Data S1

Protocols for the phase II study of personalized peptide vaccination in advanced cancer patients

Date trial started: November, 2008
Date of completion: March, 2017

There were 3 different protocols with regard to the vaccination intervals. The cancer patients who became resistant to the standard systemic therapies received 6 times of personalized peptide vaccination (PPV) at 1-week intervals (the first cycle) followed by injection 6 times by at 2-week intervals (the second cycle) (termed as PRT1, UMIN registration nos. 1482, 1839, 1844, 1847, 1850, 1854, 1855, 1856, 1875, 1881, 1882, 1883, 1884, 2282, 3590, 5631, 6249, 6295, 6493, 7493, 8126, 8823, 8824, 8825, 8826, 8827, 8828, 10068 and 19390). The cancer patients at any stages, including the early stages of cancer received vaccinations 4 times at 1-week interval and subsequently 4 times at 2-week intervals (the first cycle); they then received vaccinations for another 4 times at 2-week intervals followed by 4 times at 4 weeks intervals (the second cycle) (termed as PRT2, UMIN nos. 2906, 2907, 2908, 2984, 2985, 2987, 3027, 3028, 3029, 3059, 3060, 3081, 3082, 3083, 5329, 10290, 11593, 14855, 19802 and 19879). Alternatively, the patients received vaccinations 4 times at 4 weeks intervals (the first cycle) followed by the same schedule to the first cycle (the second cycle) (termed as PRT3 and UMIN nos. 6927 and 11230). The patients in PRT1/2 or PRT3 received 1.5 or 3.0 ml (3 or 6 mg/each peptide) emulsion by each injection. After the second cycle in all of them, the patients who wished to continue the PPV received the vaccination at 2- to 12-week intervals until the withdrawal of consent or unacceptable toxicity. The trials were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

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Background

The active specific immunotherapy for cancer patients using either tumor-associated antigens or their peptides capable of inducing cytotoxic T lymphocytes (CTLs) against tumor cells has been failing to provide sufficient clinical benefits in order to be approved despite of large numbers of clinical trials from 1990s, and the mechanisms involved in this failure are not yet clarified. The authors thus developed a new concept of immunotherapy by providing peptides for individual patients based on their secondary immune responses [termed personalized peptide vaccination (PPV)] to develop clinically effective peptide-based cancer vaccines.

Aims

The aim was to develop a novel treatment modality, and for this purpose, a phase II randomized trial of PPV was conducted for advanced cancer patients who failed the preceding chemotherapy.

Patients and methods

Study design and population. The study population included patients enrolled in the phase II clinical trials of PPV that were conducted at the Kurume University of Cancer Vaccine Center, Kurume University Hospital, the Sendai Kousei Hospital or Naito Hospital in Japan from November, 2008 to March, 2017 for the 2,588 cancer patients.

Inclusion criteria. Eligible criteria were the pathologically confirmed diagnosis of cancer, positive responses in pre-vaccination plasm to IgG responses for at least 2 of the 31 warehouse peptides, positive status for the HLA-A2, -A24, or -A3s, or -A26, ages ≥20 years, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2 and neurological PS3 for only brain tumor patients, a life expectancy of at least 12 weeks, and adequate bone marrow function, hepatic function and renal function. The exclusion criteria were acute infection, a history of severe allergic reactions, or the other systemic diseases. All patients provided written informed consent for the study participation and data collection.

Exclusion criteria. Exclusion criteria included acute infection, a history of severe allergic reactions, pulmonary, cardiac, or other systemic diseases, or other inappropriate conditions for enrollment as judged by clinicians.

Intervention. Two to 4 peptides were selected based on pre-existing peptide-specific IgG levels against 31 warehouse peptides followed by emulsification with Montanide ISA 51 incomplete Freund's adjuvant (Seppic). Each study drug in a 1.5-ml (3 mg/ml) emulsion was subcutaneously injected into thigh, abdominal, back, chest, or cervical regions. The patients in PRT1 and 2 received 1.5 ml x 2 to 4 peptide emulsions on each vaccination day, while those in PRT3 received 1.5 ml x 4 to 8 peptide emulsions on each vaccination day.

Endpoints. The primary end point is overall survival (OS), which was defined as the time from the initial assignment to death by any cause. Secondary end points are progression-free survival (PFS), 1-year survival rate, immune responses and safety. PFS was defined as the time from assignment until objective disease progression based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria, or death. Immune responses were assessed by IgG titers measured by the Luminex system and CTL activity measured by the interferon (IFN)-γ release assay using blood sampled at pre-treatment and every 6 treatments. Safety was assessed based on physical examination, vital sign measurements, clinical laboratory analyses, and adverse events (AEs) graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Statistical analysis. The Student's t-test and Chi-square test were used to compare quantitative and categorical variables, respectively. OS was calculated as the time in months from the date of study enrollment to death or to the date of last contact. Time-to-event endpoints were analyzed using the Kaplan-Meier method, and between-group comparisons for OS were conducted using the log-rank test. The clinical efficacy of individual peptides for OS was evaluated by univariate and multivariate analyses with the Cox proportional hazards regression model, and HR and 95% CI values were calculated. All reported P-values were two-sided, and P-values <0.05 were considered to indicate statistically significant differences. JMP version 12 or SAS version 9.4 software (SAS Institute Inc.) was used to perform all analyses.

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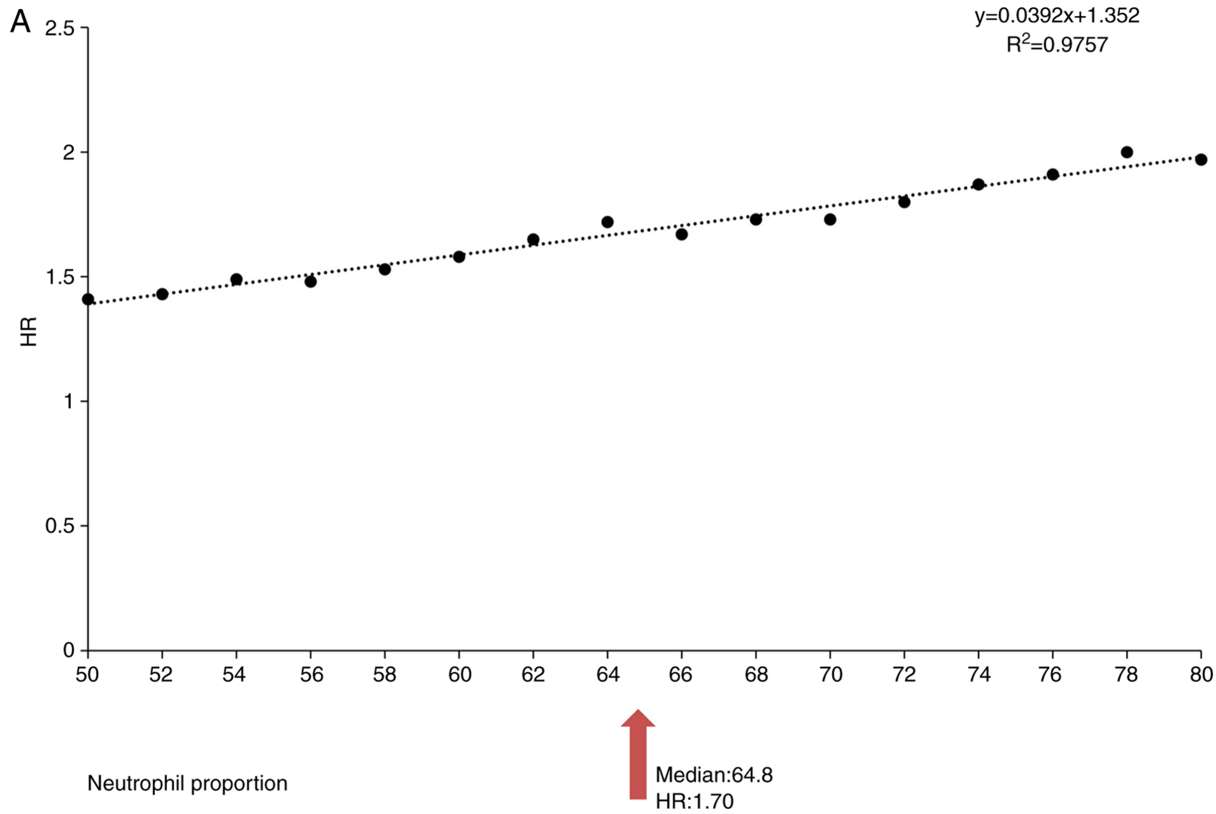
References

1. Tamura M, Nishizaka S, Maeda Y, Ito M, Harashima N, Harada M, Shichijo S and Itoh K: Identification of cyclophilin B-derived peptides capable of inducing histocompatibility leukocyte antigen-A2-restricted and tumor-specific cytotoxic T lymphocytes. Jpn J Cancer Res 92: 762-767, 2001.
2. Imai N, Harashima N, Ito M, Miyagi Y, Harada M, Yamada A and Itoh K: Identification of Lck-derived peptides capable of inducing HLA-A2-restricted and tumor-specific CTLs in cancer patients with distant metastases. Int J Cancer 94: 237-242, 2001.
3. Ito M, Shichijo S, Tsuda N, Ochi M, Harashima N, Saito N and Itoh K: Molecular basis of T cell-mediated recognition of pancreatic cancer cells. Cancer Res 61: 12038-12046, 2001.
4. Ito M, Shichijo S, Miyagi Y, Kobayashi T, Tsuda N, Yamada A, Saito N and Itoh K: Identification of SART3-derived peptides capable of inducing HLA-A2-restricted and tumor-specific CTLs in cancer patients with different HLA-A2 subtypes. Int J Cancer 88: 633-639, 2000.
5. Nakao M, Shichijo S, Imaizumi T, Inoue Y, Matsunaga K, Yamada A, Kikuchi M, Tsuda N, Ohta K, Takamori S, *et al*: Identification of a gene coding for a new squamous cell carcinoma antigen recognized by the CTL. J Immunol 164: 2565-2574, 2000.
6. Yang D, Nakao M, Shichijo S, Sasatomi T, Takasu H, Matsumoto H, Mori K, Hayashi A, Yamana H, Shirouzu K and Itoh K: Identification of a gene coding for a protein possessing shared tumor epitopes capable of inducing HLA-A24-restricted cytotoxic T lymphocytes in cancer patients. Cancer Res 59: 4056-4063, 1999.
7. Harashima N, Tanaka K, Sasatomi T, Shimizu K, Miyagi Y, Yamada A, Tamura M, Yamana H, Itoh K and Shichijo S: Recognition of the Lck tyrosine kinase as a tumor antigen by cytotoxic T lymphocytes of cancer patients with distant metastases. Eur J Immunol 31: 323-332, 2001.
8. Inoue Y, Takaue Y, Takei M, Kato K, Kanai S, Harada Y, Tobisu K, Noguchi M, Kakizoe T, Itoh K and Wakasugi H: Induction of tumor specific cytotoxic T lymphocytes in prostate cancer using prostatic acid phosphatase derived HLA-A2402 binding peptide. J Urol 166: 1508-1513, 2001.
9. Harada M, Kobayashi K, Matsueda S, Nakagawa M, Noguchi M and Itoh K: Prostate-specific antigen-derived epitopes capable of inducing cellular and humoral responses in HLA-A24+ prostate cancer patients. Prostate 57: 152-159, 2003.
10. Shomura H, Shichijo S, Komatsu N, Matsueda S, Mine T, Rikimaru T, Sato Y, Todo S and Itoh K: Identification of epidermal growth factor receptor-derived peptides recognised by both cellular and humoral immune responses in HLA-A24+ non-small cell lung cancer patients. Eur J Cancer 40: 1776-1786, 2004.
11. Yamada A, Kawano K, Koga M, Matsumoto T and Itoh K: Multidrug resistance-associated protein 3 is a tumor rejection antigen recognized by HLA-A2402-restricted cytotoxic T lymphocytes. Cancer Res 61: 6459-6466, 2001.
12. Kobayashi K, Noguchi M, Itoh K and Harada M: Identification of a prostate-specific membrane antigen-derived peptide capable of eliciting both cellular and humoral immune responses in HLA-A24+ prostate cancer patients. Cancer Sci 94: 622-627, 2003.
13. Ogata R, Matsueda S, Yao A, Noguchi M, Itoh K and Harada M: Identification of polycomb group protein enhancer of zeste homolog 2 (EZH2)-derived peptides immunogenic in HLA-A24+ prostate cancer patients. Prostate 60: 273-281, 2004.
14. Yao A, Harada M, Matsueda S, Ishihara Y, Shomura H, Noguchi M, Matsuoka K, Hara I, Kamidono S and Itoh K: Identification of parathyroid hormone-related protein-derived peptides immunogenic in human histocompatibility leukocyte antigen-A24+ prostate cancer patients. Br J Cancer 91: 287-296, 2004.
15. Minami T, Matsueda S, Takedatsu H, Tanaka M, Noguchi M, Uemura H, Itoh K and Harada M: Identification of SART3-derived peptides having the potential to induce cancer-reactive cytotoxic T lymphocytes from prostate cancer patients with HLA-A3 supertype alleles. Cancer Immunol Immunother 56: 689-698, 2007.
16. Naito M, Komohara Y, Ishihara Y, Noguchi M, Yamashita Y, Shirakusa T, Yamada A, Itoh K and Harada M: Identification of Lck-derived peptides applicable to anti-cancer vaccine for patients with human leukocyte antigen-A3 supertype alleles. Br J Cancer 97: 1648-1654, 2007.
17. Matsueda S, Takedatsu H, Yao A, Tanaka M, Noguchi M, Itoh K and Harada M: Identification of peptide vaccine candidates for prostate cancer patients with HLA-A3 supertype alleles. Clin Cancer Res 11: 6933-6943, 2005.

Figure S1. The correlation between the HR for the OS and (A) the pre-vaccination neutrophil ratio, (B) the pre-vaccination lymphocyte ratio, or (C) the neutrophil-lymphocyte ratio. HR, hazard ratio; OS, overall survival.



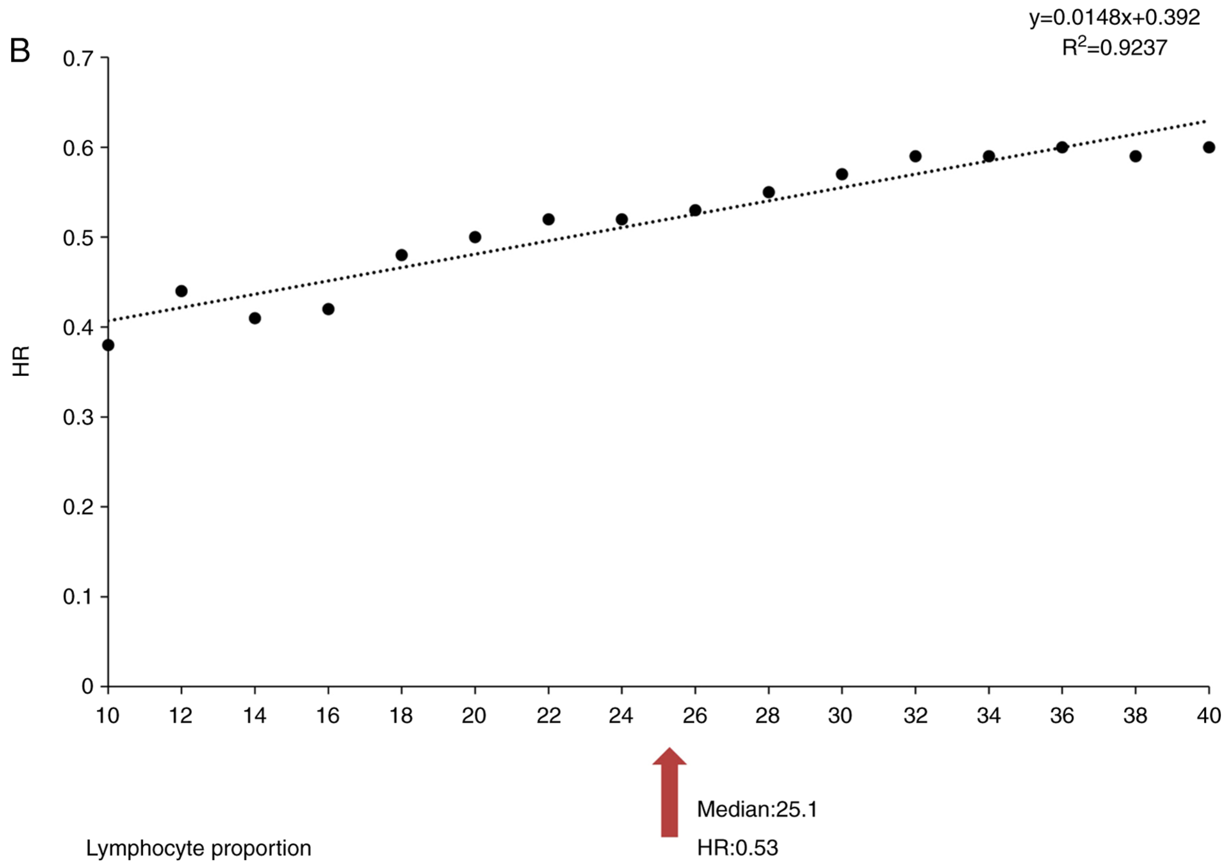


Figure S1. Continued. The correlation between the HR for the OS and (A) the pre-vaccination neutrophil ratio, (B) the pre-vaccination lymphocyte ratio, or (C) the neutrophil-lymphocyte ratio. HR, hazard ratio; OS, overall survival.

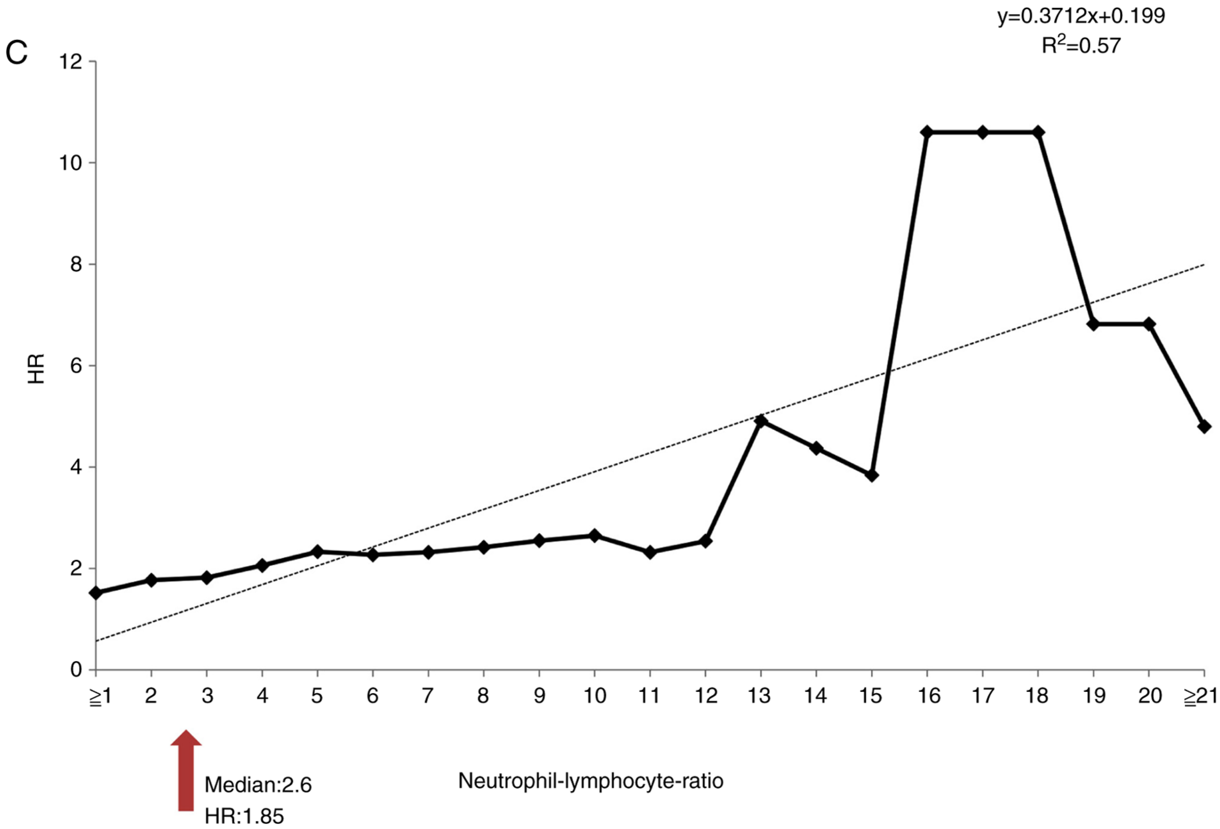


Table SI. Peptide candidates for personalized peptide vaccination.

Symbol for peptide	HLA type	Origin protein	Position of peptide	Amino acid sequence	(Refs.)
CypB-129	A2, A3sup	Cyclophilin B	129-138	KLKHYGPGWV	(1)
Lck-246	A2	$p56^{lck}$	246-254	KLVERLGAA	(2)
Lck-422	A2, A3sup	$p56^{lck}$	422-430	DVWSFGILL	(2)
ppMAPkkk-432	A2, A26	ppMAPkkk	432-440	DLLSHAFFA	(3)
WHSC2-103	A2, A3sup, A26	WHSC2	103-111	ASLDSDPWV	(3)
HNRPL-501	A2, A26	HNRPL	501-510	NVLHFFNAPL	(3)
UBE2V-43	A2	UBE2V	43-51	RLQEWCSVI	(3)
UBE2V-85	A2	UBE2V	85-93	LIADFLSGL	(3)
WHSC2-141	A2	WHSC2	141-149	ILGELREKV	(3)
HNRPL-140	A2	HNRPL	140-148	ALVEFEDVL	(3)
SART3-302	A2	SART3	302-310	LLQAEAPRL	(4)
SART3-309	A2	SART3	309-317	RLAEYQAYI	(4)
SART2-93	A24	SART2	93-101	DYSARWNEI	(5)
SART3-109	A24, A3sup, A26	SART3	109-118	VYDYNCHVDL	(6)
Lck-208	A24	$p56^{lck}$	208-216	HYTNASDGL	(7)
PAP-213	A24	PAP	213-221	LYCESVHNF	(8)
PSA-248	A24	PSA	248-257	HYRKWIKDTI	(9)
EGFR-800	A24	EGF-R	800-809	DYVREHKDNI	(10)
MRP3-503	A24	MRP3	503-511	LYAWEPSFL	(11)
MRP3-1293	A24	MRP3	1,293-1,302	NYSVRYRPGL	(11)
SART2-161	A24	SART2	161-169	AYDFLYNYL	(5)
Lck-486	A24	$p56^{lck}$	486-494	TFDYLRSVL	(7)
Lck-488	A24	$p56^{lck}$	488-497	DYLRSVLEDF	(7)
PSMA-624	A24	PSMA	624-632	TYSVSFDSL	(12)
EZH2-735	A24	EZH2	735-743	KYVGIEREM	(13)
PTHrP-102	A24	PTHrP	102-111	RYLTQETNKV	(14)
SART3-511	A3sup	SART3	511-519	WLEYYNLER	(15)
SART3-734	A3sup	SART3	734-742	QIRPIFSNR	(15)
Lck-90	A3sup	$p56^{lck}$	90-99	ILEQSGEWWK	(16)
Lck-449	A3sup	$p56^{lck}$	449-458	VIQNLERGYR	(16)
PAP-248	A3sup	PAP	248-257	GIHKQKEKSR	(17)

A3sup, HLA-A3 supertypes (A3, A11, A31, and A33); HLA, human leukocyte antigen; CypB, cyclophilin B; EGFR, epidermal growth factor-receptor; HNRPL, heterogeneous nuclear ribonucleoprotein L; Lck, $p56^{lck}$; MRP3, multidrug resistance-associated protein 3; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PTHrP, parathyroid hormone-related peptide; SART2, squamous cell carcinoma antigen2; SART3, squamous cell carcinoma antigen 3; UBE2V, ubiquitin-conjugated enzyme variant Kua; WHSC2, Wolf-Hirshhorn syndrome critical region 2.

Table SII. Association between the pre-vaccination soluble factors and OS.

Factor (no. of patients)	All cancer types HR (95%CI)	Urinary tract cancer HR (95%CI)	Prostate cancer HR (95%CI)	Biliary cancer HR (95%CI)	Breast cancer HR (95%CI)	Colon cancer HR (95%CI)	Lung cancer HR (95%CI)	Gastric cancer HR (95%CI)	Brain cancer HR (95%CI)
CRP (n=1,432)	2.13 (1.9-2.4)[a]	3.25 (1.6-6.8)[a]	1.78 (1.3-2.5)[a]	2.42 (1.6-3.8)[a]	2.77 (0.9-9.5)	2.85 (2.0-4.0)[a]	1.58 (1.2-2.1)[a]	2.96 (1.8-4.8)[a]	ND
IL-6 (n=471)	2.22 (1.8-2.7)[a]	2.83 (1.9-4.3)[a]	0.88 (0.5-1.5)	1.42 (0.7-3.7)	1.39 (0.6-3.0)	2.7 (0.6-10.5)	3.1 (1.6-6.3)[a]	1.7 (0.8-4.0)	0.79 (0.3-2.0)
BAFF (n=278)	1.58 (1.2-2.1)[a]	3.04 (1.9-4.9)[a]	ND	ND	1.8 (0.7-4.8)	ND	2.13 (1.0-5.0)[a]	1.18 (0.4-2.8)	ND
Haptoglobin (n=504)	1.58 (1.3-1.9)[a]	1.86 (1.2-2.9)[a]	1.95 (1.1-3.4)[a]	1.16 (0.7-2.0)	1.98 (0.9-4.3)	ND	2.04 (1.0-4.8)	1.59 (1.0-2.6)	0.52 (0.2-1.2)
HGF (n=250)	1.93 (1.4-2.6)[a]	1.15 (0.4-2.9)	2.34 (1.2-4.8)[a]	ND	1.49 (0.7-3.3)	ND	1.83 (0.9-3.7)	ND	1.26 (0.5-3.8)
VEGF (n=250)	3.21 (2.3-4.7)[a]	2.52 (0.9-6.3)	ND	ND	1.34 (0.4-3.4)	ND	2.75 (1.3-5.5)[a]	ND	ND
IL-2R (n=250)	1.93 (1.4-2.6)[a]	1.44 (0.6-3.8)	1.88 (1.1-3.2)[a]	ND	2.60 (1.2-5.7)[a]	ND	5.16 (2.3-13.1)[a]	ND	0.61 (0.2-1.4)
MIG (n=471)	2.30 (1.7-3.2)[a]	1.83 (0.8-4.6)	1.17 (0.7-2.1)	ND	1.98 (0.9-4.3)	ND	2.74 (1.3-6.2)*	ND	1.44 (0.6-3.6)
IL-2 (n=471)	0.53 (0.4-0.7)[a]	0.63 (0.4-1.0)[a]	2.15 (1.2-3.8)[a]	2.19 (0.5-6.3)	0.24 (0.1-0.7)[a]	0.23 (0.1-0.9)[a]	1.0 (0.5-2.1)	1.7 (0.3-5.8)	0.74 (0.3-1.7)
IL-1β (n=471)	0.58 (0.5-0.7)[a]	0.58 (0.4-0.9)[a]	1.8 (1.1-3.2)[a]	1.1 (0.5-2.0)	0.4 (0.2-0.9)[a]	0.4 (0.1-1.5)	0.7 (0.3-1.3)	2.7 (0.8-7.3)	1.39 (0.6-3.2)
GM-CSF (n=384)	0.65 (0.5-0.8)[a]	0.56 (0.4-0.9)[a]	0.93 (0.6-1.6)	ND	1.40 (0.6-4.2)	0.34 (0.1-1.3)	2.18 (1.0-5.4)[a]	2.55 (0.4-9.1)	0.75 (0.3-1.7)
IL-10 (n=471)	0.75 (0.6-0.9)[a]	0.67 (0.4-1.0)	1.50 (0.9-2.7)	2.43 (1.3-4.4)[a]	1.22 (0.5-3.6)	0.59 (0.1-2.3)	1.76 (0.8-4.4)	5.0E-10 (0.5-0.5)[a]	0.93 (0.4-2.1)
IL-8 (n=471)	1.35 (1.1-1.7)[a]	2.16 (1.4-3.3)[a]	1.37 (0.8-2.3)	1.30 (0.7-2.3)	0.56 (0.3-1.2)	0.82 (0.2-3.1)	1.49 (0.8-3.1)	1.34 (0.6-2.8)	1.35 (0.6-3.1)
MIP-1β (n=250)	1.16 (0.9-1.6)	0.67 (0.3-1.6)	1.29 (0.8-2.2)	ND	0.72 (0.3-1.5)	ND	2.43 (1.2-5.1)[a]	ND	1.46 (0.6-3.5)
IP-10 (n=461)	2.38 (1.9-2.9)[a]	3.18 (2.0-5.3)[a]	0.81 (0.4-1.4)	ND	3.59 (1.3-8.4)[a]	10.91 (2.6-55.5)[a]	0.89 (0.2-2.5)	ND	0.91 (0.2-2.7)
G-CSF (n=337)	0.67 (0.5-0.9)[a]	0.61 (0.3-1.6)	1.24 (0.7-2.2)	0.34 (0.1-1.6)	3.00 (1.0-12.7)[a]	ND	1.25 (0.6-2.7)	ND	0.89 (0.4-2.2)
MCP-1(n=338)	1.45 (1.1-1.9)[a]	0.91 (0.5-2.0)	1.14 (0.6-2.0)	ND	1.16 (0.4-2.7)	ND	0.90 (0.4-1.8)	1.01 (0.3-6.2)	0.93 (0.3-2.2)
IFN-α (n=471)	0.46 (0.3-0.6)[a]	1.35 (0.6-3.5)	1.14 (0.5-2.4)	ND	0.75 (0.3-1.8)	ND	0.42 (0.2-0.9)[a]	ND	0.36 (0.1-1.8)
IFN-γ (n=471)	0.57 (0.5-0.7)[a]	0.59 (0.4-0.9)[a]	2.70 (1.0-11.2)	3.05 (0.7-9.2)	0.77 (0.4-1.9)	0.47 (0.1-1.8)	0.66 (0.3-1.3)	ND	ND
IL-17A (n=250)	0.76 (0.6-1.0)	0.20 (0.1-0.5)[a]	1.59 (0.9-2.7)	ND	0.98 (0.5-2.3)	ND	1.35 (0.6-2.7)	ND	0.31 (0.1-0.8)[a]
Eotaxin (n=250)	0.66 (0.5-0.9)[a]	0.43 (0.2-1.1)	0.86 (0.5-1.5)	ND	1.12 (0.5-2.5)	ND	2.58 (1.1-5.9)[a]	ND	2.58 (1.1-5.9)[a]
TGF (n=277)	0.86 (0.6-1.1)	1.13 (0.7-1.8)	ND	ND	2.06 (0.8-7.1)	ND	2.04 (1.0-4.3)[a]	1.12 (0.5-2.4)	ND
IL-21 (n=277)	1.40 (1.1-1.9)[a]	1.06 (0.7-1.7)	ND	ND	0.54 (0.2-1.2)	ND	1.21 (0.5-2.7)	ND	ND
FGF-basic (n=250)	1.16 (0.9-1.6)	1.00 (0.4-2.3)	1.55 (0.9-2.7)	ND	2.10 (0.9-5.2)	ND	1.33 (0.6-2.6)	ND	0.50 (0.2-1.2)
IL-13 (n=250)	0.85 (0.6-1.2)	0.48 (0.2-1.1)	1.17 (0.7-2.0)	ND	1.45 (0.6-3.7)	ND	1.69 (0.9-3.4)	ND	0.53 (0.2-1.2)
IL-12 (n=252)	0.68 (0.5-0.9)[a]	1.41 (0.5-6.0)	1.30 (0.6-2.6)	ND	1.42 (0.7-3.3)	ND	1.13 (0.5-2.7)	ND	0.26 (0.1-1.3)
RANTES (n=250)	1.03 (0.8-1.4)	1.11 (0.5-2.8)	0.63 (0.4-1.1)	ND	1.11 (0.5-2.4)	ND	1.44 (0.7-2.9)	ND	1.90 (0.8-5.3)
MIP-1α (n=250)	1.06 (0.8-1.4)	0.80 (0.3-1.9)	0.99 (0.6-1.7)	ND	1.68 (0.8-4.1)	ND	1.25 (0.6-2.5)	ND	1.15 (0.5-2.7)
IL-15 (n=250)	0.75 (0.5-1.0)	1.24 (0.5-3.1)	1.59 (0.9-2.8)	ND	0.89 (0.4-2.3)	ND	1.50 (0.7-3.0)	ND	1.07 (0.4-2.5)
EGF (n=250)	0.51 (0.4-0.7)[a]	0.81 (0.3-1.9)	1.19 (0.6-2.2)	ND	0.64 (0.3-1.4)	ND	0.66 (0.3-1.3)	ND	0.79 (0.3-1.8)
IL-5 (n=471)	0.99 (0.8-1.2)	0.84 (0.6-1.3)	1.38 (0.8-2.5)	1.91 (1.0-3.5)	1.84 (0.9-4.2)	1.82 (0.4-6.9)	0.78 (0.3-1.6)	1.96 (0.9-4.2)	0.68 (0.3-1.8)
IL-1RA (n=471)	1.30 (1.0-1.8)	1.43 (0.6-3.3)	0.96 (0.6-1.7)	ND	1.00 (0.5-2.1)	ND	1.49 (0.8-3.0)	ND	0.53 (0.2-1.3)
TNF-α (n=471)	1.34 (1.1-1.6)[a]	1.17 (0.8-1.8)	1.40 (0.8-2.4)	1.22 (0.7-2.3)	1.37 (0.7-2.8)	2.49 (0.4-11.0)	1.54 (0.8-3.0)	0.56 (0.3-1.2)	1.34 (0.5-4.6)
IL-7 (n=250)	0.56 (0.4-0.8)[a]	0.50 (0.2-1.3)	0.87 (0.5-1.6)	ND	0.94 (0.4-2.2)	ND	0.72 (0.3-1.4)	ND	0.95 (0.4-2.2)
IL-4 (n=471)	1.27 (1.0-1.6)[a]	1.49 (1.0-2.3)	1.66 (1.0-2.9)	1.06 (0.6-1.8)	1.45 (0.7-3.3)	0.55 (0.1-3.0)	1.99 (0.9-4.1)	1.26 (0.6-2.6)	1.76 (0.6-7.5)

OS, overall survival (months); HR, hazard ratio; CI, confidence interval; ND, not determined as <20 samples were tested. [a]P<0.05, statistically significant difference.

Table SIII. Univariate and multivariate analyses of clinical blood cells and cytokines and the OS of patients at pre- and post-vaccination.

A, Clinical blood cells

	Univariate analysis		Multivariate analysis	
Factors (no. of patients)	HR (95% CI)	P-value	HR (95% CI)	P-value
Pre-vaccination cell counts				
White blood cells (2,588)	1.47 (1.35-1.60)	<0.01	1.07 (0.85-1.34)	0.55
Red blood cells (2,588)	0.61 (0.56-0.66)	<0.01	0.56 (0.46-0.68)	<0.01
Platelets (2,588)	1.29 (1.19-1.40)	<0.01	1.08 (0.89-1.32)	0.43
% Neutrophils (2,588)	1.70 (1.56-1.85)	<0.01	1.30 (0.90-1.86)	0.16
% Lymphocytes (2,588)	0.53 (0.48-0.57)	<0.01	0.67 (0.42-1.07)	0.09
Monocytes (2,575)	1.67 (1.54-1.82)	<0.01	1.40 (1.13-1.74)	<0.01
% Neutrophil-lymphocyte ratio (2,588)	1.84 (1.70-2.00)	<0.01	0.85 (0.49-1.49)	0.57
Post-vaccination IgG (FIU)	0.48 (0.43-0.52)	<0.01	1.16 (0.61-2.23)	0.65
To vaccinated peptides (2,116)				
Increased IgG levels (FIU)	0.45 (0.41-0.50)	<0.01	0.41 (0.21-0.79)	<0.01
To vaccinated peptides (2,116)				
Post-vaccination CTL	0.68 (0.56-0.82)	<0.01	0.81 (0.67-0.99)	0.04
To vaccinated peptides (IFNγ spots) (525)				

B, Cytokines

	Univariate analysis		Multivariate analysis	
Factors (no. of patients)	HR (95% CI)	P-value	HR (95% CI)	P-value
Pre-vaccination cell counts				
CRP (n=1,432)	2.13 (1.89-2.40)	<0.01	2.10 (1.16-3.85)	0.01
IL-6 (n=484)	2.19 (1.79-2.68)	<0.01	1.27 (0.64-2.42)	0.49
VEGF (n=250)	3.21 (2.33-4.47)	<0.01	2.32 (0.98-5.81)	0.05
MIG (n=250)	2.30 (1.69-3.15)	<0.01	1.14 (0.61-2.15)	0.68
IL-2 (n=471)	0.53 (0.43-0.65)	<0.01	0.98 (0.49-1.99)	0.95
IL-1β (n=471)	0.58 (0.47-0.71)	<0.01	1.34 (0.67-2.73)	0.40
GM-CSF (n=384)	0.65 (0.51-0.82)	<0.01	1.02 (0.56-1.87)	0.94
IP-10 (n=461)	2.38 (1.93-2.95)	<0.01	1.01 (0.41-2.23)	0.98
IFN-α (n=250)	0.46 (0.34-0.63)	<0.01	0.59 (0.29-1.20)	0.15
IFN-γ (n=471)	0.57 (0.47-0.70)	<0.01	0.91 (0.39-2.37)	0.85

OS, overall survival; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; VEGF, vascular endothelial growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon.