

(19) **DANMARK**

(10) **DK/EP 3066115 T3**



(12)

Oversættelse af europæisk patentskrift

Patent- og
Varemærkestyrelsen

-
- (51) Int.Cl.: **C 07 K 14/47 (2006.01)** **A 61 K 38/17 (2006.01)** **A 61 K 39/00 (2006.01)**
C 07 K 7/06 (2006.01) **C 07 K 7/08 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2019-06-11**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2019-04-03**
- (86) Europæisk ansøgning nr.: **14792834.5**
- (86) Europæisk indleveringsdag: **2014-11-03**
- (87) Den europæiske ansøgnings publiceringsdag: **2016-09-14**
- (86) International ansøgning nr.: **EP2014073588**
- (87) Internationalt publikationsnr.: **WO2015063302**
- (30) Prioritet: **2013-11-04 US 201361899680 P** **2013-11-04 GB 201319446**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **PERSONALIZED IMMUNOTHERAPY AGAINST SEVERAL NEURONAL AND BRAIN TUMORS**
- (56) Fremdragne publikationer:
EP-A1- 2 111 867
WO-A1-00/66734
WO-A1-02/06338
WO-A1-2011/051276
WO-A1-2011/051278
WO-A1-2011/128448
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Fortsættes ...

CHI-CHENG HUANG ET AL: "Concurrent Gene Signatures for Han Chinese Breast Cancers", PLOS ONE, vol. 8, no. 10, 3 October 2013 (2013-10-03), page e76421, XP055355956, DOI: 10.1371/journal.pone.0076421

DESCRIPTION

[0001] The present invention relates to a peptide, 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 a tumor-associated cytotoxic T cell (CTL) peptide epitope, alone or in combination with other tumor-associated peptides that serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses. The present invention relates to a specific peptide sequence derived from a HLA class I molecule of human tumor cells that can be used in vaccine compositions for eliciting anti-tumor immune responses as well as a method for providing optimal vaccines to persons in need.

Background of the invention

[0002] Gliomas are brain tumors originating from glial cells in the nervous system. Glial cells, commonly called neuroglia or simply glia, are non-neuronal cells that provide support and nutrition, maintain homeostasis, form myelin, and participate in signal transmission in the nervous system. The two most important subgroups of gliomas are astrocytomas and oligodendrogliomas, named according to the normal glial cell type from which they originate (astrocytes or oligodendrocytes, respectively). Belonging to the subgroup of astrocytomas, glioblastoma multiforme (referred to as glioblastoma hereinafter) is the most common malignant brain tumor in adults and accounts for approx. 40% of all malignant brain tumors and approx. 50% of gliomas. It aggressively invades the central nervous system and is ranked at the highest malignancy level (grade IV) among all gliomas. Although there has been steady progress in their treatment due to improvements in neuroimaging, microsurgery, diverse treatment options, such as temozolomide or radiation, glioblastomas remain incurable. The lethal rate of this brain tumor is very high: the average life expectancy is 9 to 12 months after first diagnosis. The 5-year survival rate during the observation period from 1986 to 1990 was 8.0%. To date, the five-year survival rate following aggressive therapy including gross tumor resection is still less than 10%. Accordingly, there is a strong medical need for an alternative and effective therapeutic method.

[0003] Tumor cells of glioblastomas are the most undifferentiated ones among brain tumors, so the tumor cells have high potential of migration and proliferation and are highly invasive, leading to very poor prognosis. Glioblastomas lead to death due to rapid, aggressive, and infiltrative growth in the brain. The infiltrative growth pattern is responsible for the unresectable nature of these tumors. Glioblastomas are also relatively resistant to radiation and chemotherapy, and, therefore, post-treatment recurrence rates are high. In addition, the immune response to the neoplastic cells is rather ineffective in completely eradicating all neoplastic cells following resection and radiation therapy.

[0004] Glioblastoma is classified into primary glioblastoma (*de novo*) and secondary

glioblastoma, depending on differences in the gene mechanism during malignant transformation of undifferentiated astrocytes or glial precursor cells. Secondary glioblastoma occurs in a younger population of up to 45 years of age. During 4 to 5 years, on average, secondary glioblastoma develops from lower-grade astrocytoma through undifferentiated astrocytoma. In contrast, primary glioblastoma predominantly occurs in an older population with a mean age of 55 years. Generally, primary glioblastoma occurs as fulminant glioblastoma characterized by tumor progression within 3 months from the state with no clinical or pathological abnormalities (Pathology and Genetics of the Nervous Systems. 29-39 (IARC Press, Lyon, France, 2000)).

[0005] Glioblastoma migrates along myelinated nerves and spreads widely in the central nervous system. In most cases surgical treatment shows only limited sustainable therapeutic effect. Malignant glioma cells evade detection by the host's immune system by producing immunosuppressive agents that impair T cell proliferation and production of the immune-stimulating cytokine IL-2.

[0006] Intracranial neoplasms can arise from any of the structures or cell types present in the CNS, including the brain, meninges, pituitary gland, skull, and even residual embryonic tissue. The overall annual incidence of primary brain tumors in the United States is 14 cases per 100,000. The most common primary brain tumors are meningiomas, representing 27% of all primary brain tumors, and glioblastomas, representing 23% of all primary brain tumors (whereas glioblastomas account for 40% of malignant brain tumor in adults). Many of these tumors are aggressive and of high grade. Primary brain tumors are the most common solid tumors in children and the second most frequent cause of cancer death after leukemia in children.

[0007] The search for effective treatment of glioblastomas in patients is still ongoing today. Immunotherapy or treatment via recruitment of the immune system, to fight these neoplastic cells has been investigated.

[0008] There is an ongoing clinical trial with IMA950, a multi-peptide vaccine conducted in the UK by immatics biotechnologies (Tübingen, Germany). The peptides in the vaccine are exclusively HLA-A*02 peptides.

[0009] WO 2011/051278 A1, WO 2011/051276 A1, WO 2012/031122 A2, and Chi-Cheng Huang et al.: "Concurrent Gene Signatures for Han Chinese Breast Cancers", PLoS ONE, vol. 8, no. 10, 3 October 2013 page e76421) disclose proteins and gene expression microarrays that were used to derive gene signatures for e.g. breast cancers, i.e. diagnosis. WO 2011/051278 A1 and WO 2011/051276 A1 disclose affinity ligands in the form of antibodies. WO 2012/031122 A2 discloses autoantibody reactivity, a mechanism different from the MHC-system.

[0010] There remains a need for new efficacious and safe treatment option for glioblastoma and medulloblastoma and other tumors which show an overexpression of the proteins of the

present invention, enhancing the well-being of the patients with other HLA alleles or combinations of alleles without using chemotherapeutic agents or other agents which may lead to severe side effects.

Summary of the invention

[0011] In a first aspect of the present invention, the present invention relates to a peptide consisting of SEQ ID No. 28, or a pharmaceutical acceptable salt thereof.

[0012] The following tables show the peptides as disclosed, their respective SEQ ID NO, and the prospective source proteins for these peptides. All peptides in Tables 1a, 1b and 1c bind to the HLA-A*02 allele, peptides in Table 1d and 1e bind to HLA-DR alleles. SEQ ID NO: 28 is according to the invention. The class II peptides in table 1d and 1e are particularly useful in the treatment of cancers over-expressing and/or over-presenting the polypeptides BCAN, BIRC5 and/or PTPRZ1.

Table 1a: Peptides as disclosed

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
1	CSRP2-001	RLGIKPESV	CSRP2
2	SLC10A4-001	ALAFKLDEV	SLC10A4
3	ELOVL2-001	YLPTFFLTV	ELOVL2
4	MTSS1L -001	GLPSGAPPGV	MTSS1L
5	PTP-013	MIWEHNVEV	PTPRZ1
6	KIF1A-001	LLWGNAIFL	KIF1A
7	PCDHGC5-001	GLDPSSGAIHV	PCDHGC5
8	GRIK3-001	LLYDAVHIV	GRIK3
9	SEZ6L-001	LLLGSPAAA	SEZ6L
10	ANKRD40-001	ALGDIREV	ANKRD40
11	NLGN4Y-001	SLDTLMTYV	NLGN4Y
12	KCN-002	ALSVRISNV	KCNJ10
13	BCA-003	FLWSDGVPL	BCAN
14	MAGI2-001	AVAPGPWKV	MAGI2
15	PTP-012	FLLPDTDGLTAL	PTPRZ1
16	SCARA3-001	SLGLFLAQV	SCARA3
17	GRI-002	VLIQDVPTL	GRIA4
18	CLU-001	KLFDSDPITVTV	CLU
19	CERS1-001	FLHDISDVQL	CERS1
20	SLC10A4-002	RVADYIVKV	SLC10A4
21	GPR98-001	ALFNKGGSVFL	GPR98

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
22	GYG2-001	KVFDEVIEV	GYG2
23	CPT1C-001	GLMEKIKEL	CPT1C
24	SLC35E1-002	GMMTAILGV	SLC35E1
25	PTP-002	FLYKVILSL	PTPRZ1
26	PTP-001	ALTTLMHQL	PTPRZ1
27	ASIC4-001	EILDYIYEV	ASIC4
28	COL20-001	FLVDGSWSI	COL20A1
29	EGFR-008	YQDPHSTAV	EGFR
30	JAK-001	KLTDIQIEL	JAKMIP2/JAKMIP3
31	WLS-002	TMMSRPPVL	WLS/MIER1
32	IRS-001	RVAS*PTSGV	IRS2
33	NAT8L-001	SLAERLFFQV	NAT8L
34	TNC-001	AMTQLLAGV	TNC
35	MAP1B-002	GLSEFTEYL	MAP1B
36	NCAN-001	VLGPPPAV	NCAN
37	ADORA3-001	ALADIAVG	ADORA3
38	NPAS3-001	LLYTGDLAL	NPAS3
39	NLGN4X-002	GLLDQIQAL	NLGN4Y/NLGN3/NLGN4X/NLGN2
40	GRI-001	NILEQIVSV	GRIA4
41	DPP3-001	FLYNEALYSL	DPP3/BBS 1
S* = optionally phosphorylated serine			

Table 1b: Additional peptides as disclosed

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
42	USP11-001	MLFGHPLLVS	USP11
43	EIF4E-001	RLISKFDTV	EIF4E
44	PLEKHA4-001	LLQDRLVS	PLEKHA4
45	CCT-001	TLLAAEFLKQV	CCT7
46	NOC4-001	LTAPPEALLMV	NOC4L
47	MAP1B-001	FLDSKFYLL	MAP1B
48	CHCHD2-005	KLCEGFNEV	CHCHD2
49	SOX-001	KLADQYPHL	SOX8/ SOX9/ SOX10

Table 1c: Additional peptides that are over-expressed in glioblastoma

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
50	PTP-005	KVFAGIPTV	PTPRZ1

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
51	BCA-002	ALWAWPSEL	BCAN
52	CDK4-001	TLWYRAPEV	CDK4/CDK6
53	MAGEF1-001	ILFPDIIARA	MAGEF1
54	PTP-003	AIIDGVESV	PTPRZ1
55	NLGN4X-001	NLDTLMTYV	NLGN4X
56	VPS13B-001	SLWGGDVVL	VPS13B
57	NRCAM-001	GLWHHQTEV	NRCAM
58	RAD54B-001	SLYKGLLSV	RAD54B
59	FABP7-001	LTFGDVVAV	FABP7
60	CSP-001	TMLARLASA	CSPG4
61	ORMDL1-002	TLTNIIHNL	ORMDL1
62	TACC3-001	KLVEFDLGA	TACC3
63	DCA-001	KLGDFFLATVV	DCLK2
64	PCNXL3-001	GVLENIFGV	PCNXL3
65	DPYSL4-001	NLLAEIHGV	DPYSL4
66	IGF2BP3-001	KIQEILTQV	IGF2BP3
67	DROSHA-001	AVVEFLTSV	DROSHA
68	ABCA13-001	ILFEINPKL	ABCA13
69	CCNB1-002	ILIDWLQVQ	CCNB1
70	CNOT1-002	SLADFMQEV	CNOT1

Table 1d: MHC class II peptides as disclosed

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
71	BCA-005	VKVNEAYRFRVALPAYPA	BCAN

Table 1e: Additional MHC class II peptides

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
72	BIR-002	TLGEFLKLDREERAKN	BIRC5
73	PTP-010	EIGWSYTGALNQKN	PTPRZ1

[0013] Tables 2a and b show additional peptides as disclosed, their respective SEQ ID NO, and the source proteins from which these peptides may arise. All peptides in tables 2 bind to the HLA A*24 alleles.

Table 2a: Additional peptides as disclosed

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
74	TMEM255A-001	YYPGVILGF	TMEM255A
75	ST8SIA5-001	VYYFHPQYL	ST8SIA5

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
76	FAM120C-001	MYPYIYHVL	FAM120C
77	GRIK3-002	YYHFIFTTL	GRIK3
78	PTP-014	YYTVRNFTL	PTPRZ1
79	PTP-019	NYTSLLVTW ⁺⁴	PTPRZ1
80	FABP7-002	EYMKALGVGF	FABP7
81	ZNF3-001	KYNDFGNSF	ZNF3
82	DOCK7-002	LYIYPQSLNF	DOCK7
83	LOC72839-001	IFTYIHLQL	LOC728392
84	PJA2-001	RYQESLGNTVF	PJA2
85	HEATR1-001	KYNEFSVSL	HEATR1
86	GPM-002	TYNYAVLKF	GPM6B
87	CRB1-001	SYFENVHGF	CRB1
88	PTP-016	VYDTMIEKF	PTPRZ1
89	PTP-015	QYVFIHDTL	PTPRZ1
90	PTP-018	NYTSLLVTW	PTPRZ1
91	OLIG2-001	IYGGHHAGF	OLIG2
92	VCAN-003	TYVDSSHTI	VCAN
93	SMOX-001	VYNLTQEFF	SMOX
94	EXOC7-001	YYQIRSSQL	EXOC7
95	LZTS 1-001	RYSDGLLRF	LZTS1
96	FADS2-003	QYQIIMTMI	FADS2
97	TMEM231-001	TYIPPLLVAF	TMEM231
98	ASCL1-001	EYIRALQQL	ASCL1
99	UNKN-003	TYIIKSVGf	TXN2
100	NKA-001	QWAPILANF	NKAIN1/NKAIN2/N KAIN4
101	PCD-002	RYGPQFTL	PCDHG-Family
102	ARHGAP21-001	RYIPLVDI	ARHGAP21
103	PNMA2-001	AYVLRLETL	PNMA2
104	FADS2-002	PYNHQHEYF	FADS2
105	APC-001	VLPDADTLLHF	APC
106	WASL-001	FYGPQVNNI	WASL/ASB15
107	SLC-002	KYFSFPGEL	SLC1A3/SLC1A6
108	TENM4-001	AYSDGHFLF	TENM4
109	ZNF749-001	RYLPSSVFL	ZNF749

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
110	EFCAB7-001	VYLTIKPLNL	EFCAB7
111	DOCK7-003	PYLDKFFAL	DOCK7
112	BMP7-001	VYQVLQEHL	BMP7
113	ITGA7-001	AFSPDSHYLLF	ITGA7
114	RPL-001	NYNDRYDEI	RPL7A
115	HS2-001	KYNLINEYF	HS2ST1
116	VIM-002	NYQDTIGRL	VIM
117	IFT17-001	AYLIDIKTI	IFT 172
118	GAB-001	AYPRLSLSF	GABRB1/ GABRB3
119	CDCA7L-001	KFAEEFYSF	CDCA7L
120	SCARA3-002	YYLDKSVSI	SCARA3
121	SSR1-001	NYKDLNGNVF	SSR1
122	NR0B 1-001	AYLKGTVLF	NR0B1
123	LNK1-001	NYIDNVGNLHF	LNK1
124	EP4-001	PFAKPLPTF	EP400
125	KIF1B-001	VYLKEANAI	KIF1B
126	RHOBTB3-001	KYFGGVLEYF	RHOBTB3
127	KIF7-001	KYFDKVVTL	KIF7
128	KIF1B-002	VYNDIGKEMLL	KIF1B
129	MAPK6-001	TYTSYLDKF	MAPK6

[0014] The peptide according to SEQ ID NO 101 can be derived from any of the following proteins: PCDHGA12, PCDHGC3, PCDHGC5, PCDHGC4, PCDHGB7, PCDHGB6, PCDHGB5, PCDHGB3, PCDHGB2, PCDHGB1, PCDHGA11, PCDHGA10, PCDHGA9, PCDHGA7, PCDHGA6, PCDHGA5, PCDHGA4, PCDHGA3, PCDHGA2, PCDHGA, PCDHGB4, or PCDHGA8. The peptide according to SEQ ID NO 109 is a frameshift of EVPSKQCVS; chr 19, 2+ frame: 57954686-57954712. W⁺⁴ : Kynurenine ((S)-2-amino-4-(2-aminophenyl)-4-oxo-butanoic acid). The peptide according to SEQ ID NO: 99 is part of the first intron of TXN2 (supported by a matching EST, BG169743.1).

Table 2b: Additional peptides that are over-expressed in glioblastoma

SEQ ID NO:	Peptide Code	Sequence	Source Protein(s)
130	ASPM-002	SYNPLWLRI	ASPM
131	SMC4-001	HYKPTPLYF	SMC4

Table 2c: Additional indications (e.g. cancers to be treated) based on the peptides as disclosed overexpressed and/or overpresented in said indications

SEQ ID NO	Sequence	Peptide Code	Additional Indication(s)
1	RLGIKPESV	CSRP2-001	Liver, Prostate
2	ALAFKLDEV	SLC10A4-001	Lung
3	YLPTFFLTV	ELOVL2-001	Kidney, Liver
4	GLPSGAPPG V	MTSS1L-001	Kidney, Liver
8	LLYDAVHIV	GRIK3-001	Leukaemia
9	LLLGSPAAA	SEZ6L-001	Pancreas
10	ALGDIREV	ANKRD40-001	Kidney, Colon, Rectum, Liver
11	SLDTLMTYV	NLGN4Y-001	Colon, Rectum, Prostate, Leukaemia
12	ALSVRISNV	KCN-002	Kidney, Liver, Pancreas
14	AVAPGPWKV	MAGI2-001	Liver
18	KLFDSDPITV TV	CLU-001	Liver
24	GMMTAILGV	SLC35E1-002	Liver
29	YQDPHSTAV	EGFR-008	Kidney, Liver
30	KLTDIQIEL	JAK-001	Prostate
32	RVASPTSGV	IRS-001	Liver
34	AMTQLLAGV	TNC-001	Lung, Colon, Rectum
35	GLSEFTEYL	MAP1B-002	Kidney, Prostate
37	ALADIAVGV	ADORA3-001	Lung, Kidney, Pancreas, Prostate
40	NILEQIVSV	GRI-001	Kidney
42	MLFGHPLL SV	USP11-001	Lung, Kidney, Liver, Pancreas, Prostate
43	RLISKFDTV	EIF4E-001	Lung, Colon, Rectum, Liver, Prostate
44	LLQDRLVSV	PLEKHA4-001	Colon, Rectum, Liver
45	TLLAAEFLK QV	CCT-001	Lung, Liver
	LTAPPEALL		

SEQ ID NO	Sequence	Peptide Code	Additional Indication(s)
46	MV	NOC4-001	Lung, Kidney, Colon, Rectum, Liver, Pancreas
47	FLDSKFYLL	MAP1B-001	Kidney, Liver, Prostate
48	KLCEGFNEV	CHCHD2-005	Colon, Rectum, Liver
52	TL WYRAPEV	CDK4-001	Lung, Kidney, Stomach, Colon, Rectum, Liver
53	ILFPDIIARA	MAGEF1-001	Lung, Kidney, Colon, Rectum, Liver, Leukaemia
56	SLWGGDVVL	VPS13B-001	Lung, Colon, Rectum, Liver, Prostate
58	SLYKGLLSV	RAD54B-001	Lung, Kidney, Colon, Rectum, Prostate
59	LTFGDVVAV	FABP7-001	Stomach
60	TMLARLASA	CSP-001	Kidney
61	TLTNIHNL	ORMDL1-002	Lung, Kidney, Liver, Leukaemia
62	KLVEFDLGA	TACC3-001	Lung, Stomach, Colon, Rectum, Liver
64	GVLENIFGV	PCNXL3-001	Lung, Kidney, Stomach, Colon, Rectum, Liver, Prostate
65	NLLAEIHGV	DPYSL4-001	Kidney
66	KIQEILTQV	IGF2BP3-001	Lung, Kidney, Stomach, Colon, Rectum, Liver, Pancreas, Leukaemia
67	AVVEFLTSV	DROSHA-001	Lung, Kidney, Stomach, Colon, Rectum, Liver, Pancreas
68	ILFEINPKL	ABCA13-001	Lung, Leukaemia
69	ILIDWLVQV	CCNB1-002	Lung, Kidney, Stomach, Colon, Rectum, Liver, Pancreas
70	SLADFMQEV	CNOT1-002	Lung, Kidney, Colon, Rectum, Pancreas
74	YYPGVILGF	TMEM255A-001	Lung
81	KYNDFGNSF	ZNF3-001	Lung, Liver
82	LYIYPQSLNF	DOCK7-002	Lung, Kidney, Liver
83	IFTYIHLQL	LOC72839-001	Liver

SEQ ID NO	Sequence	Peptide Code	Additional Indication(s)
92	TYVDSSTI	VCAN-003	Lung, Stomach, Liver
93	VYNLTQEFF	SMOX-001	Lung, Kidney, Stomach
94	YYQIRSSQL	EXOC7-001	Lung, Stomach, Liver
96	QYQIIMTMI	FADS2-003	Liver
97	TYIPPLLVAF	TMEM231-001	Lung, Kidney, Stomach, Liver
103	AYVLRLETL	PNMA2-001	Lung
104	PYNHQHEYF	FADS2-002	Lung, Liver
105	VLPDADTLL HF	APC-001	Liver
108	AYSDGHFLF	TENM4-001	Lung, Kidney, Stomach, Prostate
109	RYLPSSVFL	ZNF749-001	Lung, Stomach, Liver
110	VYLTIKPLN L	EFCAB7-001	Lung, Stomach, Liver
112	VYQVLQEHL	BMP7-001	Stomach
113	AFSPDSHYL LF	ITGA7-001	Lung, Kidney, Liver
115	KYNLINEYF	HS2-001	Lung, Kidney, Liver
116	NYQDTIGRL	VIM-002	Kidney
117	AYLIDIKTI	IFT17-001	Lung, Kidney, Liver
118	AYPRLSLSF	GAB-001	Liver
119	KFAEEFYSF	CDCA7L-001	Lung, Kidney, Stomach
122	AYLKGTVLF	NROB1-001	Lung
124	PFAKPLPTF	EP4-001	Lung, Kidney, Stomach, Liver
126	KYFGGVLEY F	RHOBTB3-001	Lung, Stomach, Liver
127	KYFDKVVTL	KIF7-001	Lung, Liver, Prostate
129	TYTSYLDKF	MAPK6-001	Lung, Liver
130	SYNPLWLRI	ASPM-002	Lung, Stomach, Liver
131	HYKPTPLYF	SMC4-001	Lung, Stomach, Liver, Prostate

[0015] Thus, another preferred aspect of the present invention relates to the use of the peptide according to the present invention for the - preferably combined - preferred immunotherapy of cancerous diseases according to the table 2c as above in analogy to the uses as described herein for, e.g., glioblastoma.

[0016] The peptides according to the present invention has the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I.

[0017] The present invention further relates to the peptide according to the present invention, wherein said peptide includes non-peptide bonds.

[0018] The present invention further relates to a nucleic acid, encoding the peptide according to the present invention.

[0019] The present invention further relates to the nucleic acid according to the present invention that is DNA, cDNA, RNA or combinations thereof.

[0020] The present invention further relates to an expression vector expressing a nucleic acid according to the present invention.

[0021] 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 a disease.

[0022] The present invention further relates to an antibody that specifically recognizes the peptide according to the present invention.

[0023] The present invention further relates to a T-cell receptor that is reactive with an HLA ligand consisting of the amino acid sequence of SEQ ID No. 28.

[0024] 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.

[0025] 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.

[0026] The present invention further relates to a method of producing a peptide according to the present invention, the method comprising culturing the host cell according to the present invention, and isolating the peptide from the host cell or its culture medium.

[0027] The present invention further relates to an in vitro method for producing activated cytotoxic T lymphocytes (CTL), the method comprising contacting in vitro CTL with antigen

loaded human class I MHC molecules expressed on the surface of a suitable antigen-presenting cell for a period of time sufficient to activate said CTL in an antigen specific manner, wherein said antigen is any peptide according to the present invention.

[0028] The present invention further relates to the method according to the present invention, wherein the antigen is loaded onto class I 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.

[0029] The present invention further relates to activated cytotoxic T lymphocytes (CTL), 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.

[0030] Disclosed is 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 cytotoxic T lymphocytes (CTL) as according to the present invention.

[0031] The present invention further relates to the peptide, the activated cytotoxic T lymphocyte, the antibody, the T cell receptor, the nucleic acid or the expression vector, or the cell for use in the treatment of a disease, wherein said disease is cancer, preferably in form of a pharmaceutical composition, e.g. vaccine.

[0032] Further, the present invention discloses a method for providing and producing vaccines for patient pool with a specific set of alleles and / or patient specific.

[0033] 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.

[0034] The present invention further relates to a method of producing a peptide according to the present invention, the method comprising culturing the host cell according to the present invention, and isolating the peptide from the host cell or its culture medium.

[0035] The present invention further relates to an in vitro method for producing activated cytotoxic T lymphocytes (CTL), the method comprising contacting in vitro CTL with antigen loaded human class I MHC molecules expressed on the surface of a suitable antigen-presenting cell for a period of time sufficient to activate said CTL in an antigen specific manner, wherein said antigen is any peptide according to the present invention.

[0036] The present invention further relates to the method according to the present invention,

wherein the antigen is loaded onto class I 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 said antigen-presenting cell comprises an expression vector expressing said peptide containing the sequence of SEQ ID No. 28.

[0037] The present invention further relates to activated cytotoxic T lymphocytes (CTL) as described herein, 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.

[0038] Further, the present invention discloses a method for producing a personalized anti-cancer vaccine comprising at least one peptide according to the present invention, a nucleic acid according to the present invention, an expression vector according to the present invention, a host cell or cell according to the present invention, or an activated cytotoxic T lymphocyte according to the present invention which has been designed and formulated for use in an individual patient, wherein said design comprises the use of a database ("warehouse") of pre-selected and/or pre-screened tumour associated peptides that are patient- and/or patient-group and/or cancer-specific

[0039] The peptides of the present invention can be used to generate, produce and develop specific antibodies against the MHC/peptide complexes of the present invention (i.e. comprising the sequence of SEQ ID No. 28). These antibodies can be used for therapy, targeting toxins or radioactive substances to a diseased tissue, e.g. a tumour. Another use of these antibodies can be targeting radionuclides to the diseased tissue for imaging purposes such as PET.

[0040] 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 being complexed with an HLA-restricted antigen (i.e. comprising the sequence of SEQ ID No. 28), the method comprising: immunizing a genetically engineered non-human mammal comprising cells expressing said human major histocompatibility complex (MHC) class I with a soluble form of a MHC class I 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 being complexed with said HLA-restricted antigen.

[0041] It is a further aspect of the invention to provide an antibody that specifically binds to a human major histocompatibility complex (MHC) class I being complexed with a HLA-restricted antigen, wherein the antibody preferably is a polyclonal antibody, monoclonal antibody, bispecific antibody and/or a chimeric antibody.

[0042] Yet another aspect of the present invention then relates to a method of producing an antibody that specifically binds to a human major histocompatibility complex (MHC) class I being complexed with an HLA-restricted antigen (i.e. comprising the sequence of SEQ ID No. 28), the method comprising: immunizing a genetically engineered non-human mammal comprising cells expressing said human major histocompatibility complex (MHC) class I with a soluble form of a MHC class I 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 bindable to said human major histocompatibility complex (MHC) class I being complexed with said HLA-restricted antigen. Respective methods for producing such antibodies and single chain class I major histocompatibility complexes, as well as other tools.

[0043] It is a further aspect of the invention to provide a method for producing a soluble T-cell receptor recognizing a specific peptide-MHC complex as disclosed. 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.

[0044] Stimulation of an immune response is dependent upon the presence of antigens recognised 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.

[0045] Specific elements of the cellular immune response are capable of specifically recognising and destroying tumor cells. The isolation of cytotoxic T-cells (CTL) from tumor-infiltrating cell populations or from peripheral blood suggests that such cells play an important role in natural immune defences against cancer. CD8-positive T-cells in particular, which recognise 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).

[0046] There are two classes of MHC-molecules: MHC class I molecules that can be found on most cells having a nucleus. MHC molecules are composed of an alpha heavy chain and beta-2-microglobulin (MHC class I receptors) or an alpha and a beta chain (MHC class II receptors), respectively. Their three-dimensional conformation results in a binding groove, which is used for non-covalent interaction with peptides. MHC class I present peptides that result from proteolytic cleavage of predominantly endogenous proteins, DRIPs and larger peptides. 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 during the course of endocytosis, and are subsequently processed. Complexes of

peptide and MHC class I molecules are recognized by CD8-positive cytotoxic T-lymphocytes bearing the appropriate TCR (T-cell receptor), 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.

[0047] CD4-positive helper T cells play an important role in inducing and sustaining effective responses by CD8-positive cytotoxic T cells (Wang and Livingstone, 2003; Sun and Bevan, 2003; Shedlock and Shen, 2003). 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 (Kobayashi et al., 2002; Qin et al., 2003; Gnjatic et al., 2003). At the tumor site, T helper cells, support a CTL friendly cytokine milieu (Qin and Blankenstein, 2000; Mortara et al., 2006) and attract effector cells, e.g. CTLs, NK cells, macrophages, (Marzo et al., 2000; Hwang et al., 2007).

[0048] 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 surprisingly been found to express MHC class II molecules (Dengjel et al., 2006).

[0049] It was shown in mammalian animal models, e.g., mice, that even in the absence of CTL effector cells (i.e., CD8-positive T lymphocytes), CD4-positive T cells are sufficient for inhibiting manifestation of tumors via inhibition of angiogenesis by secretion of interferon-gamma (IPNy). Additionally, it was shown that CD4-positive T cells recognizing peptides from tumor-associated antigens presented by HLA class II molecules can counteract tumor progression via the induction of antibody (Ab) responses (Kennedy et al., 2003).

[0050] In contrast to tumor-associated peptides binding to HLA class I molecules, only a small number of class II ligands of tumor associated antigens (TAA) have been described to date.

[0051] Since the constitutive expression of HLA class II molecules is usually limited to cells of the immune system, the possibility of isolating class II peptides directly from primary tumors was not considered possible. However, Dengjel et al. were recently successful in identifying a number of MHC Class II epitopes directly from tumors (WO 2007/028574, EP 1 760 088 B1; (Dengjel et al., 2006).

[0052] The antigens that are recognized by the tumor specific cytotoxic T lymphocytes, that is, their epitopes, 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, up-regulated in cells of the respective tumor.

[0053] 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⁺ CTLs (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.

[0054] Disclosed is also a very useful MHC class II peptide (see SEQ ID NO 71). This peptide is useful against glioblastoma and other cancers over-expressing and / or over - presenting BCAN.

[0055] For a peptide to trigger (elicit) a cellular immune response, it 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.

[0056] In the MHC class I dependent immune reaction, peptides not only have to be able to bind to certain MHC class I molecules being expressed by tumor cells, they also have to be recognized by T cells bearing specific T cell receptors (TCR).

[0057] The antigens that are recognized by the tumor specific cytotoxic T lymphocytes, that is, their epitopes, 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, up-regulated in cells of the respective tumor.

[0058] The current classification of tumor associated antigens comprises the following major groups:

1. 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 or NY-ESO-1.
2. b) Differentiation antigens: These TAAs are shared between tumors and the normal tissue from which the tumor arose; most are found in melanomas and normal melanocytes. Many of these melanocyte lineage-related proteins are involved in the 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.
3. c) Overexpressed 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 overexpression 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.

4. 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.
5. 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.
6. 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.

[0059] For proteins to be recognized by cytotoxic 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 or in another 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 a function e.g. in cell cycle control or suppression of apoptosis. Additionally, downstream targets of the proteins directly causative for a transformation may be upregulated and thus may be indirectly tumor-associated. Such indirect tumor-associated antigens may also be targets of a vaccination approach. In both cases it is essential that epitopes are present in the amino acid sequence of the antigen, since such a peptide ("immunogenic peptide") that is derived from a tumor associated antigen should lead to an in vitro or in vivo T-cell-response.

[0060] Basically, any peptide able to bind a 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

with a corresponding TCR and the absence of immunological tolerance for this particular epitope.

[0061] Therefore, TAAs are a starting point for the development of a tumor vaccine. The methods for identifying and characterizing the TAAs are based on the use of CTL 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.

[0062] 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 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").

[0063] In case of TCRs and antibodies according to the invention the immunogenicity of the underlying peptides is secondary. For TCRs and antibodies according to the invention the presentation is the determining factor.

[0064] T-helper cells 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 T_{H1} 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.

[0065] Uses against additional cancers are disclosed in the following description of the underlying polypeptide of the peptide according to the invention.

Collagen, type XX, alpha 1 (COL20A1)

[0066] COL20A1 is a collagen gene. The COL20A1 gene was mapped to the chromosome 20q13.33 (Deloukas et al., 2001). The function of this gene is still unknown. Recently, a study identified subsets of the concurrent genes associated with breast cancer recurrence, metastases, or mortality in survival analyses. A 16-gene signature, including COL20A1, was established for disease-free survival in Han Chinese breast cancer patients (Huang et al., 2013a).

[0067] Preferred is the peptide according to the invention, an activated cytotoxic T lymphocyte according to the invention, an antibody according to the invention, a T cell receptor according to the invention, the nucleic acid or the expression vector according to the invention, or the cell according to the invention for use in the treatment of cancer, wherein said cancer is selected from

astrocytoma, pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor, oligodendrogliomas, ependymoma, glioblastoma multiforme, mixed gliomas, oligoastrocytomas, medulloblastoma, retinoblastoma, neuroblastoma, germinoma, teratoma, gangliogliomas, gangliocytoma, central gangliocytoma, primitive neuroectodermal tumors (PNET, e.g. medulloblastoma, medulloepithelioma, neuroblastoma, retinoblastoma, ependymblastoma), tumors of the pineal parenchyma (e.g. pineocytoma, pineoblastoma), ependymal cell tumors, choroid plexus tumors, neuroepithelial tumors of uncertain origin (e.g. gliomatosis cerebri, astroblastoma), glioblastoma prostate tumor, breast cancer, esophageal cancer, colorectal cancer, clear cell renal cell carcinoma, lung cancer, CNS, ovarian, melanoma pancreatic cancer, squamous cell carcinoma, leukemia medulloblastoma, colon, rectum, stomach, kidney, lung, pancreatic, prostate, and skin cancer.

[0068] Another aspect of the present invention relates to a kit, comprising: (a) a container that contains a pharmaceutical composition containing a peptide according to the present invention, the nucleic acid or the 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, in solution or in lyophilized form; (b) optionally, a second container containing a diluent or reconstituting solution for the lyophilized formulation; (c) optionally, instructions for the use of the solution and/or the reconstitution and/or use of the lyophilized formulation.

[0069] Disclosed is 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 bindable to said human major histocompatibility complex (MHC) class I or II being complexed with said HLA-restricted antigen.

[0070] Yet another aspect of the present invention relates to an antibody that specifically binds to the peptide according to the present invention, preferably that specifically binds to a human major histocompatibility complex (MHC) class I being complexed with the HLA-restricted antigen and/or the peptide according to the present invention, wherein the antibody preferably is a polyclonal antibody, monoclonal antibody and/or a chimeric antibody.

[0071] The term "peptide" is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The peptides are preferably 9 amino acids in length, but can be as short as 8 amino acids in length, and as long as 10, 11, 12, 13 or 14 and in case of MHC class II peptides they can be as long as 15, 16, 17, 18, 19, 20, 21, 22 or 23 amino acids in length.

[0072] 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.

[0073] 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 oligopeptides are typically less than about 30 amino acid residues in length, and greater than about 15 amino acids in length.

[0074] 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. 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.

[0075] A peptide, oligopeptide, protein or polynucleotide coding for such a molecule is "immunogenic" (and thus is an "immunogen" as disclosed), 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.

[0076] 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 to 14 amino acids in length, and most typically 9 amino acids in length.

[0077] 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.

[0078] For therapeutic and diagnostic purposes, a peptide that binds with appropriate affinity to several different HLA class II receptors is highly desirable. A peptide binding to several different HLA class II molecules is called a promiscuous binder.

[0079] As used herein, reference to a DNA sequence includes both single stranded and double stranded DNA. Thus, 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.

[0080] The coding region can be from a non-mutated ("normal"), mutated or altered gene, or can even be 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.

[0081] The term "nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides.

[0082] 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.

[0083] As used herein the term "a nucleotide coding for 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 going to be expressed.

[0084] 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).

[0085] 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.

[0086] 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.

[0087] 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.

[0088] The term "promoter" means a region of DNA involved in binding of RNA polymerase to initiate transcription.

[0089] 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.

[0090] 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 contemplated. The polypeptides can be in aqueous solution and the purity is then defined by compound purity not taking in account water and determinants used for the solution.

[0091] 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.

[0092] The term "active fragment" means a fragment that generates an immune response (i.e., has immunogenic activity) when administered, alone or optionally with a suitable adjuvant, to an animal, such as a mammal, for example, a rabbit or a mouse, and also including a human, such immune response taking the form of stimulating a T-cell response within the recipient animal, such as a human. Alternatively, the "active fragment" may also be used to

induce a T-cell response in vitro.

[0093] 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.

[0094] In accordance with the present invention, the term "percent identity" or "percent identical", when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The Percent Identity is then determined according to the following formula:

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

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence, wherein

1. (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
2. (ii) each gap in the Reference Sequence and
3. (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 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.

[0095] 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.

[0096] The original peptides 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 these 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."

[0097] 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).

[0098] 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.

[0099] 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, amino acids possessing non-standard R groups (i.e., R groups other than those found in the common 20 amino acids of natural proteins) may also be used for substitution purposes to produce immunogens and immunogenic polypeptides according to the present invention.

[0100] 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 simultaneously be substituted.

[0101] The peptides as disclosed can be elongated by up to four amino acids, meaning 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 can be taken from the following table 3:

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

[0102] The amino acids for the elongation can be the peptides of the original sequence of the protein or any other amino acid. The elongation can be used to enhance the stability or solubility of the peptides.

[0103] 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 CTLs, 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.

[0104] Preferably, when the CTLs specific for a peptide of SEQ ID No. 1 to SEQ ID No. 49, SEQ ID No. 71, and SEQ IDs No. 74 to 129 are tested against (compared with) the substituted peptides, the peptide concentration at which the substituted peptides achieve half the maximal increase in lysis relative to background is no more than about 1 mM, preferably no more than about 1 μ M, more preferably no more than about 1 nM, and still more preferably no more than about 100 pM, and most preferably no more than about 10 pM. It is also preferred that the substituted peptide be recognized by CTLs from more than one individual, at least two, and more preferably three individuals.

[0105] Thus, the epitopes as disclosed may be identical to naturally occurring tumor-associated or tumor-specific epitopes or may include epitopes that differ by no more than 4 residues from the reference peptide, as long as they have substantially identical antigenic activity. Substantially identical antigenic activity means stimulation of T cells in comparable frequencies or numbers with comparable avidity, effector or memory phenotype, similar response pattern.

[0106] 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 now 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 explored for cancer immunotherapy.

[0107] Specific elements of the cellular immune response are capable of specifically

recognizing and destroying tumor cells. The isolation of cytotoxic T-cells (CTL) from tumor-infiltrating cell populations or from peripheral blood suggests that such cells play an important role in natural immune defences 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 12 residues derived from proteins or defect ribosomal products (DRIPS) located in the cytosols, play an important role in this response. The MHC-molecules of the human are also designated as human leukocyte-antigens (HLA).

[0108] MHC class I molecules can be found on most cells having a nucleus which present peptides that result from proteolytic cleavage of mainly endogenous, cytosolic or nuclear proteins, 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.

[0109] 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-positive CTLs (MHC class I molecule) or by CD4-positive CTLs (MHC class II molecule) is important in the development of tumor vaccines. It is therefore an object of the present invention, to provide compositions of peptides that contain peptides binding to MHC complexes of either class.

[0110] Considering the severe side-effects and expense associated with treating cancer better prognosis and diagnostic methods are desperately needed. Therefore, there is a need to identify other factors representing biomarkers for cancer in general and glioblastoma in particular. Furthermore, there is a need to identify factors that can be used in the treatment of cancer in general and glioblastoma in particular.

[0111] The present invention provides a peptide that is useful in treating cancers / tumors, preferably brain cancers, even more preferably glioblastomas that over- or exclusively present the peptides of the invention. This peptide was shown by mass spectrometry to be naturally presented by HLA molecules on primary human glioblastoma samples (see example 1, and figure 1).

[0112] The source gene/protein (also designated "full-length protein" or "underlying protein") from which the peptides are derived were shown to be highly overexpressed in glioblastoma compared with normal tissues (see example 2, and figure 2 for glioblastoma) demonstrating a high degree of tumor association of the source genes. Moreover, the peptides themselves are strongly over-presented on tumor tissue but not on normal tissues (see example 1 and figure 3).

[0113] HLA-bound peptides can be recognized by the immune system, specifically T lymphocytes/T cells. T cells can destroy the cells presenting the recognized HLA/peptide complex, e.g. glioblastoma cells presenting the derived peptides.

[0114] The peptides as disclosed have been shown to be capable of stimulating T cell responses and / or are over-presented and can be used for the production of antibodies and/or sTCRs according to the present invention (see example 3 and 1 and figure 4 and 3). Thus, the peptides 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 as disclosed are not presented on normal tissues in comparable copy numbers, preventing the risk of undesired autoimmune reactions against normal cells in the patient.

[0115] The pharmaceutical compositions comprise the peptides either in the free form or in the form of a pharmaceutically acceptable salt. 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.

[0116] In an especially preferred embodiment, the pharmaceutical compositions comprise the peptides as salts of acetic acid (acetates), tri-fluor acetates or hydrochloric acid (chlorides).

[0117] The peptide 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, disclosed is a method for producing a recombinant antibody specifically binding to a human major histocompatibility complex (MHC) class I 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 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 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 being complexed with a HLA-restricted antigen, wherein the antibody preferably is a polyclonal antibody, monoclonal antibody, bispecific antibody and/or a chimeric antibody.

[0118] Disclosed is a method of producing said antibody specifically binding to a human major histocompatibility complex (MHC) class I 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 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 bindable to said human major histocompatibility complex (MHC) class I being complexed with said HLA-restricted antigen. 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 Cohen CJ, Denkberg G, Lev A, Epel M, Reiter Y. Recombinant antibodies with MHC-restricted, peptide-specific, T-cell receptor-like specificity: new tools to study antigen presentation and TCR-peptide-MHC interactions. *J Mol Recognit.* 2003 Sep-Oct;16(5):324-32.; Denkberg G, Lev A, Eisenbach L, Benhar I, Reiter Y. Selective targeting of melanoma and APCs using a recombinant antibody with TCR-like specificity directed toward a melanoma differentiation antigen. *J Immunol.* 2003 Sep 1;171(5):2197-207; and Cohen CJ, Sarig O, Yamano Y, Tomaru U, Jacobson S, Reiter Y. Direct phenotypic analysis of human MHC class I antigen presentation: visualization, quantitation, and in situ detection of human viral epitopes using peptide-specific, MHC-restricted human recombinant antibodies. *J Immunol.* 2003 Apr 15;170(8):4349-61.

[0119] 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.

[0120] Disclosed is a method for producing a soluble T-cell receptor 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 N, Bossi G, Adams KJ, Lissina A, Mahon TM, Hassan NJ, et al. Monoclonal TCR-redirected tumor cell killing. *Nat Med* 2012 Jun;18(6):980-987). 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 (see Boulter

JM, et al. Stable, soluble T-cell receptor molecules for crystallization and therapeutics. *Protein Eng* 2003 Sep;16(9):707-711.; Card KF, Price-Schiavi SA, Liu B, Thomson E, Nieves E, Belmont H, et al. A soluble single-chain T-cell receptor IL-2 fusion protein retains MHC-restricted peptide specificity and IL-2 bioactivity. *Cancer Immunol Immunother* 2004 Apr;53(4):345-357; and Willcox BE, Gao GF, Wyer JR, O'Callaghan CA, Boulter JM, Jones EY, et al. Production of soluble alphabeta T-cell receptor heterodimers suitable for biophysical analysis of ligand binding. *Protein Sci* 1999 Nov;8(11):2418-2423). The T-cell receptor can be linked to toxins, drugs, cytokines (see 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.

[0121] 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 (J. Pinheiro, D. Bates, S. DebRoy, Sarkar D., R Core team. *nlme: Linear and Nonlinear Mixed Effects Models*. 2008) adjusting for multiple testing by False Discovery Rate (Y. Benjamini and Y. Hochberg. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, Vol.57 (No.1):289-300, 1995).

[0122] In order to identify and to relatively quantify 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 glioblastoma 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 tumor tissue obtained from glioblastoma patients.

[0123] The proprietary discovery pipeline XPRESIDENT® v2.1 (see for example US Patent Application No.: 13/640,989) 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.

[0124] 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.

[0125] HLA-peptide complexes from 32 HLA-A*02-restricted and 13 HLA-A*24-restricted shock-frozen glioblastoma tumor tissue samples were purified and HLA-associated peptides were isolated and analyzed by LC-MS.

[0126] All TUMAPs contained in the application at hand were identified with this approach on primary glioblastoma tumor samples confirming their presentation on primary glioblastoma. TUMAPs identified on multiple glioblastoma tumor 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.

[0127] The present invention further relates to the peptide previously described, having the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I.

[0128] The present invention further relates to the peptide previously described, wherein the peptide includes non-peptide bonds.

[0129] Disclosed is a fusion protein, in particular comprising N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii).

[0130] The present invention further relates to a nucleic acid, encoding the peptide previously described.

[0131] The present invention further relates to the nucleic acid previously described that is DNA, cDNA, RNA or combinations thereof.

[0132] The present invention further relates to an expression vector expressing a nucleic acid previously described.

[0133] The present invention further relates to a peptide as described before, a nucleic acid as described before or an expression vector as described before for use in the treatment of a disease.

[0134] The present invention further relates to a host cell comprising a nucleic acid as described before or an expression vector as described before.

[0135] The present invention further relates to the host cell described that is an antigen

presenting cell.

[0136] The present invention further relates to the host cell described wherein the antigen presenting cell is a dendritic cell.

[0137] The present invention further relates to a method of producing a peptide as described, the method comprising culturing the host cell described and isolating the peptide from the host cell or its culture medium.

[0138] The present invention further relates to an in vitro method for producing activated cytotoxic T lymphocytes (CTL), the method comprising contacting in vitro CTL with antigen loaded human class I MHC molecules expressed on the surface of a suitable antigen-presenting cell for a period of time sufficient to activate said CTL in an antigen specific manner, wherein said antigen is a peptide as described.

[0139] The present invention further relates to the method as described, wherein the antigen is loaded onto class I 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.

[0140] The present invention further relates to the method as described, wherein the antigen-presenting cell comprises an expression vector capable of expressing said peptide containing SEQ ID No. 28.

[0141] The present invention further relates to activated cytotoxic T lymphocytes (CTL), produced by the method described, which selectively recognise a cell which aberrantly expresses a polypeptide comprising an amino acid sequence as described.

[0142] Disclosed is a method of killing target cells in a patient which target cells aberrantly express a polypeptide comprising any amino acid sequence described, the method comprising administering to the patient an effective number of cytotoxic T lymphocytes (CTL) as defined.

[0143] The present invention further relates to the use of any peptide described, a nucleic acid as described, an expression vector as described, a cell as described, or an activated cytotoxic T lymphocyte as described for use in the treatment of cancer.

[0144] The present invention further relates to a use as described, wherein said cancer is glioblastoma or other brain tumor.

[0145] The term "antibodies" is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term "antibodies" are fragments or polymers of those immunoglobulin molecules and humanized versions of immunoglobulin molecules, so long as they exhibit any of the desired properties (e.g., specific binding of an glioblastoma marker polypeptide, delivery of a toxin to an glioblastoma cell expressing a glioblastoma marker gene at an increased level, and/or

inhibiting the activity of a glioblastoma marker polypeptide) described herein.

[0146] The antibodies of the invention may also be generated using well-known methods. The skilled artisan will understand that either full length glioblastoma 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.

[0147] For example, a cDNA encoding PTPRZ1, BCAN, and FABP7, or any other polypeptide according to SEQ ID No. 1 to SEQ ID No. 49, SEQ ID No. 71, and SEQ IDs No. 74 to 129 or a variant sequence thereof which is at least 90% homolog to SEQ ID No. 1 to SEQ ID No. 49, SEQ ID No. 71, and SEQ IDs No. 74 to 129, or a 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 glioblastoma marker polypeptide used to generate the antibody.

[0148] One of skill in the art will know 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., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988). For example, the antibodies may be tested in ELISA assays, Western blots, immunohistochemical staining of formalin-fixed glioblastoma 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.

[0149] 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 (U.S. Pat. No.4,816,567).

[0150] 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.

[0151] The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No.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).

[0152] 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 published Dec. 22, 1994 and U.S. Pat. No.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 Fe fragment. Pepsin treatment yields a fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0153] 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.

[0154] 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.

[0155] 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 (U.S. Pat. No.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.

[0156] 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.

[0157] 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.

[0158] The antibodies can be administered to the subject, patient, or cell by injection (e.g., intravenous, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. The antibodies may also be administered by intratumoral or peritumoral routes, to exert local as well as systemic therapeutic effects. Local or intravenous injection is preferred.

[0159] 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 ($\mu\text{g/kg}$ to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. Following administration of an antibody for treating glioblastoma, 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 glioblastoma in a subject receiving treatment may be monitored using standard tumor imaging techniques. A therapeutically-administered antibody that arrests tumor growth, results in tumor shrinkage, and/or prevents the development of new tumors, compared to the disease course that would occur in the absence of antibody administration, is an efficacious antibody for treatment of glioblastoma.

[0160] Because the glioblastoma markers PTPRZ1, BCAN, FABP7 as disclosed are highly expressed in glioblastoma cells and are expressed at extremely low levels in normal cells, inhibition of PTPRZ1, BCAN, FABP7 expression or polypeptide activity may be integrated into any therapeutic strategy for treating or preventing glioblastoma.

[0161] In the methods described above, which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or the nucleic acids can be in a vector for delivering the nucleic acids to the cells for inhibition of gastric tumor marker protein expression. The vector can be a commercially available preparation, such as an adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada). Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO- 25 BRL, Inc., Gaithersburg, Md.), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, Wis.), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered in vivo by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, Calif.) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, Arizona).

[0162] The present invention thus provides a peptide consisting of SEQ ID No. 28, or a pharmaceutical acceptable salt thereof.

[0163] The peptides of the invention has the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I.

[0164] In the present invention, the term "homologous" refers to the degree of identity 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. The sequences to be compared herein may have an addition or deletion (for example, gap and the like) in the optimum alignment of the two sequences. 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 analysis tools provided by public databases.

[0165] 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 (Fong et al., 2001; (Zaremba et al., 1997; Colombetti et al., 2006; Appay et al., 2006).

[0166] These CTL 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 (Rammensee et al., 1997) and databases (Rammensee et al., 1999), 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.

[0167] Of course, the peptide according to the present invention will have the ability to bind to a molecule of the human major histocompatibility complex (MHC) class I. Binding of a peptide or a variant to a MHC complex may be tested by methods known in the art.

[0168] Disclosed is 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.

[0169] In addition, the peptide 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.

[0170] 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) J. Immunol. 159, 3230-3237. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. 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.

[0171] A non-peptide bond is, for example, -CH₂-NH-, -CH₂S-, -CH₂CH₂-, -CH=CH-, -COCH₂-, -CH(OH)CH₂-, and -CH₂SO-. United States Patent 4,897,445 provides a method for the solid phase synthesis of non-peptide bonds (-CH₂-NH) in polypeptide chains which involves

polypeptides synthesized by standard procedures and the non-peptide bond synthesized by reacting an amino aldehyde and an amino acid in the presence of NaCNBH_3 .

[0172] 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.

[0173] A peptide wherein the peptide includes non-peptide bonds is a preferred embodiment of the invention. Generally, peptides (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 Lu et al (1981) and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group.

[0174] 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-dicyclohexylcarbodiimide/lhydroxybenzotriazole 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 ethandithiol, 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).

[0175] 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 lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from e.g. Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 2QJ, UK.

[0176] Purification may be performed by any one, or a combination of, techniques such as recrystallization, size exclusion chromatography, ion-exchange chromatography, hydrophobic

interaction chromatography and (usually) reverse-phase high performance liquid chromatography using e.g. acetonitril/water gradient separation.

[0177] 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.

[0178] A further aspect of the invention provides a nucleic acid (for example a polynucleotide) encoding a peptide of the invention. The polynucleotide may be, for example, DNA, cDNA, 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 expressing a polypeptide according to the invention.

[0179] 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.

[0180] 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.

[0181] A desirable method of modifying the DNA encoding the polypeptide of the invention employs the polymerase chain reaction as disclosed by (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.

[0182] 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 of the invention. Thus, the DNA encoding the peptide 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 in US Patent Nos. 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.

[0183] 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.

[0184] 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.

[0185] Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

[0186] 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.

[0187] 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.

[0188] 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.

[0189] 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 pMBI (derivative of pBR322) origin for replication in bacterial cells, the b-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the fl origin. Vectors containing the preprotrypsin 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.

[0190] 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.

[0191] 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 (1972) Proc. Natl. Acad. Sci. USA 69, 2110, and Sambrook et al (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Transformation of yeast cells is described in Sherman et al (1986) Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor, NY. The method of Beggs (1978) Nature 275,104-109 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.

[0192] 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.

[0193] 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.

[0194] 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) are currently under investigation for the treatment of prostate cancer (Sipuleucel-T) (Small et al., 2006; Rini et al., 2006).

[0195] A further aspect of the invention provides a method of producing a peptide, the method comprising culturing a host cell and isolating the peptide from the host cell or its culture medium.

[0196] 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 (Brunsvig et al., 2006; Staehler et al., 2007).

[0197] 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.

[0198] 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.

[0199] 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 Karre et al 1985.

[0200] Preferably, the host cell before transfection 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 costimulator molecules are publicly available from the GenBank and EMBL databases.

[0201] In case of a MHC class I epitope being used as an antigen, the T cells are CD8-positive CTLs.

[0202] 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. 28.

[0203] A number of other methods may be used for generating CTL *in vitro*. For example, the methods described in Peoples et al (1995) and Kawakami et al (1992) use autologous tumor-infiltrating

lymphocytes in the generation of CTL. Plebanski et al (1995) makes use of autologous peripheral blood lymphocytes (PLBs) in the preparation of CTL. Jochmus et al (1997) describes the production of autologous CTL by pulsing dendritic cells with peptide or polypeptide, or via infection with recombinant virus. Hill et al (1995) and Jerome et al (1993) make use of B cells in the production of autologous CTL. In addition, macrophages pulsed with peptide or polypeptide, or infected with recombinant virus, may be used in the preparation of autologous CTL. S. 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 this study, 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.

[0204] Allogeneic cells may also be used in the preparation of T cells and a method is described in detail in WO 97/26328. 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 (1994)) which describes the development of cowpea mosaic virus as a high-yielding system for the presentation of foreign peptides.

[0205] 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.

[0206] 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. 28.

[0207] 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.

[0208] 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)).

[0209] The T cells of the present invention may be used as active ingredients of a therapeutic composition. Thus, disclosed is 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.

[0210] 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.

[0211] T cells may be obtained by methods known in the art, e.g. those described above.

[0212] Protocols for this so-called adoptive transfer of T cells are well known in the art. Reviews can be found in (Gattinoni et al., 2006) and (Morgan et al., 2006).

[0213] Any molecule of the invention, i.e. the peptide, nucleic acid, antibody, expression vector, cell, activated CTL, 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).

[0214] Preferably, the medicament of the present invention is 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 peptides as disclosed are expected to stimulate CD4 or CD8 T cells. However, stimulation of CD8 CTLs is more efficient in the presence of help provided by CD4 T-helper cells. Thus, for MHC Class I epitopes that stimulate CD8 CTL 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.

[0215] In one aspect, the vaccine comprises at least one peptide consisting of the amino acid sequence set forth in SEQ ID No. 28, 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.

[0216] The polynucleotide 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. (Pascolo 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.

[0217] 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 CTLs and helper-T (T_H) 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, aluminium 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; 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) and acting as immunoadjuvants (e.g., IL-12, IL-15, IL-23, IL-7, IFN-alpha. IFN-beta) [Gabrilovich 1996].

[0218] 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 T_{H1} cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T cell help. The T_{H1} 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 T_{H2} 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 Pat. No. 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.

[0219] Other examples for useful adjuvants include, but are not limited to, chemically modified CpGs (e.g. CpR, Idera), dsRNA analogues such as Poly(I:C) and derivatives thereof (e.g. AmpliGen®,

[0220] 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.

[0221] Preferred adjuvants are imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, interferon-alpha, CpG oligonucleotides and derivatives, poly-(I:C) and derivatives, RNA, sildenafil, and particulate formulations with PLG or virosomes.

[0222] 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), imiquimod, resiquimod, and interferon-alpha.

[0223] 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), imiquimod and resiquimod.

[0224] In a preferred embodiment of the pharmaceutical composition according to the invention, the adjuvant is imiquimod or resiquimod.

[0225] 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, flavours, 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, 3. Ed. 2000, American Pharmaceutical Association and pharmaceutical press. The composition can be used for a prevention, prophylaxis and/or therapy of adenomatous or cancerous diseases. Exemplary formulations can be found in EP2113253. Nevertheless depending on the number and the physico-chemical characteristics of the peptides of the invention further research is needed to provide formulations for specific combinations of peptides that are stable for more than 12 - 18 months.

[0226] The present invention provides a medicament that useful in treating cancer, in particular non-small cell lung carcinoma, gastric cancer, renal cell carcinoma, colon cancer,

adenocarcinoma, prostate cancer, benign neoplasm and malignant melanoma.

[0227] The present invention further includes a kit comprising: (a) a container comprising a pharmaceutical composition containing a peptide according to the present invention, an activated cytotoxic T lymphocyte according to the present invention, an antibody according to the present invention, a T cell receptor according to the present invention, the nucleic acid or the expression vector according to the present invention, or the cell according to the present invention, in solution or in lyophilized form; (b) 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.

[0228] The kit may further comprise one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, or (vii) a syringe or at least one or more peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 27, and SEQ ID No. 29 to SEQ ID No. 131. 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.

[0229] 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.

[0230] 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).

[0231] 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.

[0232] 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.

[0233] 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, a 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.

[0234] 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.

[0235] 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.

[0236] Since the peptide of the invention derived from SEQ ID No. 28 was isolated from glioblastoma, the medicament of the invention is preferably used to treat glioblastoma.

[0237] The present invention will now be described in the following examples that describe preferred embodiments thereof, nevertheless, without being limited thereto.

Figure 1: Exemplary mass spectrum from IGF2BP3-001 demonstrating its presentation on primary tumor sample glioblastoma. NanoESI-LCMS was performed on a peptide pool eluted from the glioblastoma sample 6010. The mass chromatogram for m/z 536.3229 $\pm$ 0.001 Da, $z=2$ shows a peptide peak at the retention time 48.76 min. B) The detected peak in the mass chromatogram at 48.76 min revealed a signal of m/z 536.3229 in the MS spectrum. C) A collisionally induced decay mass spectrum from the selected precursor m/z 536.3229 recorded in the nanoESI-LCMS experiment at the given retention time confirmed the presence of IGF2BP3-001 in the glioblastoma 6010 tumor sample. D) The fragmentation pattern of the synthetic IGF2BP3-001 reference peptide was recorded and compared to the generated natural TUMAP fragmentation pattern shown in C for sequence verification.

Figure 2: Expression profiles of mRNA of selected proteins in normal tissues and in 22 glioblastoma cancer samples. a) CSRP2 (Probeset ID: 211126_s_at); b) PTPRZ1 (Probeset ID: 204469_at).

Figure 3: Presentation profiles for selected HLA class I peptides. A presentation profile was calculated for each peptide showing the mean sample presentation as well as replicate variations. The profile juxtaposes samples of the tumor entity of interest to a baseline of normal tissue samples. a) CSRP2-001 (HLA-A*02); b) PTP-012 (HLA-A*02); c) TMEM255A-001 (HLA-A*24); d) PJA2-001 (HLA-A*24).

Figure 4: Exemplary results of peptide-specific in vitro immunogenicity of class I TUMAPs for HLA*A02 and HLA*A24. Specific CD8+ T cells were stained with HLA multimers each linked to two different fluorochromes. Dot plots show MHC multimer-double-positive populations for the stimulating peptides (left panels) and the respective negative control stimulations (right panels).

EXAMPLES

[0238] EXAMPLE 1:

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

[0239] Patients' tumor tissues were provided by Universities of Heidelberg, University of Tübingen, both Germany, University of Geneva, Switzerland. Written informed consents of all patients had been given before surgery. Tissues were shock-frozen in liquid nitrogen immediately after surgery and stored until isolation of TUMAPs at -80°C.

Isolation of HLA peptides from tissue samples

[0240] HLA peptide pools from shock-frozen tissue samples were obtained by immune precipitation from solid tissues according to a slightly modified protocol (Falk et al., 1991; Seeger et al., 1999) using the HLA-A*02-specific antibody BB7.2, the HLA-A, -B, -C-specific antibody W6/32, CNBr-activated sepharose, acid treatment, and ultrafiltration.

Methods

[0241] The HLA peptide pools as obtained were separated according to their hydrophobicity by reversed-phase chromatography (Acquity UPLC system, Waters) and the eluting peptides were analyzed in an LTQ-Orbitrap hybrid mass spectrometer (ThermoElectron) equipped with an ESI source. Peptide pools were loaded directly onto the analytical fused-silica micro-

capillary column (75 μm i.d. x 250 mm) packed with 1.7 μm C18 reversed-phase material (Waters) applying a flow rate of 400 nL per minute. Subsequently, the peptides were separated using a two-step 180 minute-binary gradient from 10% to 33% B at a flow rate of 300 nL per minute. The gradient was composed of Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A gold coated glass capillary (PicoTip, New Objective) was used for introduction into the nanoESI source. The LTQ-Orbitrap mass spectrometer was operated in the data-dependent mode using a TOP5 strategy. In brief, a scan cycle was initiated with a full scan of high mass accuracy in the orbitrap ($R = 30\,000$), which was followed by MS/MS scans also in the orbitrap ($R = 7500$) on the 5 most abundant precursor ions with dynamic exclusion of previously selected ions. Tandem mass spectra were interpreted by SEQUEST and additional manual control. The identified peptide sequence was assured by comparison of the generated natural peptide fragmentation pattern with the fragmentation pattern of a synthetic sequence-identical reference peptide. Fig 1 shows an exemplary spectrum obtained from tumor tissue for the MHC class I associated peptide IGF2BP3-001 and its elution profile on the UPLC system.

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

[0242] Presentation profiles of exemplary over-presented peptides are shown in Figure 3.

EXAMPLE 2

Expression profiling of genes encoding the peptides as disclosed

[0243] Not all peptides identified as being presented on the surface of tumor cells by MHC molecules are suitable for immunotherapy, because the majority of these peptides are derived from normal cellular proteins expressed by many cell types. Only few of these peptides are tumor-associated and likely able to induce T cells with a high specificity of recognition for the tumor from which they were derived. In order to identify such peptides and minimize the risk for

autoimmunity induced by vaccination the inventors focused on those peptides that are derived from proteins that are over-expressed on tumor cells compared to the majority of normal tissues.

[0244] The ideal peptide will be derived from a protein that is unique to the tumor and not present in any other tissue. To identify peptides that are derived from genes with an expression profile similar to the ideal one the identified peptides were assigned to the proteins and genes, respectively, from which they were derived and expression profiles of these genes were generated.

RNA sources and preparation

[0245] Surgically removed tissue specimens were provided by several institutions as listed in Example 1 after written informed consent had been obtained from each patient. Tumor tissue specimens were snap-frozen in liquid nitrogen immediately after surgery and later homogenized with mortar and pestle under liquid nitrogen. Total RNA was prepared from these samples using TRI Reagent (Ambion, Darmstadt, Germany) followed by a cleanup with RNeasy (QIAGEN, Hilden, Germany); both methods were performed according to the manufacturer's protocol.

[0246] Total RNA from healthy human tissues was obtained commercially (Ambion, Huntingdon, UK; Clontech, Heidelberg, Germany; Stratagene, Amsterdam, Netherlands; BioChain, Hayward, CA, USA). The RNA from several individuals (between 2 and 123 individuals) was mixed such that RNA from each individual was equally weighted.

[0247] Quality and quantity of all RNA samples were assessed on an Agilent 2100 Bioanalyzer (Agilent, Waldbronn, Germany) using the RNA 6000 Pico LabChip Kit (Agilent).

Microarray experiments

[0248] Gene expression analysis of all tumor and normal tissue RNA samples was performed by Affymetrix Human Genome (HG) U133A or HG-U133 Plus 2.0 oligonucleotide microarrays (Affymetrix, Santa Clara, CA, USA). All steps were carried out according to the Affymetrix manual. Briefly, double-stranded cDNA was synthesized from 5-8 µg of total RNA, using SuperScript RTII (Invitrogen) and the oligo-dT-T7 primer (MWG Biotech, Ebersberg, Germany) as described in the manual. In vitro transcription was performed with the BioArray High Yield RNA Transcript Labelling Kit (ENZO Diagnostics, Inc., Farmingdale, NY, USA) for the U133A arrays or with the GeneChip IVT Labelling Kit (Affymetrix) for the U133 Plus 2.0 arrays, followed by cRNA fragmentation, hybridization, and staining with streptavidin-phycoerythrin and biotinylated anti-streptavidin antibody (Molecular Probes, Leiden, Netherlands). Images were scanned with the Agilent 2500A GeneArray Scanner (U133A) or the Affymetrix Gene-Chip

Scanner 3000 (U133 Plus 2.0), and data were analyzed with the GCOS software (Affymetrix), using default settings for all parameters. For normalisation, 100 housekeeping genes provided by Affymetrix were used. Relative expression values were calculated from the signal log ratios given by the software and the normal kidney sample was arbitrarily set to 1.0.

[0249] Exemplary expression profiles of source genes as disclosed that are highly over-expressed or exclusively expressed in glioblastoma are shown in Fig. 2.

EXAMPLE 3

***In vitro* immunogenicity for glioblastoma MHC class I presented peptides**

[0250] In order to obtain information regarding the immunogenicity of the TUMAPs as disclosed, we performed investigations using an *in vitro* T-cell priming assay based on repeated stimulations of CD8⁺ T cells with artificial antigen presenting cells (aAPCs) loaded with peptide/MHC complexes and anti-CD28 antibody. This way we could show immunogenicity for 69 HLA-A*0201 and 58 HLA-A*24 restricted TUMAPs as disclosed so far, demonstrating that these peptides are T-cell epitopes against which CD8⁺ precursor T cells exist in humans.

***In vitro* priming of CD8⁺ T cells**

[0251] In order to perform *in vitro* stimulations by artificial antigen presenting cells loaded with peptide-MHC complex (pMHC) and anti-CD28 antibody, we first isolated CD8⁺ T cells from fresh HLA-A*02 leukapheresis products via positive selection using CD8 microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) of healthy donors obtained from the Transfusion Medicine Tuebingen after informed consent.

[0252] Isolated CD8⁺ lymphocytes or PBMCs were incubated until use in T-cell medium (TCM) consisting of RPMI-Glutamax (Invitrogen, Karlsruhe, Germany) supplemented with 10% heat inactivated human AB serum (PAN-Biotech, Aidenbach, Germany), 100 U/ml Penicillin / 100 µg/ml Streptomycin (Cambrex, Cologne, Germany), 1 mM sodium pyruvate (CC Pro, Oberdorla, Germany), 20 µg/ml Gentamycin (Cambrex). 2.5 ng/ml IL-7 (PromoCell, Heidelberg, Germany) and 10 U/ml IL-2 (Novartis Pharma, Nürnberg, Germany) were also added to the TCM at this step. Generation of pMHC/anti-CD28 coated beads, T-cell stimulations and readout was performed in a highly defined *in vitro* system using four different pMHC molecules per stimulation condition and 8 different pMHC molecules per readout condition. All pMHC complexes used for aAPC loading and cytometric readout were derived from UV-induced MHC ligand exchange with minor modifications. In order to determine the amount of pMHC monomer obtained by exchange we performed streptavidin-based sandwich ELISAs according to (Rodenko et al., 2006). The purified co-stimulatory mouse IgG2a anti

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

[0253] pMHC used as controls for high immunogenic and low immunogenic stimulations were A*0201/MLA-001 (peptide ELAGIGILTV from modified Melan-A/MART-1) and A*0201/DDX5-001 (YLLPAIVHI from DDX5), respectively.

[0254] 800.000 beads / 200 µl were coated in 96-well plates in the presence of 4 x 12.5 ng different biotin-pMHC, washed and 600 ng biotin anti-CD28 were added subsequently in a volume of 200 µl. Stimulations were initiated in 96-well plates by co-incubating 1×10^6 CD8+ T cells with 2×10^5 washed coated beads in 200 µl TCM supplemented with 5 ng/ml IL-12 (PromoCell) for 3-4 days at 37°C. Half of the medium was then exchanged by fresh TCM supplemented with 80 U/ml IL-2 and incubating was continued for 3-4 days at 37°C. This stimulation cycle was performed for a total of three times, with 12 individual wells per condition. For the pMHC multimer readout using 8 different pMHC molecules per condition, a two-dimensional combinatorial coding approach was used as previously described (Andersen et al., 2012) with minor modifications encompassing coupling to 5 different fluorochromes. Finally, multimeric analyses were performed by staining the cells with Live/dead near IR dye (Invitrogen, Karlsruhe, Germany), CD8-FITC antibody clone SK1 (BD, Heidelberg, Germany) and fluorescent pMHC multimers. For analysis, a BD LSRII SORP cytometer equipped with appropriate lasers and filters was used. Peptide specific cells were calculated as percentage of total CD8+ cells. Evaluation of multimeric analysis was done using the FlowJo software (Tree Star, Oregon, USA). In vitro priming of specific multimer+ CD8+ lymphocytes was detected by comparison to irrelevant control stimulations. Immunogenicity for a given antigen was detected if at least one evaluable in vitro stimulated well of one healthy donor was found to contain a specific CD8+ T-cell line after in vitro stimulation (i.e. this well contained at least 1% of specific multimer+ among CD8+ T-cells and the percentage of specific multimer+ cells was at least 10x the median of the irrelevant control stimulations).

In vitro immunogenicity for glioblastoma peptides

[0255] For tested HLA class I peptides, in vitro immunogenicity could be demonstrated by generation of peptide specific T-cell lines. Exemplary flow cytometry results after TUMAP-specific multimer staining for two peptides as disclosed are shown in figure 4 together with corresponding negative controls. Results for 69 HLA-A*0201 and 58 HLA-A*24 peptides as disclosed are summarized in Table 5a and b.

Table 5a: In vitro immunogenicity of HLA-A*02class I peptides as disclosed

Exemplary results of in vitro immunogenicity experiments conducted by the applicant for the peptides as disclosed.

<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++

SEQ ID NO:	Peptide Code	Wells	Donors
68	ABCA13-001	+	++
37	ADORA3-001	+	++
10	ANKRD40-001	+	+++
27	ASIC4-001	+	++
51	BCA-002	++++	++++(100%)
13	BCA-003	+	++
69	CCNB1-002	+	+++
45	CCT-001	+	+++
52	CDK4-001	++	++++
48	CHCHD2-005	+	+++
18	CLU-001	+	++
70	CNOT1-002	+	++
28	COL20-001	+	++
23	CPT1C-001	+	++
60	CSP-001	+	+++
1	CSRP2-001	++	++++(100%)
63	DCA-001	+	++
41	DPP3-001	+	+++
65	DPYSL4-001	+	++
67	DROSHA-001	+	++
29	EGFR-008	+	++
43	EIF4E-001	+	+++
3	ELOVL2-001	+	++++
59	FABP7-001	+	++++
21	GPR98-001	+	++
40	GRI-001	+	++
17	GRI-002	+	++
8	GRIK3-001	+	++++
22	GYG2-001	+	++
66	IGF2BP3-001	+	+++
32	IRS-001	+	+++
30	JAK-001	+	++

Exemplary results of in vitro immunogenicity experiments conducted by the applicant for the peptides as disclosed.

<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++

SEQ ID NO:	Peptide Code	Wells	Donors
12	KCN-002	+	+++
6	KIF1A-001	++	+++
53	MAGEF 1-001	++	++++
14	MAGI2-001	+	++
47	MAP1B-001	+	+++
35	MAP1B-002	+	++
4	MTSS1L-001	+++	++++(100%)
33	NAT8L-001	+	++++
36	NCAN-001	+	++++(100%)
55	NLGN4X-001	++	++++(100%)
39	NLGN4X-002	+	++
11	NLGN4Y-001	+	++++
46	NOC4-001	+	+++
33	NPAS3-001	+	++
57	NRCAM-001	+	++++(100%)
61	ORMDL1-002	+	+++
7	PCDHGC5-001	+	++++(100%)
64	PCNXL3-001	+	++
44	PLEKHA4-001	+	+++
26	PTP-001	+	++
25	PTP-002	+	+++
54	PTP-003	+	++++
50	PTP-005	++	++++
15	PTP-012	+	++
5	PTP-013	+	++++
58	RAD54B-001	++	++++(100%)
16	SCARA3-001	+	++
9	SEZ6L-001	+	++++
2	SLC10A4-001	++	+++
20	SLC10A4-002	+	+++
24	SLC35E1-002	+	++
49	SOX-001	+	++++

Exemplary results of in vitro immunogenicity experiments conducted by the applicant for the peptides as disclosed.			
<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++			
SEQ ID NO:	Peptide Code	Wells	Donors
62	TACC3-001	+	++
34	TNC-001	+	++
42	USP11-001	++	++++
56	VPS13B-001	++	++++(100%)
31	WLS-002	+	+++

Table 5b: In vitro immunogenicity of HLA-A*24 class I peptides as disclosed

Exemplary results of in vitro immunogenicity experiments conducted by the inventors for the peptides as disclosed.			
<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++			
SEQ ID NO:	Peptide Code	Wells	Donors
74	TMEM255A-001	+	++
75	ST8SIA5-001	++	++++
76	FAM120C-001	++	++++(100%)
77	GRIK3-002	+	++++
78	PTP-014	+	++
79	PTP-019	+	++
80	FABP7-002	+	++
81	ZNF749-001	+	++
82	DOCK7-002	+	+++
83	LOC72839-001	+	+++
84	PJA2-001	+	++
85	HEATR1-001	+	+++
86	GPM-002	+	+++
87	CRB1-001	+	++
88	PTP-016	+	++
89	PTP-015	+	++
90	PTP-018	+	++++
91	OLIG2-001	+	++
92	VCAN-003	+	+++
93	SMOX-001	+	++
94	EXOC7-001	+	++
95	LZTS1-001	+	++

Exemplary results of in vitro immunogenicity experiments conducted by the inventors for the peptides as disclosed.

<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++

96	FADS2-003	+	+++
97	TMEM231-001	+	+++
98	ASCL1-001	+	++
99	UNKN-003	+	++
100	NKA-001	+	++
101	PCD-002	+	++
102	ARHGAP21-001	+	++
103	PNMA2-001	+	++
104	FADS2-002	+	++++
105	APC-001	+	++
106	WASL-001	+	++++
107	SLC-002	+	++
108	TENM4-001	+	++
109	ZNFS3-001	++	+++
110	EFCAB7-001	+	++
111	DOCK7-003	+	++
112	BMP7-001	+	++
113	ITGA7-001	+	++
114	RPL-001	+	++
115	HS2-001	+	++
116	VIM-002	+	++
117	IFT 17-001	+	+++
118	GAB-001	+	++
119	CDCA7L-001	+	++
120	SCARA3-002	+	++
121	SSR1-001	+	++
122	NROB1-001	+	++
123	LNK1-001	+	++
124	EP4-001	+	++
125	KIF1B-001	+	++
126	RHOBTB3-001	+	++
127	KIF7-001	+	++
128	KIF1B-002	+	++

Exemplary results of in vitro immunogenicity experiments conducted by the inventors for the peptides as disclosed.			
<20 % = +; 20 % - 49 % = ++; 50 % - 70 % = +++; > 70 % = ++++			
129	MAPK6-001	+	++
130	ASPM-002	+	+++
131	SMC4-001	+	++

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PATENTKRAV

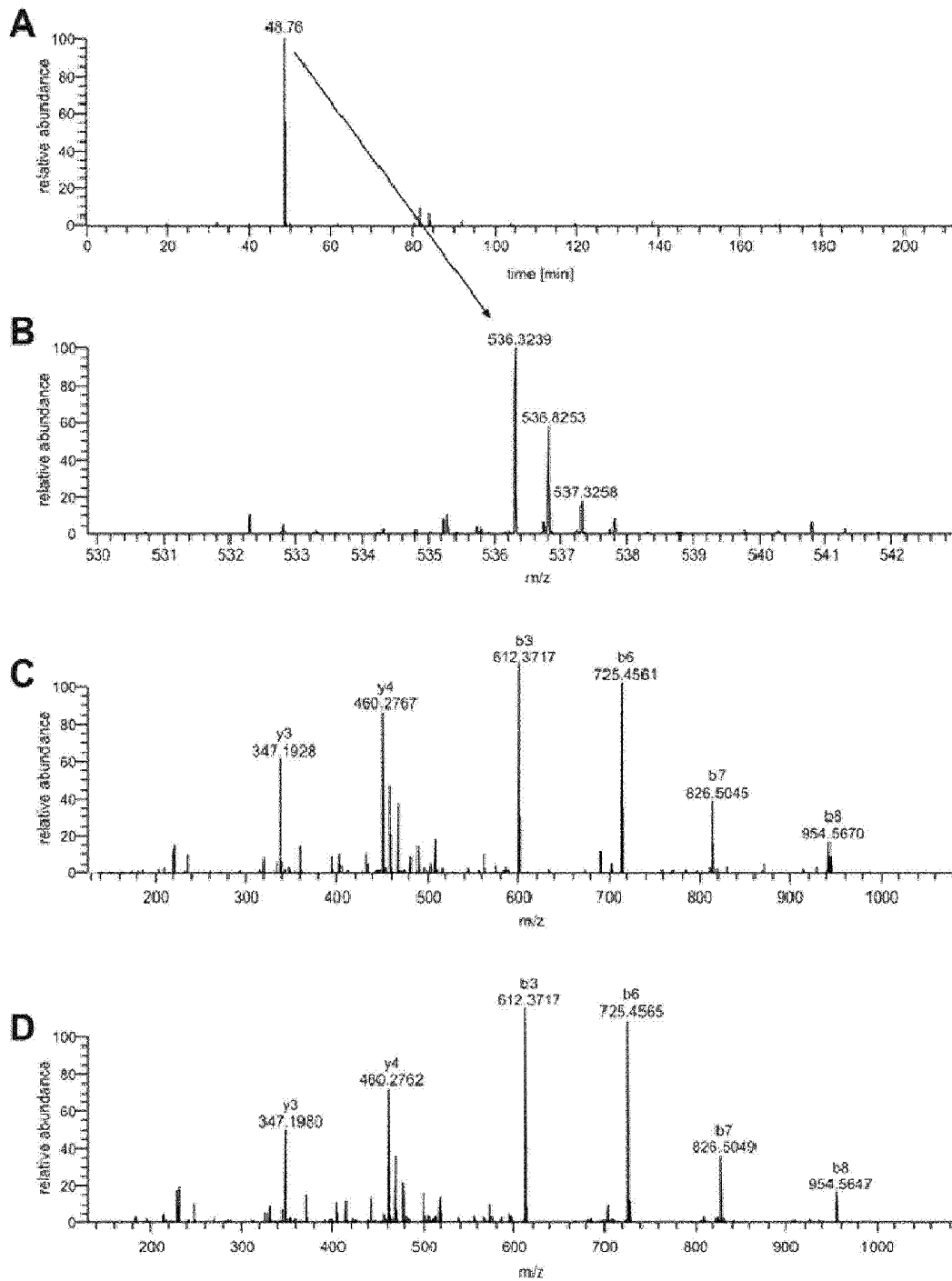
1. Peptid, som består af SEQ ID NO: 28, eller et farmaceutisk acceptabelt salt deraf.
2. Peptid ifølge krav 1, hvor peptidet indbefatter ikke-peptidbindinger.
3. *In vitro*-fremgangsmåde til frembringelse af aktiverede cytotoksiske T-lymfocytter (CTL), hvilken fremgangsmåde omfatter etablering af *in vitro*-kontakt mellem CTL og antigenbærende humane klasse I-MHC-molekyler udtrykt på overfladen af en egnet antigenpræsenterende celle eller en kunstig konstruktion, der er en efterligning af en antigenpræsenterende celle, i et tidsrum, som er tilstrækkeligt til at aktivere nævnte CTL på en antigenspecifik måde, hvor antigenet er et peptid ifølge krav 1.
4. Aktiveret cytotoksisk T-lymfocyt (CTL), som er frembragt ved fremgangsmåden ifølge krav 3, hvilken CTL selektivt genkender en celle, der har abnorm ekspresion af et polypeptid, som omfatter en aminosyresekvens ifølge krav 1.
5. Antistof, som specifikt genkender peptidet ifølge krav 1 eller 2 eller peptidet, når det er bundet til et MHC-molekyle.
6. T-cellereceptor, som reagerer med en HLA-ligand, der består af aminosyresekvensen ifølge SEQ ID NO: 28.
7. Nukleinsyre, som koder for et peptid ifølge et hvilket som helst af kravene 1 eller 2, en aktiveret cytotoksisk T-lymfocyt ifølge krav 4, et antistof ifølge krav 5 eller en T-cellereceptor ifølge krav 6, og som eventuelt er bundet til en heterolog promotorsekvens, eller ekspressionsvektor, der udtrykker nukleinsyren.
8. Værtscelle, som omfatter nukleinsyren eller ekspressionsvektoren ifølge krav 7.
9. Værtscelle ifølge krav 8, hvor værtscellen er en antigenpræsenterende celle eller en dendritisk celle.
10. Peptid ifølge et hvilket som helst af kravene 1 eller 2, aktiveret cytotoksisk T-lymfocyt ifølge krav 4, antistof ifølge krav 5, T-cellereceptor ifølge krav 6, nukleinsyre eller

ekspressionsvektor ifølge krav 7 eller celle ifølge krav 8 eller 9 til anvendelse ved behandling af en sygdom.

11. Peptid, aktiveret cytotoxisk T-lymfocyt, antistof, T-cellereceptor, nukleinsyre eller ekspressionsvektor eller celle til anvendelse ifølge krav 10, hvor sygdommen er cancer, fortrinsvis i form af en farmaceutisk sammensætning, f.eks. vaccine.
12. Farmaceutisk sammensætning til anvendelse ifølge krav 11, hvor canceren er valgt blandt astrocytom, pilocytisk astrocytom, dysembryoplastisk neuroepitelial tumor, oligodendrogliomer, ependymom, glioblastoma multiforme, blandede gliomer, oligoastrocytomer, medulloblastom, retinoblastom, neuroblastom, germinom, teratom, gangliogliomer, gangliocytom, centralt gangliocytom, primitive neuroektodermale tumorer (PNET, f.eks. medulloblastom, medulloepiteliom, neuroblastom, retinoblastom, ependymblastom), pineal-parenkymale tumorer (f.eks. pineocytom, pineoblastom), ependymcelletumorer, plexus chorioideus-tumorer, neuroepiteliale tumorer af uvis oprindelse (f.eks. gliomatosis cerebri, astroblastom), glioblastom, prostatatumor, brystcancer, øsofagus cancer, kolorektal cancer, clear cell-renalcellekarcinom, lungecancer, CNS-, ovarie-, melanom-, pancreascancer, pladecellekarcinom, leukæmi, medulloblastom, colon-, rectum-, mave-, nyre-, lunge-, pancreas-, prostata- og hudcancer.
13. Kit, som omfatter:
 - (a) en beholder, der omfatter en farmaceutisk sammensætning, som indeholder et peptid ifølge et hvilket som helst af kravene 1 eller 2, en aktiveret cytotoxisk T-lymfocyt ifølge krav 4, et antistof ifølge krav 5, en T-cellereceptor ifølge krav 6, nukleinsyren eller ekspressionsvektoren ifølge krav 7 eller cellen ifølge krav 8 eller 9 i opløsning eller i lyofiliseret form;
 - (b) en anden beholder, der indeholder et fortyndingsmiddel eller en rekonstitueringsopløsning til den lyofiliserede formulering; og
 - (c) eventuelt anvisninger vedrørende (i) anvendelse af opløsningen eller (ii) rekonstituering og/eller anvendelse af den lyofiliserede formulering.
14. Kit ifølge krav 13, som endvidere omfatter:
 - (d) mindst et eller flere peptider valgt fra gruppen bestående af SEQ ID NO: 1 til SEQ ID NO: 27 og SEQ ID NO: 29 til SEQ ID NO: 131.

DRAWINGS

Figure 1:



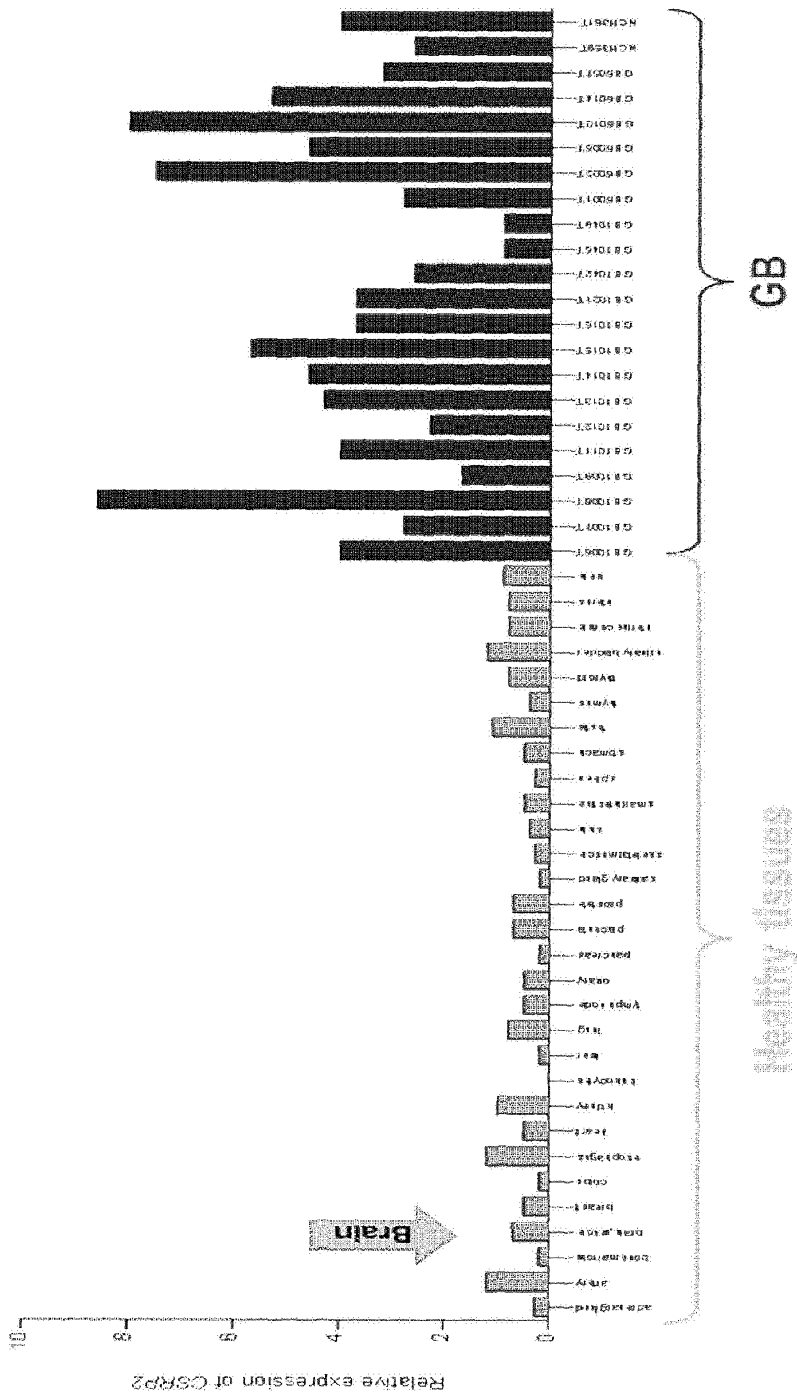


Figure 2a)

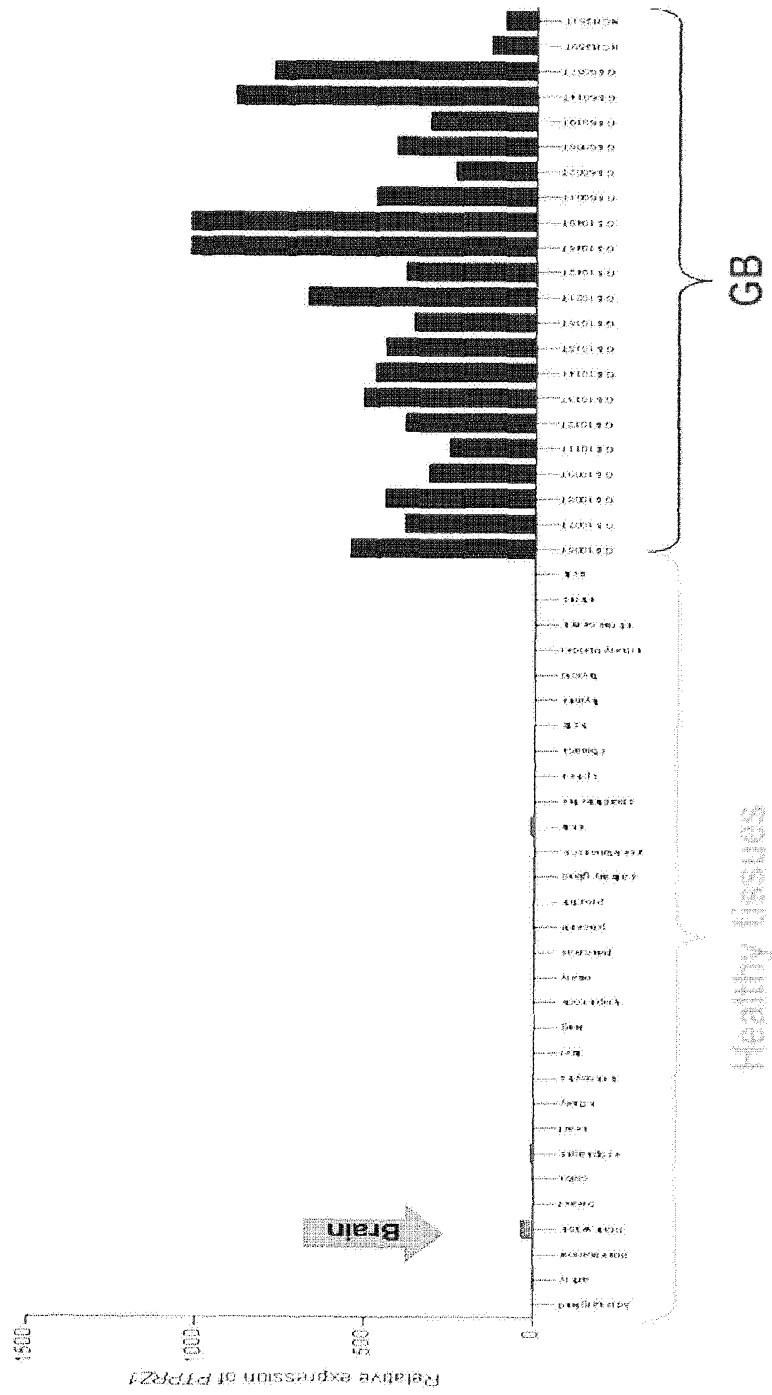


Figure 2b)

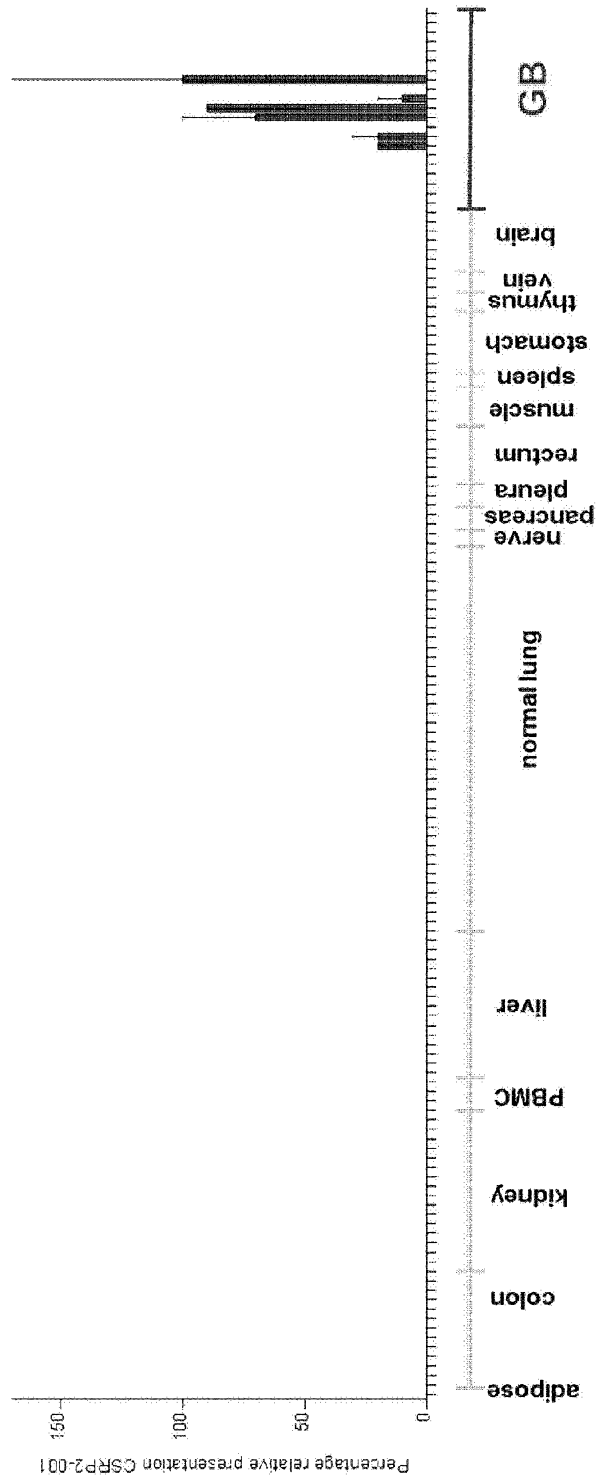


Figure 3a)

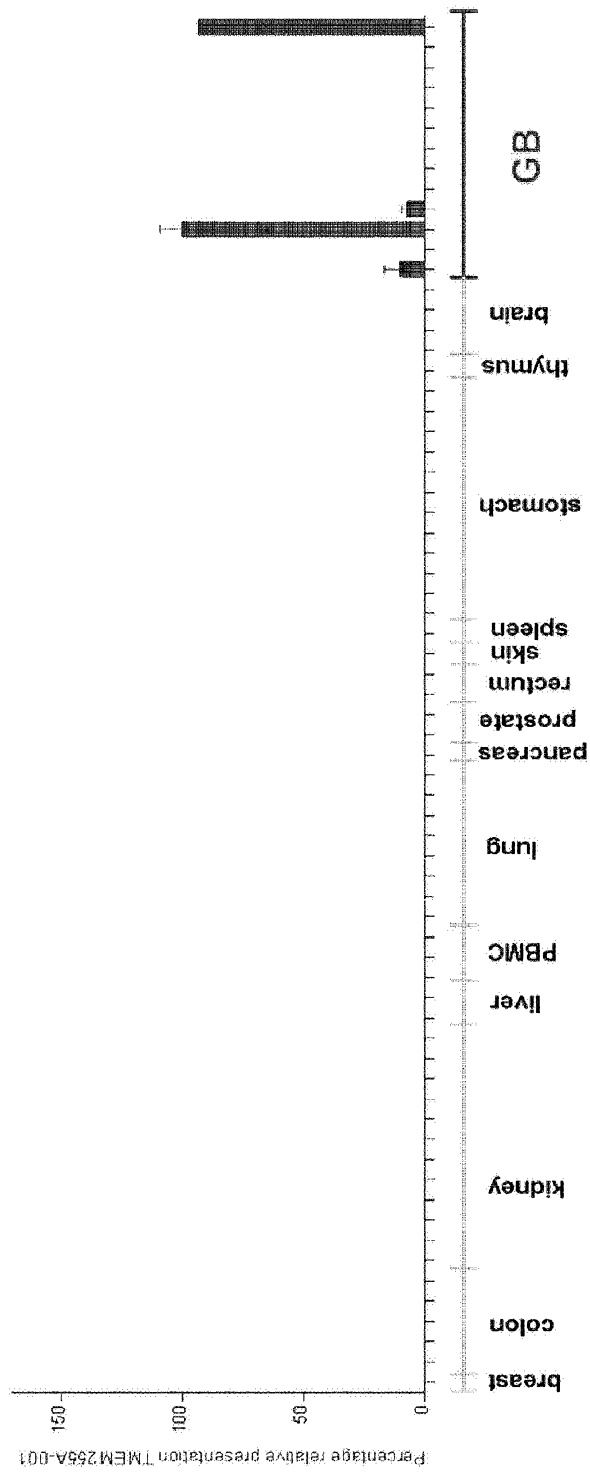


Figure 3c)

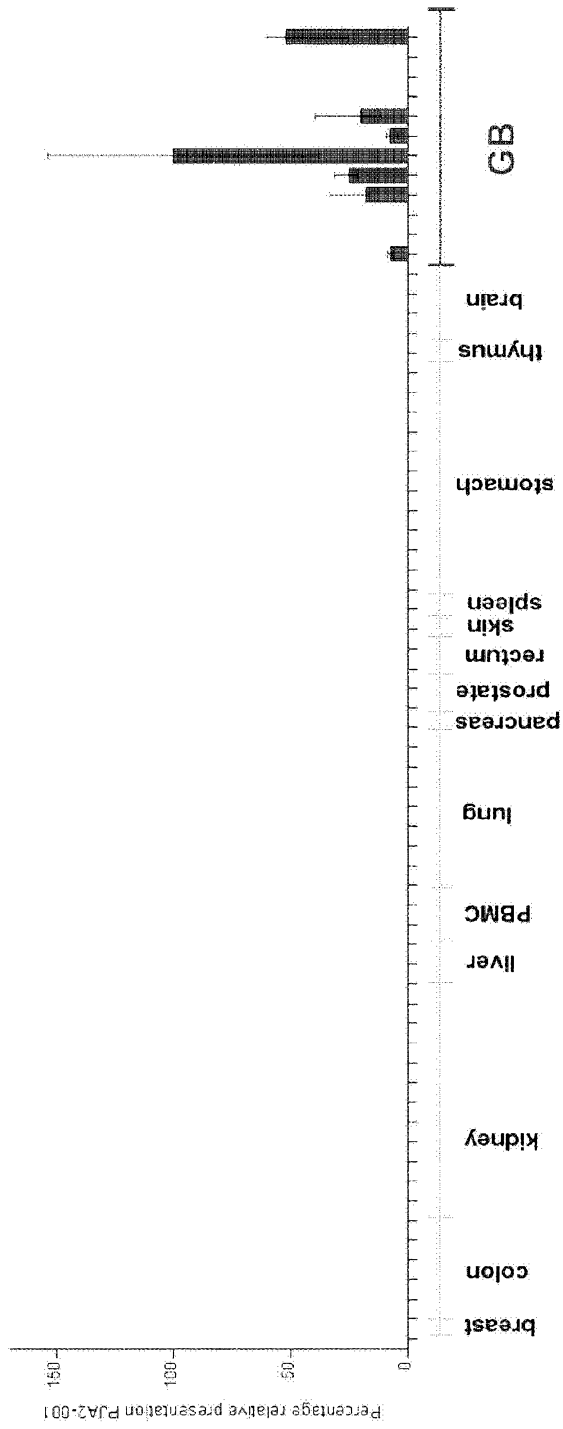


Figure 3d)

Figure 4

