors used label free quantification to determine the source of each identified HLA ligand in a total of 5 EOCs (for a representative example see Figure 3). As expected, MUC16 derived HLA ligands, identified on (4/5) EOC samples, were always found to be overrepresented on enriched cancer cells with a median 5 fold overrepresentation (range 1.8-135 fold) dependent on the efficiency of the enrichment.

The same held true for several other frequently presented TOP100 antigens like DDR1, SOX9, CRABP1/2, EYA2, LAMC2, MUC1 or KLK10. However a number of other antigens especially those known to be upregulated by interferon such as toll like receptors (TLR3, TLR7) or 2'-5'-oligoadenylate synthase-like protein synthase (OASL) could not be unambiguously shown to be presented by tumor cells but rather displayed strong overrepresentation on CD45+ leukocytes and/or stroma cells. Apart from tumor associated antigens the inventors also recognized ligands from source proteins with cell type specific expression. For example ligands derived from CD8, CD132 or lymphocyte specific protein 1 (LSP1) were found highly overrepresented on CD45+ cells and van Willebrand factor (vWF) most likely expressed by endothelial cells in the stroma was found highly overrepresented within the stromal subset emphasizing the strength of this cell type specific approach.

Example 4: Immunogenicity analysis of MUC16 derived ligands

For the applicability of peptide vaccines immunogenicity is a major imperative. In order to evaluate the immunogenic potential of the identified HLA ligands the inventors used a T-cell priming protocol involving artificial antigen presenting cells and T cells isolated from blood of healthy donors. The results of this analysis for the number one EOC associated antigen MUC16 are presented in Table 11. Among 23 different peptides tested so far, 18 were shown to be immunogenic in at least 1/3 donors. This nearly 80% recognition rate verifies the presence of naïve MUC16 recognizing T cells in the human population. Similar results have been obtained for other TOP100 antigens (e.g. IDO1, LGALS1).

Table 11: Immunogenicity analysis of EOC presented HLA ligands from MUC16 /CA-125

HLA	Sequence	SEQ ID	positive / tested donors
A*01	STETSTVLY	2	0 / 2
A*02	IITEVITRL	547	3 / 10
A*02	KMISAIPTL	548	4 / 6
A*03	SVLADLVTTK	63	0 / 1

A*11	STSQEIHSATK	62	2 / 6
A*11	GTSGTPVSK	23	0 / 5
A*24	TYSEKTLLF	549	2 / 2
A*24	AVTNVRTSI	5	1 / 3
A*25	ETILTFHAF	11	2 / 2
A*25	EVITSSRTTI	13	1 / 1
A*25	EVTSSGRTSI	14	2 / 3
A*25	EVISSRGTSM	12	1 / 3
B*07	SPHPVTALL	48	0 / 1
B*07	SPQNLRNTL	50	1 / 1
B*07	LPHSEITTL	34	0 / 2
B*07	SPSKAFASL	53	2 / 2
B*07	VPRSAATTL	77	1 / 2
B*07	TPGNRAISL	72	2 / 2
B*15	SQGFSHSQM	56	4 / 5
B*15	FQRQGQTAL	18	1 / 6
B*27	ERSPVIQTL	10	1 / 2
B*51	DALVLKTV	7	1 / 3
B*51	DPYKATSAV	8	3 / 3
8 / 10 allotypes	18/23 HLA ligands		34 / 73

Example 5: Biomarkers for HLA ligand presentation

Antigen specific cancer immunotherapy (e.g. peptide vaccination, adoptive T-cell transfer) requires a stringent selection of candidate antigens within a short timeframe. HLA ligandome analysis however, is not always possible due to the lack of appropriate material. A feasible alternative would be the use of biomarkers to predict the presence of HLA ligands on the tumor cells. In order to evaluate whether, protein expression analyzed by immunohistochemistry (immunoreactivity score, IRS) could serve as a surrogate marker for HLA ligand presentation, the inventors analyzed the TOP100 MHC class I antigens MUC16 and IDO1 as well as the TOP100 MHC class II antigen MSLN by immunohistochemistry and correlated the staining intensity (Figure 4A) to the

presence or absence of HLA ligands on the same tumors. For both MUC16 and MSLN, staining scores were significantly higher on tumors, which presented HLA ligands of respective source proteins (Figure 4C). The same was true for CA-125 serum levels determined at the day of surgery (Figure 4D), indicating that these parameters could be used for a proper selection of candidate antigens for peptide vaccination. In contrast, IDO1 did not show a significant association with ligand presentation.

Example 6: Prognostic relevance of the MUC16/MSLN axis

Because of their importance as targets for immunotherapy the inventors wanted to assess whether MSLN and MUC16 are also of prognostic relevance in a patients similar to our immunopeptidome collective. For this purpose the inventors analyzed the expression of both antigens as well as the extent of T-cell infiltration by immunohistochemistry in a tissue microarray (TMA) of high grade serous ovarian cancers (FIGO stage II-III). In order to avoid prognostically relevant confounders the inventors restricted our analysis to 71 patients with optimally debulked cancers (residual mass below < 1 cm).

While the inventors did not observe any prognostic effect for MUC16 staining, strong MSLN staining was associated with a notable borderline significant ($p=0.0572$) decrease of median overall survival from 50 to 28 months (Figure 5A). Despite their different prognostic relevance, staining scores for MUC16 and MSLN showed a direct and highly significant correlation (Spearman correlation coefficient $r = 0.5237$; 95% c.i. = 0.3159-0.6835, two tailed significance $p < 0.001$).

For the evaluation of T-cell infiltration the inventors assessed the number of CD3 T cells in the intraepithelial compartment of the tumor (CD3E) and the fibrovascular stroma (CD3S) separately. Notably only the number of intraepithelial T cells showed a significant ($p<0.0063$) prognostic impact, whereas infiltration of the surrounding stroma alone had no prognostic relevance (Figure 5B). Only in a subgroup analysis combining MSLN and CD3 staining a significant prognostic benefit for tumors with low MSLN and high T-cell infiltration could be observed (Figure 5C) for both CD3E ($p < 0.001$) and

CD3S ($p < 0.0049$). Most strikingly, the combination of high intratumoral T-cell infiltration (CD3E) and low MSLN staining defined a subset of long term cancer survivors (10/11 patients with confirmed survival beyond 3 years).

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CLAIMS

1. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549, and variant sequences thereof which are at least 88% homologous to SEQ ID No. 1 to SEQ ID No. xxx, 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. 549, and in particular of amino acids according to any one of SEQ ID No. 1 to SEQ ID No. 319.

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. 549, and in particular of an amino acid sequence according to any one of SEQ ID No. 1 to SEQ ID No. 319.

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

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

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

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

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

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

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

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

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

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

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

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

17. The use according to claim 16, wherein said cancer is selected from the group of ovarian cancer, non-small cell lung cancer, small cell lung cancer, kidney cancer, brain cancer, colon or rectum cancer, stomach cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, esophageal cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, bile duct cancer and other tumors that show an overexpression of a protein from which a peptide SEQ ID No. 1 to SEQ ID No. 549 is derived from, in particular ovarian cancer.

18. A kit comprising:

(a) a container comprising a pharmaceutical composition containing the peptide(s) or the variant according to any one of claims 1 to 6, the nucleic acid(s) according to claim

7, the expression vector(s) according to claim 8, the cell(s) according to claim 10, the activated T lymphocyte(s) according to claim 13 or the antibody according to claim 15, in solution or in lyophilized form;

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

(c) optionally, at least one more peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549, and

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

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

20. The kit according to claim 18 or 19, wherein said peptide is selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549.

21. A method for producing a personalized anti-cancer vaccine for 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 sid personalized vaccine based on step c).

22. The method according to claim 21, wherein said TUMAPs are identified by a method comprising:

a1) comparing expression data from the tumor sample to expression data from a sample of normal tissue corresponding to the tissue type of the tumor sample to identify proteins that are over-expressed or aberrantly expressed in the tumor sample; and
a2) correlating the expression data with sequences of MHC ligands bound to MHC class I and/or class II molecules in the tumor sample to identify MHC ligands derived from proteins over-expressed or aberrantly expressed by the tumor.

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

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

25. The method according to any of claims 21 to 24, wherein the peptides included in the warehouse are identified based on the following steps:
aa. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;
ab. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step aa, and
ac. Determining an induction of in vivo T-cell responses by the peptides as selected comprising *in vitro* immunogenicity assays using human T cells from healthy donors or said patient; or
ba. Identifying HLA ligands from said tumor sample using mass spectrometry;
bb. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;
bc. Comparing the identified HLA ligands to said gene expression data;

bd. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step bc;
be. Re-detecting of selected TUMAPs from step bd on tumor tissue and lack of or infrequent detection on healthy tissues and confirming the relevance of over-expression at the mRNA level; and
bf. Determining an induction of in vivo T-cell responses by the peptides as selected comprising *in vitro* immunogenicity assays using human T cells from healthy donors or said patient.

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

27. The method according to any of claims 21 to 26, wherein said warehouse comprises a plurality of peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549.

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

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

30. A T-cell receptor, preferably a recombinant soluble or membrane-bound T-cell receptor, that is reactive with an HLA ligand, wherein said ligand has at least 75% identity to an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549.

31. The T-cell receptor according to claim 30, wherein said amino acid sequence is at least 88% identical to SEQ ID No. 1 to SEQ ID No. 549.

32. The T-cell receptor according to claim 30 or 31, wherein said amino acid sequence consists of any of SEQ ID No. 1 to SEQ ID No. 549.

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

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

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

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

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

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

a) a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549;

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

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

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

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

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

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

h) an aptamer recognizing a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549 and/or a complex of a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 549 with an MHC molecule,

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

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

Figure 1

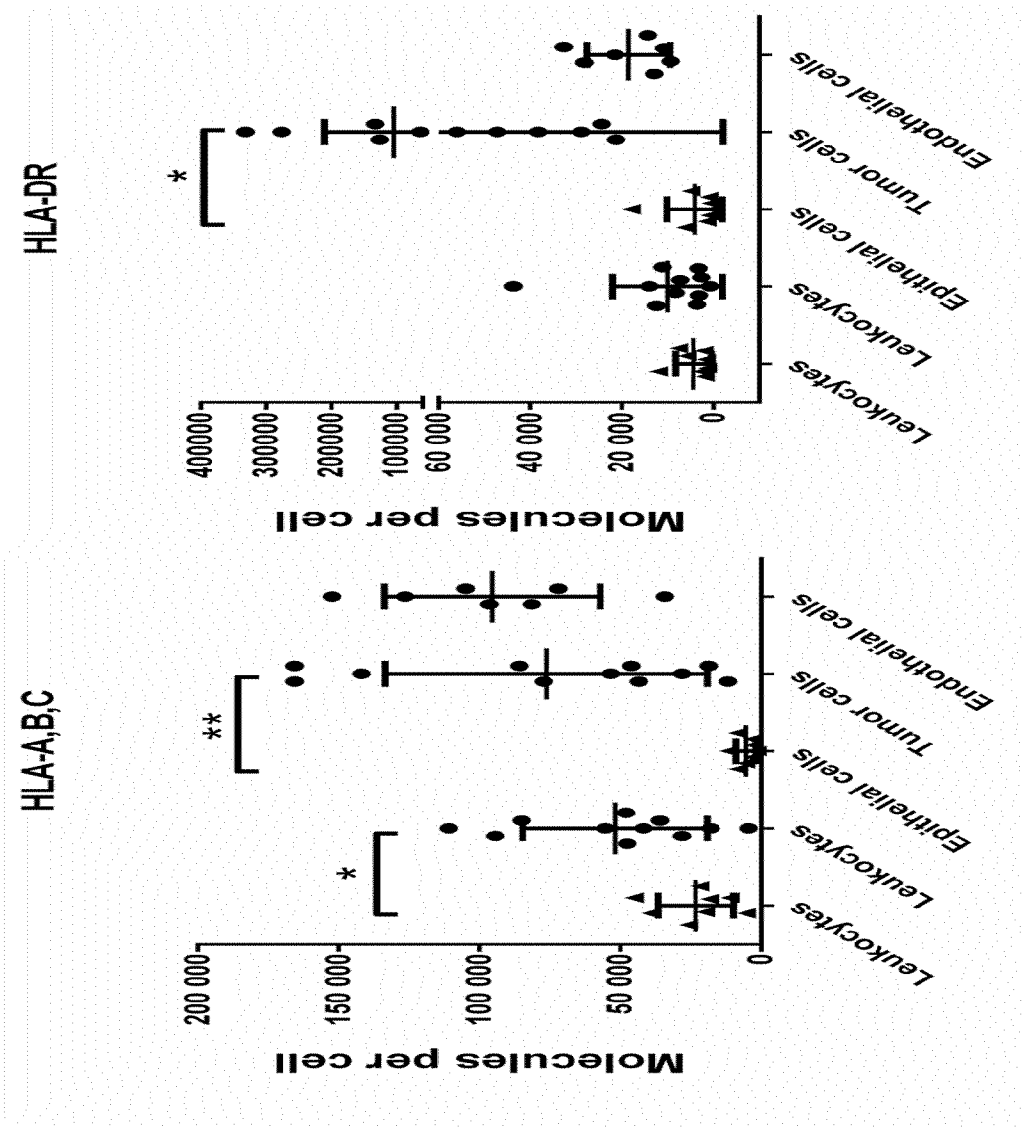
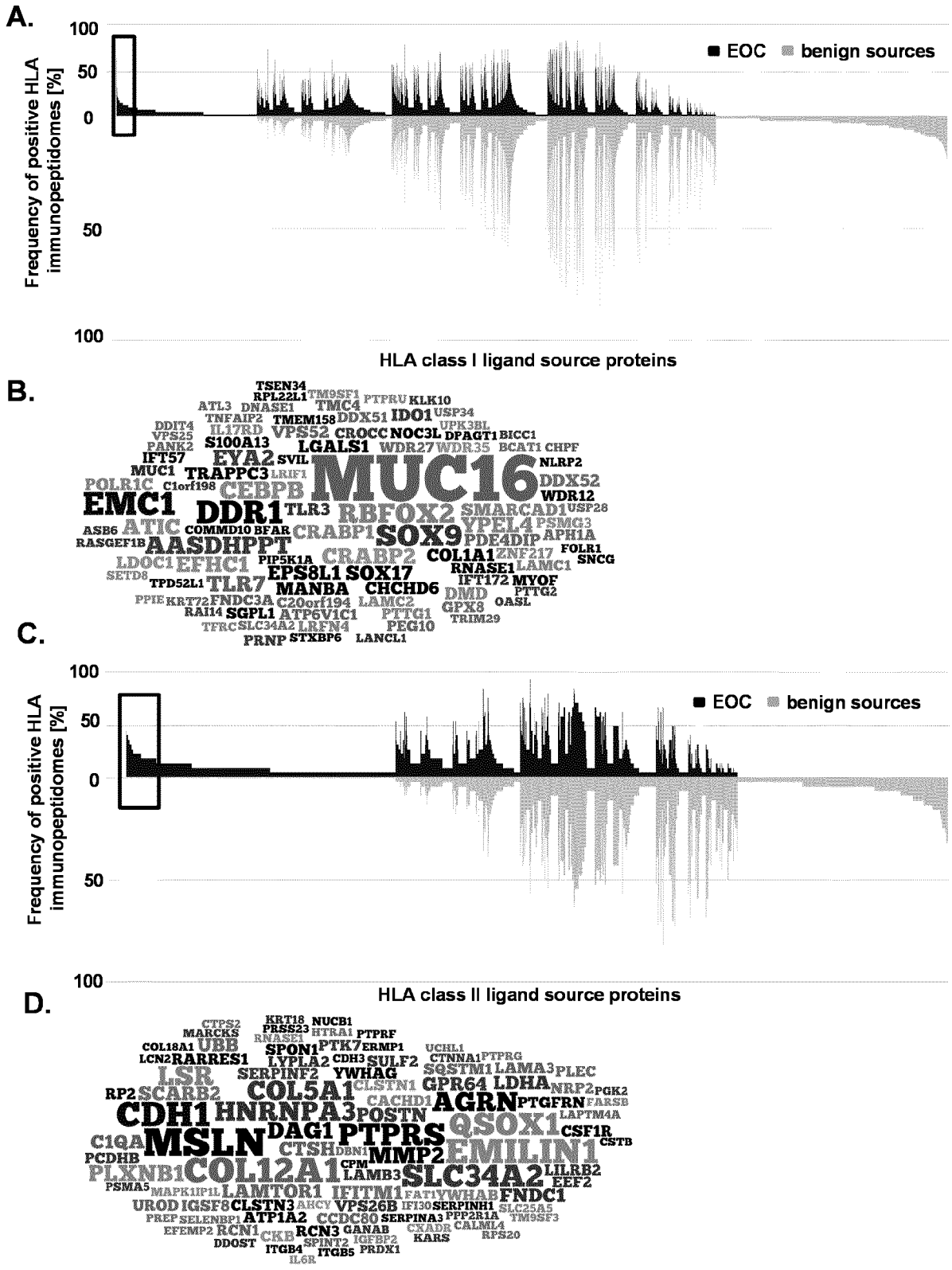

A
HLA-A,B,C
Molecules per cell
200 000
150 000
100 000
50 000
0
*
**
Leukocytes
Leukocytes
Epithelial cells
Tumor cells
Endothelial cells
B
HLA-DR
Molecules per cell
400000
300000
200000
100000
60 000
40 000
20 000
0
*
Leukocytes
Leukocytes
Epithelial cells
Tumor cells
Endothelial cells
benign ovary/
fallopian tube
ovarian cancer

Figure 2


A.
100
50
0
50
100
Frequency of positive HLA immunopeptidomes [%]
EOC
benign sources
HLA class I ligand source proteins
B.
TSEN34
RPL22L1
TM9SF1
PTFRU
KLK10
ATL3
DNASE1
TMC4
TNFAIP2
TMEM158
DDX51
IDO1
USP34
DDIT4
VPS25
IL17RD
VPS52
CROCC
NOC3L
DPAGT1
BICC1
PANK2
S100A13
LGALS1
WDR27
WDR35
BCAT1
CHPF
IFT57
EYA2
SVIL
NLRP2
MUC1
TRAPPC3
LRIF1
MUC16
POLR1C
C1orf198
CEBPB
DDX52
WDR12
EMC1
DDR1
TLR3
RBFOX2
SMARCAD1
USP28
ASB6
ATIC
COMMD10
BFAR
CRABP1
SOX9
YPEL4
PSMG3
RASGEF1B
AASDHPPT
PDE4DIP
APH1A
LDOC1
EFHC1
PIP5K1A
CRABP2
COL1A1
ZNF217
FOLR1
SNCG
SETD8
EPS8L1
SOX17
RNASE1
LAMC1
TPD52L1
TLR7
MANBA
CHCHD6
IFT172
MYOF
PPIE
KRT72
FNDC3A
C20orf194
LAMC2
DMD
PTTG2
GPX8
OASL
RAI14
SGPL1
ATP6V1C1
PTTG1
TRIM29
TFRC
SLC34A2
LRFN4
PEG10
PRNP
STXBP6
LANCL1
C.
100
50
0
50
100
Frequency of positive HLA immunopeptidomes [%]
EOC
benign sources
HLA class II ligand source proteins
D.
CTPS2
KRT18
NUCB1
MARCKS
PRSS23
HTRA1
PTPRF
RNASE1
COL18A1
UBB
SPON1
PTK7
ERMP1
UCHL1
LCN2
RARRES1
LYPLA2
CDH3
SULF2
CTNNA1
PTPRG
LSR
SERPINF2
YWHAG
SQSTM1
LAMA3
PLEC
RP2
SCARB2
COL5A1
CLSTN1
GPR64
LDHA
NRP2
PGK2
CDH1
HNRNPA3
POSTN
CACHD1
AGRN
PTGFRN
FARSB
LAPTM4A
QSOX1
CSF1R
C1QA
MSLN
DAG1
PTPRS
CSTB
CTSH
DBN1
EMILIN1
PCDHB
MMP2
PLXNB1
COL12A1
CPM
LAMB3
SLC34A2
LILRB2
EEF2
PSMA5
MAPK1IP1L
LAMTOR1
IFITM1
FAT1
YWHAB
FNDC1
UROD
IGSF8
CLSTN3
AHCY
VPS26B
IFI30
SERPINH1
SLC25A5
PREP
SELENBP1
ATP1A2
CCDC80
SERPINA3
PPP2R1A
TM9SF3
EFEMP2
RCN1
CKB
RCN3
GANAB
CXADR
CALML4
DDOST
KARS
RPS20
ITGB4
SPINT2
IGFBP2
ITGB5
PRDX1
IL6R

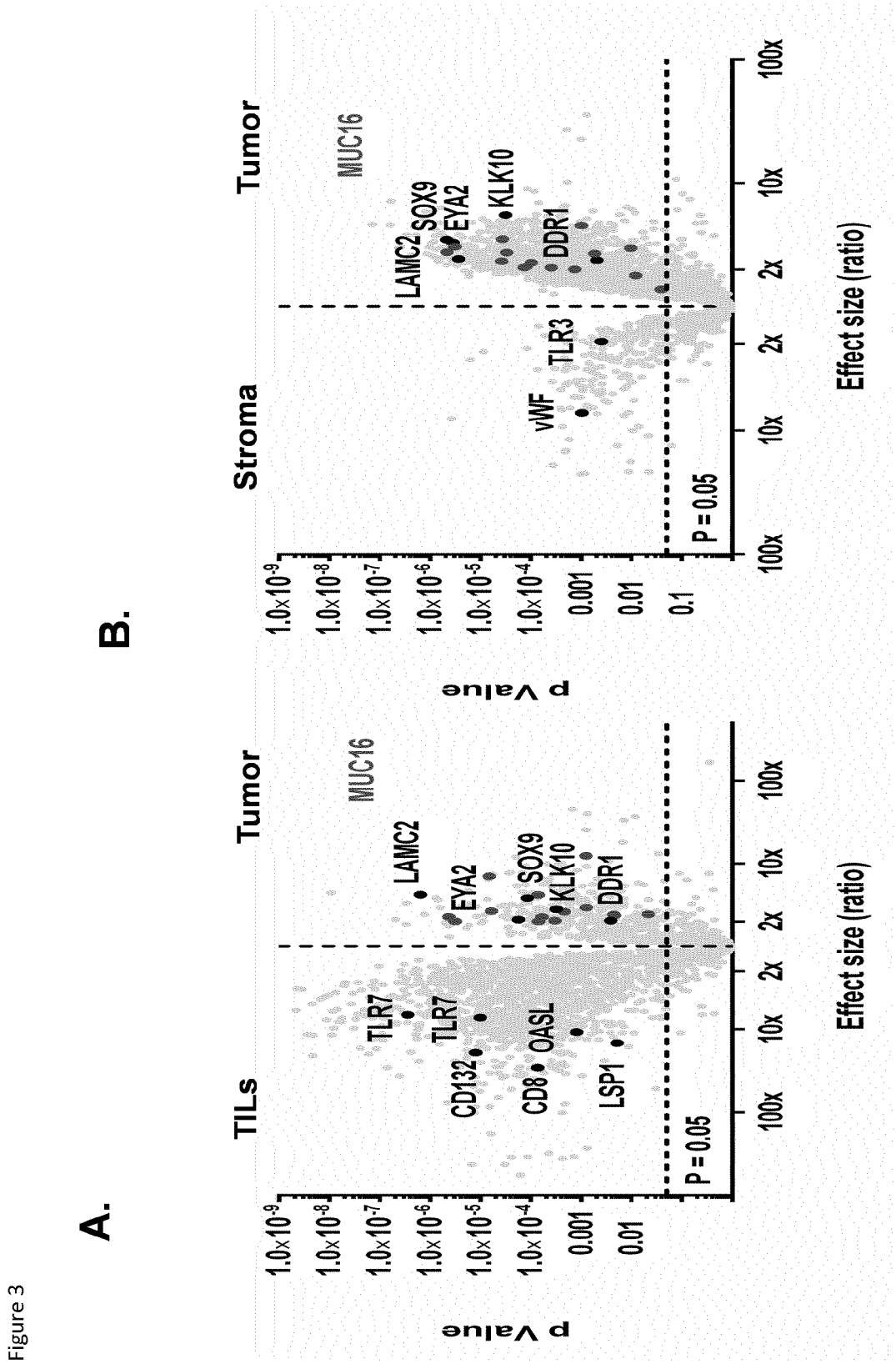

A.
TILs
Tumor
MUC16
p Value
Effect size (ratio)
P = 0.05
TLR7
TLR7
CD132
CD8
OASL
LSP1
LAMC2
EYA2
SOX9
KLK10
DDR1
B.
Stroma
Tumor
MUC16
vWF
TLR3
LAMC2
SOX9
EYA2
KLK10
DDR1

Figure 3

Figure 4

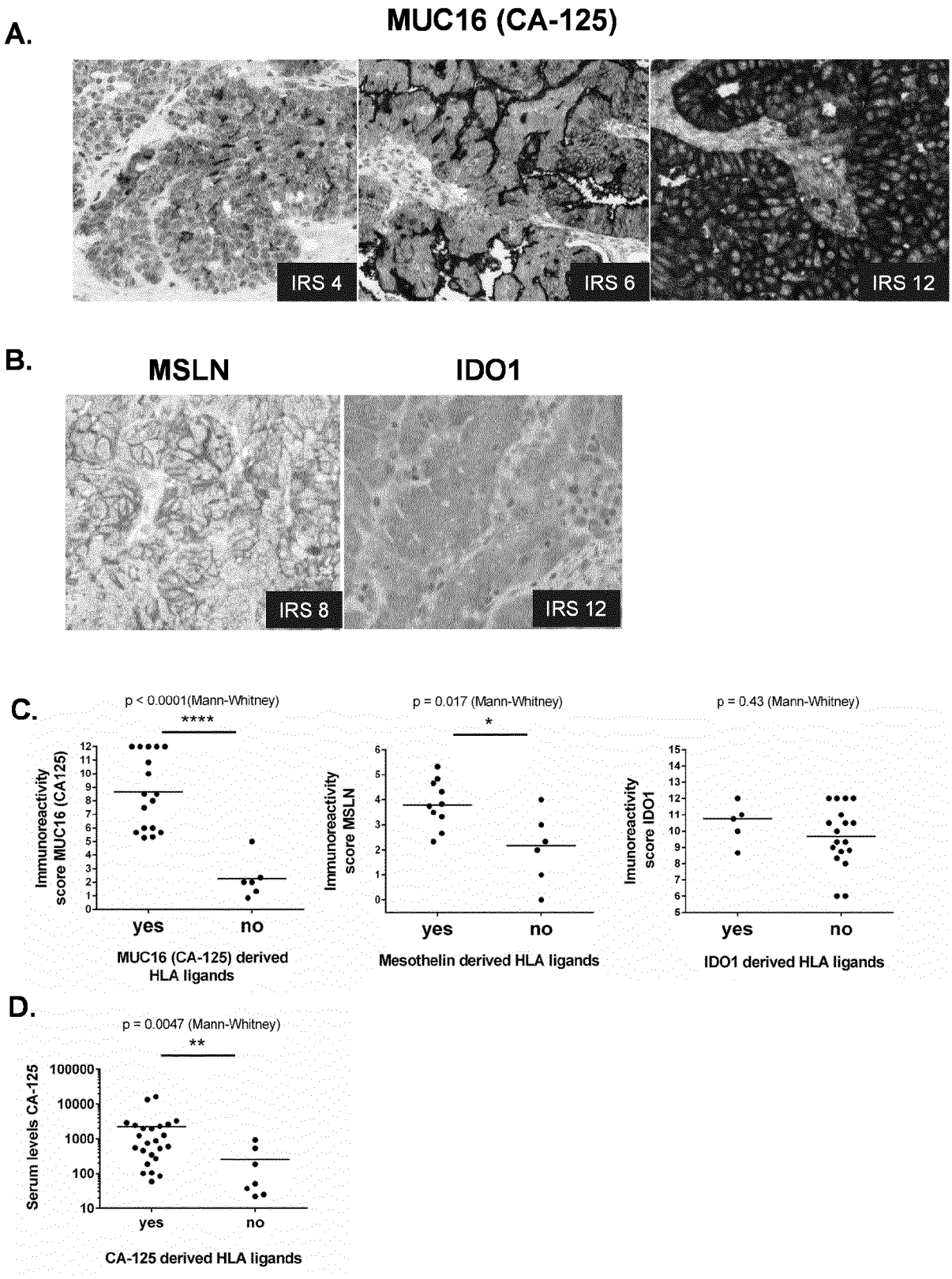

A.
MUC16 (CA-125)
IRS 4
IRS 6
IRS 12
B.
MSLN
IDO1
IRS 8
IRS 12
C.
p < 0.0001(Mann-Whitney)
****
Immunoreactivity score MUC16 (CA125)
yes
no
MUC16 (CA-125) derived HLA ligands
p = 0.017 (Mann-Whitney)
*
Immunoreactivity score MSLN
yes
no
Mesothelin derived HLA ligands
p = 0.43 (Mann-Whitney)
Imunoreactivity score IDO1
yes
no
IDO1 derived HLA ligands
D.
p = 0.0047 (Mann-Whitney)
**
Serum levels CA-125
yes
no
CA-125 derived HLA ligands

Figure 5

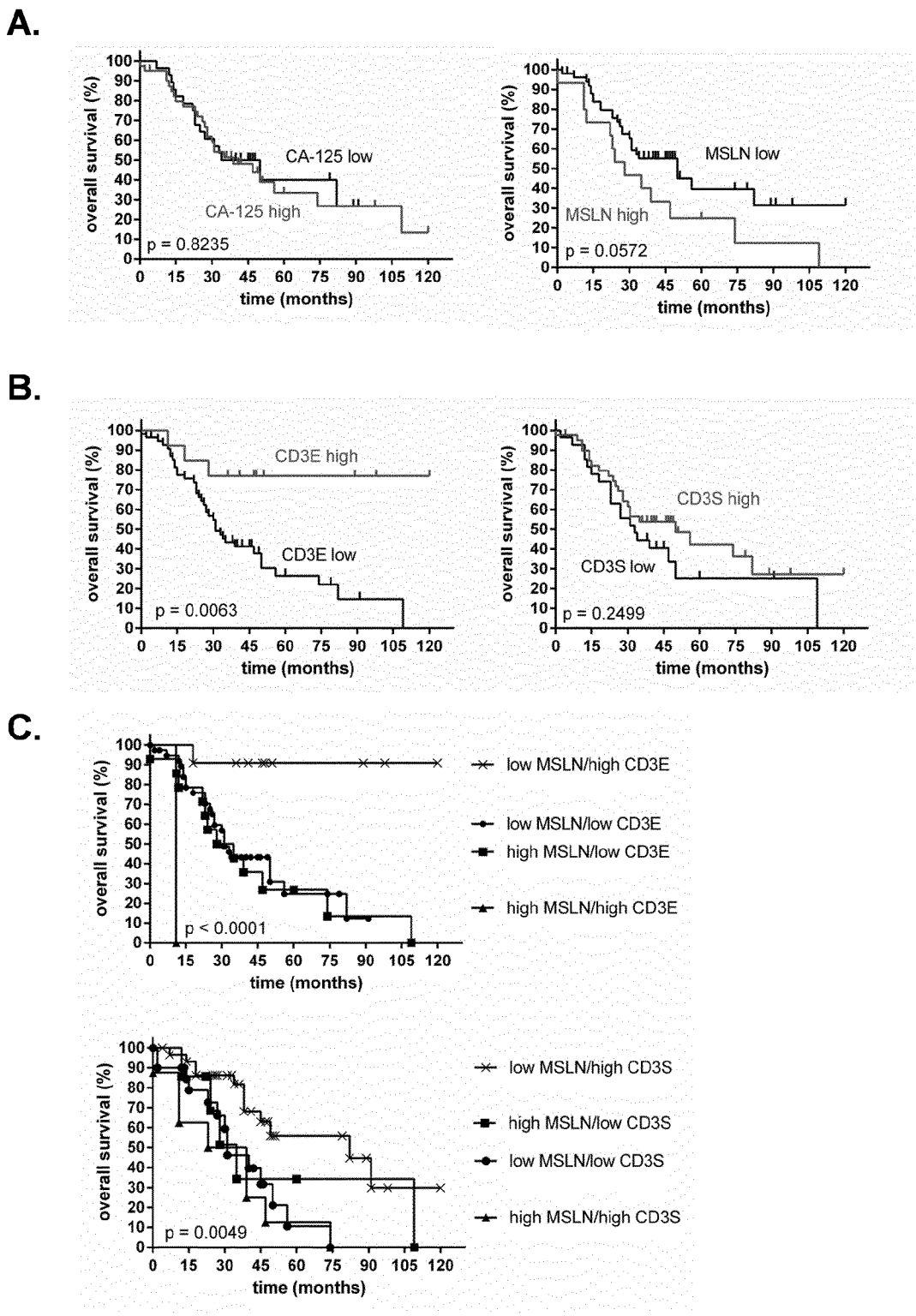

A.
overall survival (%)
CA-125 low
CA-125 high
p = 0.8235
time (months)
MSLN low
MSLN high
p = 0.0572
B.
CD3E high
CD3E low
p = 0.0063
CD3S high
CD3S low
p = 0.2499
C.
low MSLN/high CD3E
low MSLN/low CD3E
high MSLN/low CD3E
high MSLN/high CD3E
p < 0.0001
low MSLN/high CD3S
high MSLN/low CD3S
low MSLN/low CD3S
high MSLN/high CD3S
p = 0.0049

Figure 6

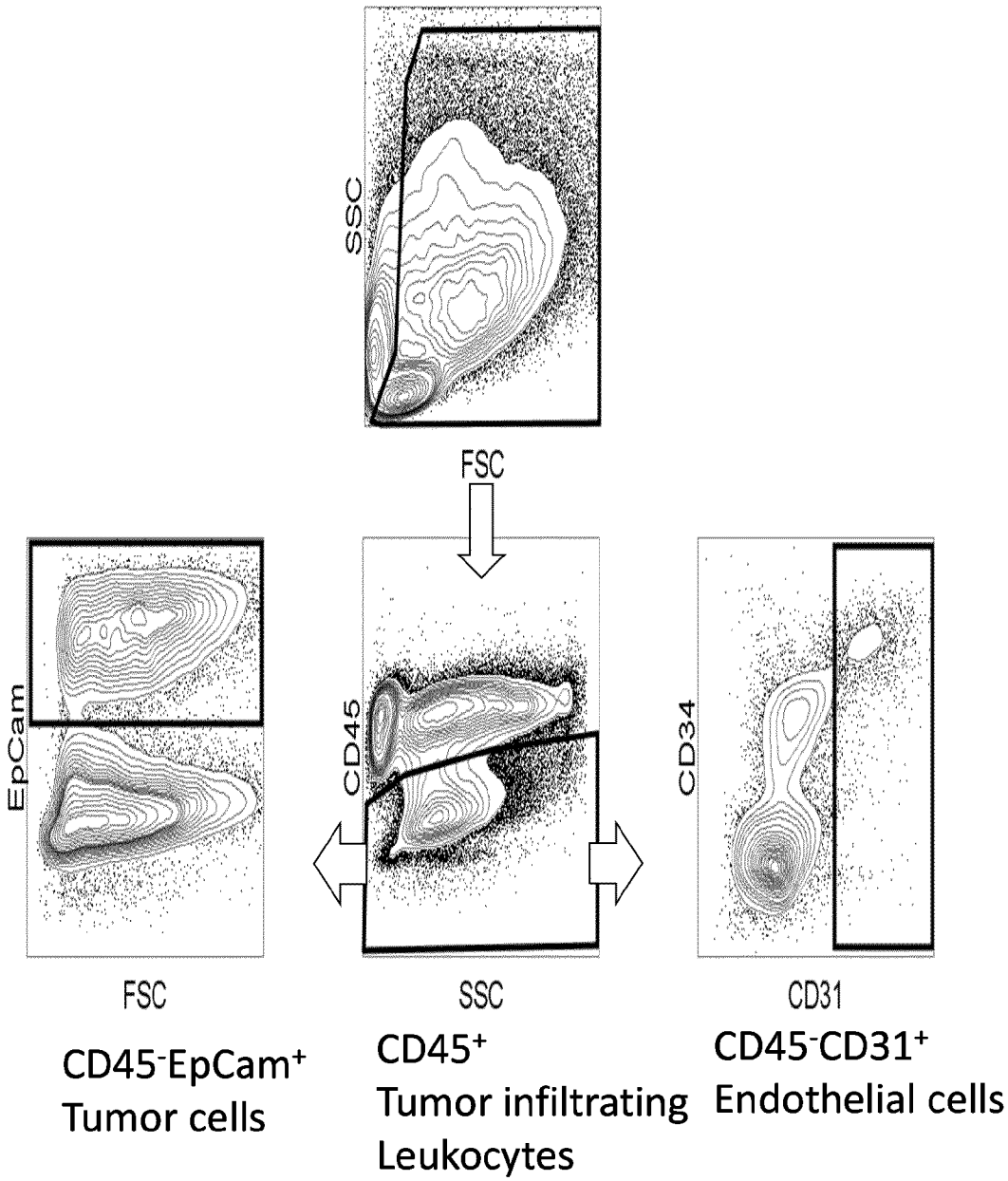

SSC
FSC
EpCam
CD45
CD34
FSC
SSC
CD31
CD45-EpCam+
Tumor cells
CD45+
Tumor infiltrating
Leukocytes
CD45-CD31+
Endothelial cells

Figure 7

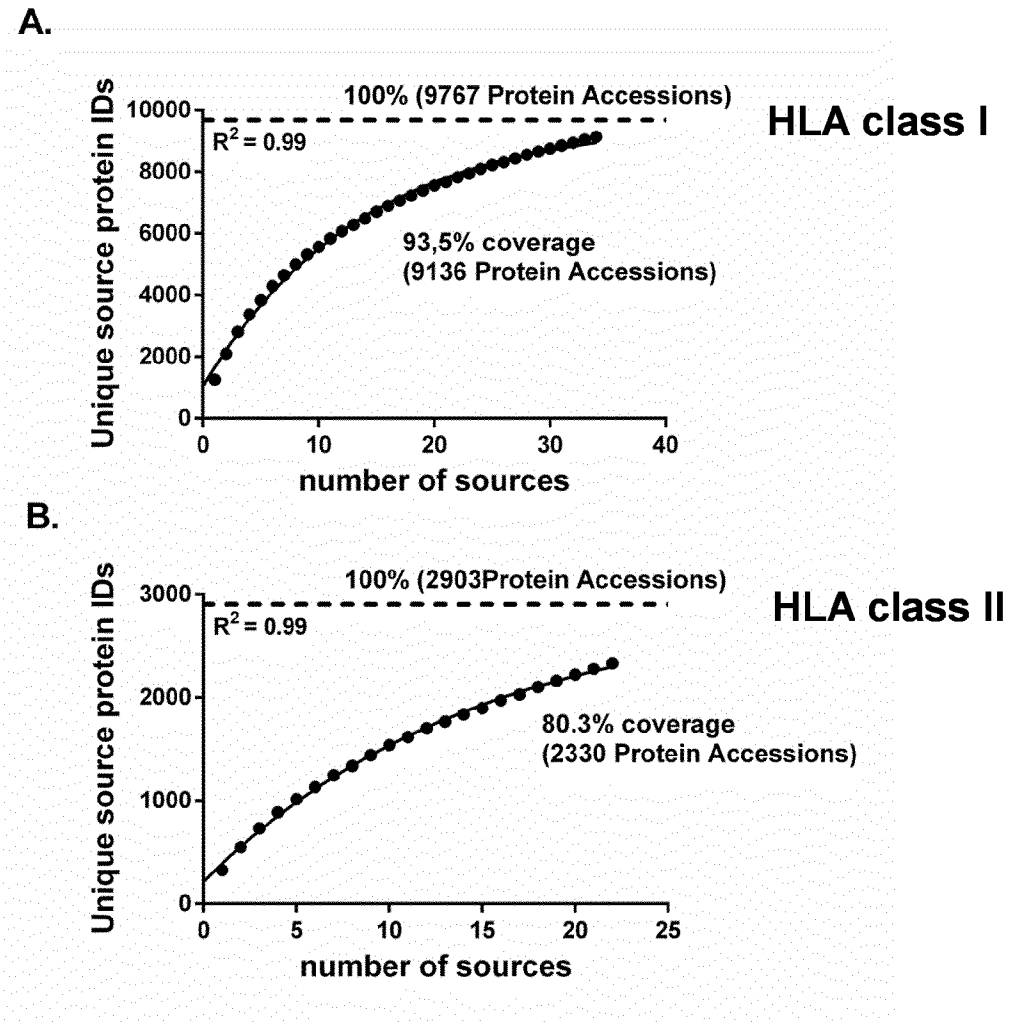
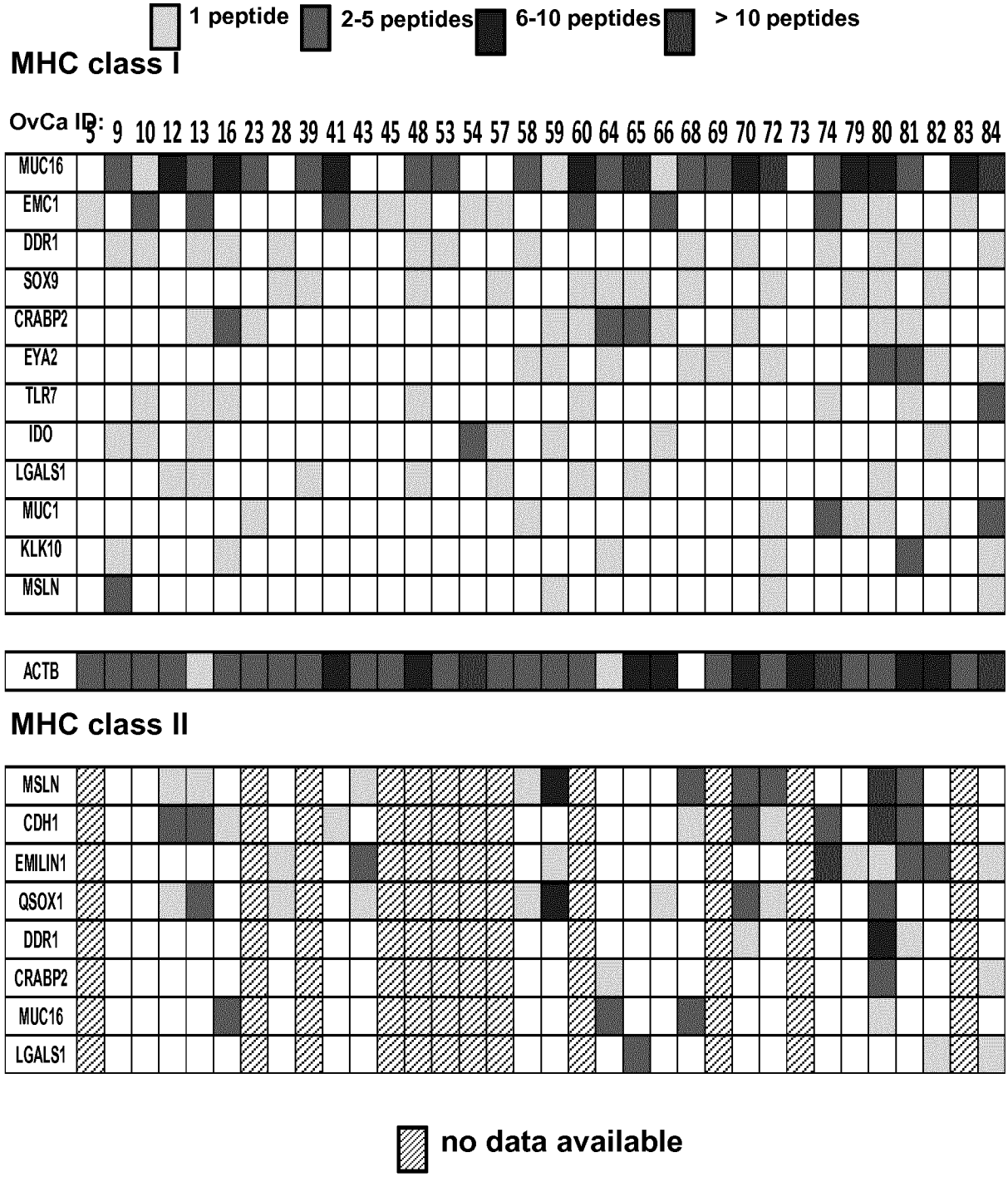

A.
100% (9767 Protein Accessions)
HLA class I
R^2 = 0.99
93,5% coverage
(9136 Protein Accessions)
Unique source protein IDs
number of sources
B.
100% (2903Protein Accessions)
HLA class II
R^2 = 0.99
80.3% coverage
(2330 Protein Accessions)
Unique source protein IDs
number of sources

Figure 8


1 peptide
2-5 peptides
6-10 peptides
> 10 peptides
MHC class I
OvCa ID:
5 9 10 12 13 16 23 28 39 41 43 45 48 53 54 57 58 59 60 64 65 66 68 69 70 72 73 74 79 80 81 82 83 84
MUC16
EMC1
DDR1
SOX9
CRABP2
EYA2
TLR7
IDO
LGALS1
MUC1
KLK10
MSLN
ACTB
MHC class II
MSLN
CDH1
EMILIN1
QSOX1
DDR1
CRABP2
MUC16
LGALS1
no data available

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066706

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

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MICHAL BASSANI-STERNBERG ET AL: "Mass Spectrometry of Human Leukocyte Antigen Class I Peptidomes Reveals Strong Effects of Protein Abundance and Turnover on Antigen Presentation", MOLECULAR & CELLULAR PROTEOMICS, vol. 14, no. 3, 2 March 2015 (2015-03-02), pages 658-673, XP055272560, US ISSN: 1535-9476, DOI: 10.1074/mcp.M114.042812	1-4
Y	page 661, column 1, paragraph 3; table S1; sequence 13448	5-20, 30-39

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2016	22/12/2016

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

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/028573 A1 (IMMATICS BIOTECHNOLOGIES GMBH [DE]; SINGH HARPREET [DE]; EMMERICH NIEL) 15 March 2007 (2007-03-15) claims 1-36 -----	5-20, 30-39
Y	WO 2014/011465 A2 (EINSTEIN COLL MED [US]) 16 January 2014 (2014-01-16) claims 1,13 -----	39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/066706

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-20, 30-39(all partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-20, 30-39(all partially)

A peptide comprising an amino acid sequence consisting of SEQ ID No. 1 and variant sequences thereof which are at least 88% homologous to SEQ ID No. 1, 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. Subject-matter related thereto.

2-549. claims: 1-20, 30-39(all partially)

Idem to invention 1 but relating to each of SEQ ID NO: 2-549

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550. claims: 21-29

A method for producing a personalized anti-cancer vaccine for 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 tissuesc) selecting at least one peptide from the warehouse that matches a TUMAP identified in the patient; andd) manufacturing and/or formulating sid personalized vaccine based on step c).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/066706

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007028573 A1	15-03-2007	AT 440107 T	15-09-2009
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		US 2009274714 A1	05-11-2009
		WO 2007028573 A1	15-03-2007
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		WO 2014011465 A2	16-01-2014