

Supplementary Material

Evaluation	Method
Cytotoxic T cells (CTLs) were counted by SRL Laboratories (Kanagawa, Japan) using an enzyme-linked immune absorbent spot kit (ELISpot^{PLUS} for Human IFN-γ; Mabtech AB, Stockholm, Sweden) according to the manufacturer's instructions.	• Peripheral blood mononuclear cells (PBMC, 100,000 cells/well) from samples were co-cultured with peptide (10 μg/mL) corresponding to HLA type (HIV SLYNTYATL, HIV RLRDLLLVTR, HIV RYLRQQLGI [negative control]; LCK p246-254, WHSC2 (known as NELFA) p103-111, SART3 p302-310, SART2 (known as DSE) p93-101, SART3 p109-118, MRP3 p503-511, LCK p488-497, SART3 p734-742, LCK p90-99, TMEM189 p43-51 (also referred to as KUA), EGFR p800–809, and PTHRP p102-111; Toray Research Center, Inc., Kanagawa, Japan) in 96-well plates for 6 days. • Peptide-stimulated PBMC (20,000 cells/well), peptide (10 μg/mL), and antigen-presenting (T2[174xCEM.T2]) cells (10,000 cells/well) from ATCC, Manassas, VA, USA; C1R-A0301, and C1R-A2402 from Taiho Pharmaceutical Co., Ltd, Japan^a; and C1R-A1101, C1R-A3101, and C1R-A3303 from Kurume University, Kurume, Japan were co-cultured overnight (18 ± 0.5 hours) on an ELISpot plate. • After washing, an antibody detection solution, alkaline phosphatase, and substrate solution were added sequentially, and the number of spots was analyzed by an immunospot analyzer.

	• The mean number of spots, and the coefficient of variation (%) were calculated.
IgG was evaluated by Sekisui Medical Co., Ltd. (Ibaraki, Japan) using a Bio-Plex Pro magnetic COOH bead multiplex fluoroimmunoassay (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions.	• Peptides (LCK p246-254, WHSC2 (known as NELFA) p103-111, SART3 p302-310, SART2 (known as DSE) p93-101, SART3 p109-118, MRP3 p503-511, LCK p488-497, SART3 p734-742, LCK p90-99, TMEM189 p43-51, EGFR p800–809, and PTHRP p102-111 from American Peptide Co., Inc., Sunnyvale, CA, USA; TAS0314, TAS0315 and TAS0316 acetate from Bachem, Bubendorf, Switzerland) were attached to Bio-Plex Pro magnetic COOH beads. • The peptide-bound beads were added to diluted plasma and incubated for 60 minutes. • IgG antibodies to individual peptides were then measured by a Luminexassay (Luminex Corporation, Austin, TX, USA) after reacting to a biotinylated antibody (biotinylated anti-human IgG, from Vector Laboratories, Inc., Burlingame, CA, USA).

Supplementary Table 1 Experimental Procedures for CTL and IgG Evaluation.

EGFR, epidermal growth factor receptor; LCK, tyrosine-protein kinase LCK; MRP3, multidrug resistance-associated protein 3 (MRP3); PTHRP, parathyroid hormone-related protein; SART, squamous cell carcinoma antigen recognized by T-cells; TMEM189, transmembrane protein 189; WHSC2, Wolf–Hirschhorn syndrome candidate 2 protein.

^aThe parent cell line (C1R) was supplied by ATCC, and Taiho established the stable expression strains HLA-A*03:01 and A*24:02. Human HLA-A*03:01 cDNA and Human HLA-A*24:02:01 cDNA were provided by the RIKEN BRC through the National Bio-Resource Project of the

MEXT, Japan (1).

1. Akatsuka Y, Goldberg TA, Kondo A, *et al* (2002). Efficient cloning and expression of HLA class I cDNA in human B-lymphoblastoid cell lines. *Tissue Antigens*. 59:502–11.

Adverse Events	All Grade	Grade ≥3	Grade				
			1	2	3	4	5
Dermatological injection site reactions	9 (90.0)	0	7 (70.0)	2 (20.0)	0	0	0
Injection site abscess	1 (10.0)	0	0	1 (10.0)	0	0	0
Injection site erythema	2 (20.0)	0	2 (20.0)	0	0	0	0
Injection site pain	1 (10.0)	0	0	1 (10.0)	0	0	0
Injection site pruritus	2 (20.0)	0	1 (10.0)	1 (10.0)	0	0	0
Injection site reaction	8 (80.0)	0	7 (70.0)	1 (10.0)	0	0	0
Injection site swelling	1 (10.0)	0	1 (10.0)	0	0	0	0
Injection site injury	1 (10.0)	0	0	1 (10.0)	0	0	0

Supplementary Table 2 Treatment-Related Adverse Events Associated with Dermatological Injection Site Reactions in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313

Pt No.	Cycle	Specific IgG (pg/mL)														
		TA1	TA2	TA4	TA5	TA6	TA7	TA8	TA9	TA10	TA13	TA15	TA18	TAS0314	TAS0315	TAS0316
B-27-001	BL	4427.33	23447.44	93507.37	8103.23	4084.7	33638.8	42606.1	113976.4	27129.2	7587.52	16371.01	<27.34	20390.43	<384.38	250625.9
	2	17683.39 ^a	29302986 2.74 ^a	72660478 .56 ^a	2222995. 72 ^a	41850.11 ^a	357564.9 6 ^a	61065.21 ^a	4756763. 03 ^a	22522640 .87 ^a	40204.69 ^a	1235797. 34 ^a	2065.22 ^a	11008553 8.94 ^a	67421328 2.21 ^a	29422393 0.82 ^a
	3	35180.21 ^a	22721478 1.66 ^a	17972233 3.69 ^a	3199732. 84 ^a	67730.35 ^a	207619.6 4 ^a	65509.25 ^a	15860489 .40 ^a	34044487 .26 ^a	41800.30 ^a	1962348. 10 ^a	29716.06 ^a	25911139 8.52 ^a	10236154 71.38 ^a	42103011 5.90 ^a
B-27-002	BL	<59.77	20677.97	122716.0 3	4911.45	1179.34	<566.41	17043.46	49902.32	12402.41	<73.44	3847.1	<27.34	50754.26	<384.38	2494473.6 9
	2	14000.66 ^a	94124406 .21 ^a	42887018 8.85 ^a	3892148. 06 ^a	4542.21 ^a	206145.5 8 ^a	132933.2 0 ^a	1376494. 16 ^a	23752653 .86 ^a	91404.40 ^a	1172747. 66 ^a	12352.98 ^a	28167622 8.42 ^a	57936811 8.28 ^a	50768338 8.00 ^a
	3	40547.28 ^a	33312836 9.42 ^a	41231947 2.61 ^a	14199532 .21 ^a	5894.02 ^a	374161.9 5 ^a	286356.6 7 ^a	2738351. 79 ^a	30875319 .95 ^a	151382.7 8 ^a	6261681. 96 ^a	25049.12 ^a	36754170 9.51 ^a	14644965 34.27 ^a	13472276 90.54 ^a
B-27-003	BL	<59.77	11699.83	21261.17	10145.34	8700.19	<566.41	41424.1	155186	23616.69	5848.56	8651.12	<27.34	15946.87	102842.3 8	98594.15
	2	5595.81 ^a	90234695 .07 ^a	25838247 8.85 ^a	7361912. 69 ^a	51327.74 ^a	91045.58 ^a	134246.0 2 ^a	5300254. 03 ^a	460543.1 4 ^a	317033.6 9 ^a	1130823. 28 ^a	6229.38 ^a	31306366 2.87 ^a	37920909 9.92 ^a	28426957 4.08 ^a
	3	3978.70 ^a	81250188 .87 ^a	31865737 2.74 ^a	6451632. 29 ^a	49427.44 ^a	217480.8 2 ^a	193977.4 9 ^a	13839914 .45 ^a	730889.4 0 ^a	91728.56 ^a	1079607. 95 ^a	6933.60 ^a	39144189 1.26 ^a	34500241 5.23 ^a	25081838 0.19 ^a

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B-27-004 ^b	BL	1748.75	88524.04	78241.15	6404.04	3334.06	<566.41	18346.45	53945.56	14987.35	<73.44	2901.76	<27.34	65756.3	909199.8	2193715.2
															8	3
	2	12442.28 ^a	44302450.99 ^a	31717488.56 ^a	7721543.10 ^a	39929.43 ^a	1152732.84 ^a	102104.59 ^a	39043478.678 ^a	893409.80 ^a	1188087.88 ^a	1024818.28 ^a	241548.67 ^a	60707159.09 ^a	33033261.3531 ^a	82124983.583 ^a
B-27-005	3	23031.33 ^a	75638381.45 ^a	10376870.125 ^a	8949753.73 ^a	44284.11 ^a	2555365.20 ^a	262952.57 ^a	35154120.256 ^a	1996874.56 ^a	767881.03 ^a	1458778.56 ^a	358443.78 ^a	12186667.612 ^a	23713034.8724 ^a	64815987.656 ^a
	BL	<59.77	<45.31	3224.37	4846.55	2908.5	<566.41	24198.69	<1922.66	<59.77	6051.74	4056.97	<27.34	<31.64	<384.38	214142.24
	2	<59.77	16347872.812 ^a	24893472.587 ^a	3620417.42 ^a	10592.80 ^a	<566.41	40880.39 ^a	3140433.15 ^a	199275.69 ^a	15368.66 ^a	268168.88 ^a	3501.95 ^a	28589397.227 ^a	89730004.946 ^a	39314217.383 ^a
B-27-006		2289.82 ^a	59883330.73 ^a	26153951.241 ^a	3748165.93 ^a	10506.78 ^a	21722.92 ^a	42602.55 ^a	7068446.74 ^a	705600.77 ^a	7765.07	482716.30 ^a	6598.47 ^a	20249813.986 ^a	30745606.119 ^a	16378333.366 ^a
	BL	<59.77	<45.31	2214.57	5747.48	1289.46	<566.41	21138.4	<1922.66	<59.77	<73.44	<39.84	<27.34	3762.26	<384.38	173760.52
	2	2006.86 ^a	41080235.34 ^a	87990729.34 ^a	637163.17 ^a	3829.26 ^a	<566.41	33671.03 ^a	4022531.36 ^a	22502133.84 ^a	18204.37 ^a	622822.05 ^a	4344.93 ^a	67521532.92 ^a	40416308.118 ^a	92556482.22 ^a
B-27-007 ^b	BL	<59.77	9710.43	251141	6540.99	3721.88	<566.41	27750.49	<1922.66	19740.44	4660.33	3399.72	<27.34	143798.4	<384.38	276737.09
														8		
	2	45933.25 ^a	63309720.915 ^a	68193068.158 ^a	13798251.56 ^a	8081.80 ^a	202478.99 ^a	43252.43 ^a	25535546.97 ^a	3569997.50 ^a	25987.24 ^a	2029894.63 ^a	34833.72 ^a	74225293.533 ^a	15190712.0551 ^a	15363477.9335 ^a

	3	37243.23 ^a	57083842 2.83 ^a	78567403 0.99 ^a	13804909 .59 ^a	10231.40 ^a	566332.9 8 ^a	87043.53 ^a	51083187 .27 ^a	4741747. 54 ^a	42155.61 ^a	2893171. 99 ^a	92624.37 ^a	75887607 2.85 ^a	10124707 14.88 ^a	13879567 38.61 ^a
B-27-008	BL	1835.41	12850.88	8528.59	7890.14	5020.99	<566.41	30441.6	<1922.66	20906.94	5645.41	11982.21	<27.34	4802.7	<384.38	83360.78
	2	4314.58 ^a	55453008 .01 ^a	32656340 6.32 ^a	838805.8 6 ^a	5127.11	315529.8 7 ^a	76875.01 ^a	1151594. 30 ^a	96517.19 ^a	14983.50 ^a	245741.0 5 ^a	2598.39 ^a	27351569 0.79 ^a	17051208 1.10 ^a	27437562 7.09 ^a
B-27-009	BL	<59.77	13496.79	44805.28	6997.33	4138.42	<566.41	25796.77	8232880. 82	12772.04	4345.17	<39.84	1643.41	29306.72	<384.38	153521.37
	2	5022.40 ^a	11640111. 19 ^a	12008658 9.09 ^a	701854.6 2 ^a	5912.40 ^a	158450.7 1 ^a	47258.97 ^a	11568125 .16 ^a	805571.8 1 ^a	71850.99 ^a	688382.7 4 ^a	6116.23 ^a	13132461 5.35 ^a	27881054 8.13 ^a	24592823 6.01 ^a
B-27-010	BL	<59.77	<45.31	425430.9 1	4126.93	1649.92	<566.41	16026.72	<1922.66	<59.77	4135.48	2728.44	<27.34	159854.6 8	<384.38	90744.86
	2	4002.90 ^a	15573034 5.16 ^a	26397131 3.98 ^a	2751251. 49 ^a	53447.16 ^a	241774.3 3 ^a	54092.21 ^a	4602223. 75 ^a	214125.4 0 ^a	69727.20 ^a	1748710. 82 ^a	1972.26 ^a	23123381 1.99 ^a	21924769 2.80 ^a	53212534 4.98 ^a

Supplementary Table 3 Induction of Specific IgG Levels in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313.

BL, baseline; IgG, immunoglobulin G; PFS, progression-free survival.

^a≥30% increase from baseline; ^bPatients achieved long-term PFS.

IgG Antibody	Antigen	Cut-off Criteria (pg/mL)	Sensitivity	Specificity
TA1	Lck	≥ 1748.75	0.400	0.800
TA2	WHSC2	≥ 9710.43	1.000	0.600
TA4	SART3	≤ 93507.37	0.800	0.400
TA5	SART2	≥ 6404.04	1.000	0.800
TA6	SART3	≥ 3334.06	1.000	0.800
TA7	MRP3	≥ 33638.80	0.200	1.000
TA8	Lck	≥ 25796.77	0.800	0.800
TA9	SART3	≥ 53945.56	0.800	1.000
TA10	Lck	≥ 12772.04	1.000	0.800
TA13	TMEM189	≥ 4345.17	0.800	0.600

Supplementary Table 4 Sensitivity and Specificity of IgG Cutoff Criteria.

Pt No.	MRP3	SART2	EGFR	LCK	PTHRP	SART3	KUA	WHSC2	HLA-A	3 × standard deviation of negative control
B27-001	1748.94	790.47	448.04	19.20	61.61	2315.39	608.85	844.87	6209.31	26.043
B27-002	860.26	536.54	36824.04	13.05	163.08	3246.16	604.22	746.10	9073.10	11.973
B27-003	111.22	711.79	1878.84	11.86	65.25	1288.65	521.98	381.11	2122.04	17.639
B27-004	1325.23	1785.73	687.18	50.73	170.41	2301.71	847.29	657.06	6923.35	11.536
B27-005	212.70	529.06	5316.72	7.21	53.18	2391.13	420.90	688.59	1968.42	12.466
B27-006	1560.86	182.56	22109.59	8.46	85.84	893.48	205.54	301.05	4194.14	5.768
B27-007	3538.45	675.20	117.43	70.46	62.63	1225.15	520.59	393.38	2595.12	13.677
B27-008	2551.57	764.08	2088.49	9.26	344.99	2213.52	571.90	588.11	4322.85	9.071
B27-009	565.84	1016.10	820.14	28.62	110.09	1201.04	592.26	345.67	1412.41	9.613
B27-010	306.07	379.42	139.12	12.65	32.88	1350.74	561.54	179.59	2286.65	6.414

Supplementary Table 5 Baseline mRNA Expression Level of Immunological Factors and Target Cancer-Associated Antigens in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313

EGFR, epidermal growth factor receptor; HLA, human leukocyte antigen; KUA, transmembrane protein 189 LCK, tyrosine-protein kinase LCK; mRNA, messenger ribonucleic acid; MRP3, multidrug resistance-associated protein 3; PTHRP, parathyroid hormone-related protein; SART2, squamous cell carcinoma antigen recognized by T-cells 2; SART3, squamous cell carcinoma antigen recognized by T-cells 3; WHSC2, Wolf-Hirschhorn Syndrome-Associated Genes.

Pt No.	IL-1 β	IL-1RA	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8	IL-9	IL-10
B27-001	1.45	87.14	<7.71	1.93	<19.99	<1.68	19.60	6.81	314.98	<5.59
B27-002	1.67	86.05	<7.62	1.99	<19.45	<1.61	18.13	7.18	334.08	<5.39
B27-005	1.54	<152.80	<7.62	1.48	<19.45	<1.61	11.93	<3.76	286.69	<5.39
B27-006	1.11	<152.80	<7.62	1.94	<21.04	<1.61	17.15	6.71	317.17	<5.39
B27-007	1.89	94.63	<7.62	1.67	<19.45	<1.61	15.09	4.62	319.22	<5.39
B27-008	1.79	97.15	<7.71	2.02	<19.99	1.94	22.21	6.81	323.07	<5.59
B27-009	1.93	188.11	<7.71	1.63	<19.99	2.25	15.41	5.62	286.50	<5.59
B27-010	2.90	102.85	<7.62	1.54	<19.45	<1.61	26.14	4.54	310.64	<5.39

Supplementary Table 6 Baseline Cytokine Levels (pg/mL) in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313

IL, interleukin; RA, receptor antagonist.

Pt No.	IL-12p70	IL-13	IL-15	IL-17A	Eotaxin	Basic FGF	G-CSF	GM-CSF	IFN- γ	IP-10
B27-001	<6.82	<1.31	<37.14	16.43	53.34	38.73	41.56	<1.85	<5.15	497.61
B27-002	<6.66	<1.28	<203.37	17.61	62.25	37.88	38.14	<1.84	<5.06	293.52
B27-005	<6.66	<1.28	<203.37	14.63	44.87	31.38	26.95	<1.84	<5.06	185.36
B27-006	<6.66	<1.28	<203.55	19.26	54.19	45.99	48.23	<1.78	5.24	999.13
B27-007	<6.66	<1.28	<203.37	16.26	41.97	35.91	31.97	<1.84	<5.06	325.31
B27-008	<6.82	<1.31	<37.14	18.52	56.90	39.09	36.27	<1.85	<5.15	391.31
B27-009	<6.82	<1.31	<37.14	17.33	36.10	37.30	39.98	<1.85	5.38	273.12
B27-010	<6.66	<1.28	<203.37	15.44	40.28	37.23	<25.26	<1.84	<5.06	326.74

Supplementary Table 7 Baseline Cytokine Levels (pg/mL) in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313

FGF, fibroblast growth factor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IL, interleukin; IP-10, interferon gamma-induced protein 10.

Pt No.	MCP-1	MIP-1α	PDGF-BB	MIP-1β	RANTES	TNF-α	VEGF
B27-001	12.88	<1.37	1014.87	253.32	5709.50	65.31	<108.63
B27-002	10.03	<1.39	732.04	226.74	5815.62	56.96	<18.61
B27-005	7.87	<1.39	478.83	213.48	5065.51	48.75	<18.61
B27-006	14.27	<1.38	505.60	251.79	6109.02	78.96	<93.88
B27-007	7.74	<1.39	1409.06	239.44	6341.34	55.57	<18.61
B27-008	16.27	<1.37	1018.16	236.27	6927.21	76.45	<108.63
B27-009	20.58	<1.37	1582.27	234.37	5252.00	66.31	<108.63
B27-010	9.06	<1.39	764.60	206.41	6467.89	52.17	<18.61

Supplementary Table 8 Baseline Cytokine Levels (pg/mL) in Patients with Glioblastoma Treated with Cancer Peptide Vaccine TAS0313

MIP-1 α , macrophage inflammatory protein-1; PDGF, platelet-derived growth factor-BB; RANTES, Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.