INTRODUCTION

Most studies predicting the clinical efficacy of cancer chemotherapies and immunotherapies have focused on tumor and somatic genomic features\(^1,2\), however, the mechanisms by which germline genetics affect the response to therapy remain unknown.

Approximately 8%–9% of the human genome is endogenous retroviral DNA\(^3\), and human endogenous retroviruses (HERVs) may benefit hosts\(^4,5\). HERVs have a similar genetic organization to that of exogenous retroviruses, such as human immunodeficiency virus (HIV), with two long terminal repeats encompassing the internal coding sequence of the four basic retroviral genes (\textit{gag}, \textit{pro}, \textit{pol}, and \textit{env})\(^6,7\). Therefore, HERVs are another class of germline-encoded elements in cancer biology. However, most of the peptides representing regions of similarity between HIV and different HERVs have unknown functions. Furthermore, the relationship between HERV function and response to therapy in cancer remains largely unexplored.

The major histocompatibility complex (MHC) class I (human leukocyte antigen [HLA]-A, -B, and -C in humans) displays 8–11 amino acid-long peptide fragments of proteins on the cell surface of tumor-infiltrating CD8\(^+\) cytotoxic T lymphocytes (CTLs)\(^8\), and positively responds to CD8\(^+\) CTL, leading to an improvement in overall survival for patients with cancer\(^9-12\). Sim JH et al. reported that the chromatin accessibility of baseline circulating CD8\(^+\) T cells could help predict PD-1 blockade responders in patients with gastric cancer\(^13\). However, the role of circulating lymphocyte subpopulations in the treatment response in cancer patients has not been extensively investigated. Fortunately, the correlation between HIV and HLA is the most studied field in CTL.
had a good prognosis, and those administered with ineffective therapies defined by baseline germline HLA information into four categories because of homogeneous Japanese populations. However, we did not elucidate the mechanisms underlying these findings.

In this pooled analysis of 2,049 GC patients, we adopted a novel approach to confirm the clinical significance of baseline HLA examination: We compared the training set at Tokai University Hospital and the validation set at the other Hospitals using candidate HLAs relevant to clinical outcomes and therapeutic response. To elucidate the mechanisms underlying our findings, we focused on the similarity between HERV and HIV gene-derived peptides and predicted HLA-restricted CD8+ T cell epitopes i.e. HERV and HIV genes derived-peptides that are recognized by T cells using bioinformatics. We hypothesized that patients whose HERV peptides might have induced CTL by administering therapies, such as epigenetic stimulation, showed increased CD8+ cells after gastrectomy and survived longer than CTL (-) patients. Finally, we compared the results of these two analyses.

PATIENTS AND METHODS

Clinical Trials and Patients

We assessed the efficacy of polysaccharide k (PSK)8, a Toll-like receptor (TLR) 2 agonist. Specifically, three randomized trials (cohort 1 study, cohort 2 study, and cohort 3 study) were conducted throughout Japan at member hospitals of the Japanese Society of Strategies for Cancer Research and Therapy (Appendix Table A1), and one observational study (cohort 4 study) was conducted at Tokai University Hospital. The detailed treatment and therapeutic regimens in the cohort studies are provided in Appendix Table A2. A total of 2049 patients were enrolled in the following manner in this study: the training set (n = 1501) at Tokai University Hospital, and a validation set (n = 548) at member hospitals.

Cohort studies (TRIAL REGISTRATION: UMIN ID: UMIN000037472, UMIN000037475, UMIN000037483, UMIN000037487) were conducted according to the principles of the Helsinki Declaration. All patients provided written informed consent before participating in the study. Ethical approval was received from the committee of the Japanese Society of Strategies for Cancer Research, which was proposed in our official Japanese journal “W”Waves” in 20039, which based on the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Health, Labor and Welfare in Japan in 2001 http://www.mhlw.go.jp/houdou/0103/ h0329-3.htm1 2001) and from Tokai University (11R-127, 11R-128).

Treatment regimens

PSK has the potential to suppress immunosuppressive factors and downregulate several invasion-related factors, namely, transforming growth factor-beta, urokinase plasminogen activator, matrix metalloproteinase (MMP)-2, MMP-9, and hypoxia-inducible factor-Iα 

Fluoropyrimidines are a group of anti-metabolites that impede DNA and RNA synthesis by blocking enzymes. Mitomycin C (M) is an antibiotic and a carcinostatic agent that inhibits DNA synthesis by cross-linking DNA. Cisplatin (DDP) (C) is an antineoplastic agent that binds to specific DNA base sequences and produces intrastrand and interstrand DNA cross-links.

HLA Examination and Phenotypic Analysis of Lymphocyte Subpopulations

Peripheral blood samples were collected from the enrolled patients before surgery, and HLA examination was conducted. In some patients, phenotypic analyses of lymphocyte subpopulations (CD4+, CD8+, CD16+, and CD57+ cells [%]) were performed on the same blood samples by using the flow cytometry at the LSI Medience Corporation (Tokyo, Japan) (formerly known as Mitsubishi Chemical Medicine Corporation). CD8+ cells were performed in 1241 (953 and 288 in the training set and the validation set, respectively) subjects at baseline and 492 (449 and 43 in the training set and the validation set, respectively) at baseline and 1 year after gastrectomy.

Since 1977, HLAs have been examined serologically using the National Institutes of Health standard microlymphocytotoxicity method for HLA-A, -B, -C, -DR, and -DQ antigens at the Department of Transplantation Immunology, Tokai University, followed by the LSI Medience Corporation from May 1987. HLA examination was followed by PCR sequence-based typing (SBT) from June 2006 at the LSI Medience Corporation. Some HLA profiles of patients who received treatment between 1977 and 1987 and survived until 2006 were later re-examined using PCR-SBT.

In this study, all data refer to HLA phenotypes, despite the use of PCR-SBT. HLAs that were evaluated as independent parameters were as follows: A loci: 1, 2, 3, 9 (23, 24), 10 (25, 26, 34, 66), 11, A19 (29, 31, 32, 33, 74), 28 (68, 69), 36, 43, 80, and blank; B loci: 5 (51, 52), 7, 8, 12 (44, 45), 13, 14 (64, 65), 15 (62, 63), 16 (38, 39), 17, 18, 21 (49, 50), 22 (54, 55, 56), 27, 35, 37, 40 (60, 61), 41, 42, 46, 47, 48, 53, 59, 67, and blank; C loci: w1, w2, w3 (w9, w10), w4, w5, w6, w7, w8, and blank; DR loci: 1, 2 (15, 16), 3 (17, 18), 4, 5 (11, 12), 6 (13, 14), 7, 8, 9, 10, 52, 53, and blank; and DQ loci: 1 (5, 6), 3 (7, 8, 9), 4, and blank.

Effective and Ineffective Therapy

In the training set, we defined the following two sets

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of patients in the training set: those who received effective therapies and were alive over 10 years, and those who received ineffective therapies and died within 5 years after gastrectomy (Table 1). Effective and ineffec-

| HLA category Type | HLA antigen | Initial therapy: effective therapy-10Y | Patients survived all at 10 years after gastrectomy |
|------------------|-------------|----------------------------------------|---------------------------------------------------|
| Type I           | A33 PSK     | Yes                                    |                                                   |
|                  | A33 F       | Yes                                    |                                                   |
|                  | B15 PSK     | Yes                                    |                                                   |
|                  | B22 PSK     | Yes                                    |                                                   |
|                  | B54 PSK     | Yes                                    |                                                   |
|                  | A2 F        | Yes                                    |                                                   |
|                  | A10 PSK     | Yes                                    |                                                   |
|                  | B5 PSK      | Yes                                    |                                                   |
|                  | B15 PSK     | Yes                                    |                                                   |
|                  | B16 PSK     | Yes                                    |                                                   |
|                  | B22 PSK     | Yes                                    |                                                   |
|                  | B46 PSK     | Yes                                    |                                                   |
| Type II          | A33 PSK     | Yes                                    |                                                   |
|                  | B15 PSK     | Yes                                    |                                                   |
|                  | B22 PSK     | Yes                                    |                                                   |
|                  | B46 PSK     | Yes                                    |                                                   |
|                  | B51 PSK     | Yes                                    |                                                   |
|                  | B52 PSK     | Yes                                    |                                                   |
|                  | B54 PSK     | Yes                                    |                                                   |
|                  | B61 PSK     | Yes                                    |                                                   |
|                  | Cw1 PSK     | Yes                                    |                                                   |
|                  | Cw4 PSK     | Yes                                    |                                                   |
|                  | DR2 M       | Yes                                    |                                                   |
|                  | DR4 PSK     | Yes                                    |                                                   |
|                  | DR5 M       | Yes                                    |                                                   |
|                  | DR8 PSK     | Yes                                    |                                                   |
|                  | DR9 MFPSK   | Yes                                    |                                                   |
| Type III         | A10 PSK     | Yes                                    |                                                   |
|                  | B15 PSK     | Yes                                    |                                                   |
|                  | B16 PSK     | Yes                                    |                                                   |
|                  | B35 PSK     | Yes                                    |                                                   |
|                  | B51 PSK     | Yes                                    |                                                   |
|                  | B52 PSK     | Yes                                    |                                                   |
|                  | B61 MFPSK   | Yes                                    |                                                   |
|                  | Cw4 PSK     | Yes                                    |                                                   |
|                  | DR5 PSK     | Yes                                    |                                                   |
|                  | DR9 MFPSK   | Yes                                    |                                                   |
| Type IV          | A10 MFPSK   | Yes                                    |                                                   |
|                  | A33 PSK     | Yes                                    |                                                   |
|                  | B12 PSK     | Yes                                    |                                                   |
|                  | B16 F       | Yes                                    |                                                   |
|                  | B22 MF      | Yes                                    |                                                   |
|                  | B35 PSK     | Yes                                    |                                                   |
|                  | B46 PSK     | Yes                                    |                                                   |
|                  | B51 PSK     | Yes                                    |                                                   |
|                  | B52 PSK     | Yes                                    |                                                   |
|                  | B54 PSK     | Yes                                    |                                                   |
|                  | B61 PSK     | Yes                                    |                                                   |
|                  | Cw4 PSK     | Yes                                    |                                                   |
|                  | DR2 MFPSK   | Yes                                    |                                                   |
|                  | DR1 MFPSK   | Yes                                    |                                                   |
|                  | DR3 MFPSK   | Yes                                    |                                                   |
|                  | DR4 MFPSK   | Yes                                    |                                                   |
|                  | DR5 MFPSK   | Yes                                    |                                                   |
|                  | DR6 MFPSK   | Yes                                    |                                                   |
|                  | DR8 MFPSK   | Yes                                    |                                                   |
|                  | DR9 MFPSK   | Yes                                    |                                                   |
|                  | DR52 MFPSK  | Yes                                    |                                                   |

Abbreviations: PSK: Krestin, F: Fluoropyrimidines, M: Mitomycin C, CDDP: Cisplatin (C), FPSK: a combination PSK and F, MF: a combination M and F, MFPSK: a combination PSK and F and M
tive therapies derived from the training set were applied to each patient in the validation set by using HLA category types with specific HLA-A, -B, -C, -DQ, and -DR phenotypes. Four HLA category types were based on a prior study by using “Quantification method III”, which was developed in Japan by Hayashi in the 1950s. This method involves the simultaneous grouping of individuals and categories and is used for the taxonomy of individuals and categories based on response patterns, which is mathematically equivalent to the correspondence analysis of “Analyse Factorielle des Correspondances”, developed in the 1960s. Subsequently, “Quantification method III” was introduced for correspondence analysis in English-speaking countries. Therefore, we classified patients into the following four groups: those administered with one of the effective therapies, those administered with one of the ineffective therapies, those administered with one of neither effective nor ineffective therapies but received other therapy, and unclassified patients who presented with no specific HLAs required for the classification of HLA category types. The details are provided in Appendix Methods.

HLA-restricted HERV-derived Epitope Peptides on HLA Alleles and Prediction of Cytotoxic T Lymphocyte Epitopes

We first focused on HLA alleles with serospecificity for 11 candidate HLAs (Appendix Table A3), associated with survival outcomes and therapies. To investigate the relationship between clinical outcomes and putative HERV and HIV gene-derived peptides, we used the NetMHCpan - 4.1 epitope prediction search and calculated binding affinity (% rank < .5) in peptides of 8–15 amino acids in length. We used the HLA allele sequences with HLA specificity listed in the Immuno Polymorphism Database (IPD) - international ImMunoGene Tics project (IMGT)/HLA Database (https://www.ebi.ac.uk/ipd/imgt/hla/index.html). HLA alleles used in the NetMHCpan 4.1 prediction epitope search were shown in Appendix Table A4.

We used the sequences of 12 HERV genes and 9 HIV-1 group M subtype B (isolated HXB2) genes listed in the UniProt Knowledgebase (UniProtKB) (https://www.uniprot.org/) (Appendix Table A5). We used the HLA-restricted HIV-1 epitope peptides listed in the Los Alamos National Laboratory HIV immunology database (https://www.hiv.lanl.gov/content/immunology/tables/ctl_summary.html; last updated at 2020-03-27).

In the NetMHCpan - 4.1 epitope prediction search, we identified putative 1617 HLA-restricted HERV-derived epitope peptides on HLA alleles; 768 peptides good for F therapy; 1193 peptides poor for F therapy; 667 peptides good for FPSK therapy; 613 peptides poor for FPSK therapy; 221 peptides for good M therapy; 413 peptides poor for M therapy; 655 peptides poor for MF therapy; 413 peptides good for FPSK therapy; 436 peptides poor for FPSK therapy; and 697 peptides good for PSK therapy, and 973 HLA-restricted HIV-derived epitope peptides; 416 peptides good for F therapy, 698 peptides poor for F therapy, 361 peptides good for FPSK therapy, 458 peptides poor for FPSK therapy, 176 peptides good for M therapy, 161 peptides poor for M therapy, 341 peptides poor for MF therapy, 150 peptides good for FPSK therapy, 248 peptides poor for FPSK therapy, and 445 peptides good for PSK therapy.

Global alignment of identity and similarity was performed using the online version of Needle (ebi.ac.uk/Tools/psa/emboss_needle). EBLOSUM62 (true) matrix (gap penalty of 10 and extended penalty of 1.0) and EBLOSUM50 (false) matrix (gap penalty of 10 and extended penalty of 1.0) were used to identify regions between two HERV-derived and HIV-derived peptide sequences. Ninety-five HERV-derived peptides similar to HLA-restricted HIV-1 gene-derived peptides (henceforth called HERV-HIV peptides) were similar to HIV-1 peptides and included the same three or more amino acids. We used data on the HLA of the HLA-restricted HIV peptide derived from the Los Alamos National Laboratory HIV immunology database and then linked a HERV-HIV peptide to 2049 patients based on the patient's HLA information. These peptides could be linked to 2041 subjects. We examined the association among 2041 patients with 95 HERV-HIV peptides, HLAs, therapies, and outcomes (all patients survived during the follow-up period) by sex. We identified 31 new specific HLAs associated with specific therapy and sex. Next, we evaluated the beneficial survival outcomes of all combinations (n = 2697) of the new 31 HLAs and 95 HERV-HIV peptides by sex. Finally, we analyzed the association between clinical outcomes (females or males survived all during the follow-up period), HLA, and therapies in 84 HLA-restricted HERV-HIV peptides. We performed two analyses to identify patients whose HERV peptides might have induced CTLs in response to the administered therapy. Finally, we compared the outcomes of CTL (+) or CTL (-) patients to those administered with one of the effective therapies or one of the ineffective therapies, those who received neither one of the effective nor ineffective therapies, but other therapy, and unclassified patients.

Outcomes and Statistical Analysis

Cumulative survival time was calculated using the Kaplan–Meier method and compared with overall survival (OS), defined as the time from the date of gastrectomy to death due to any cause, including death in the hospital. A log-rank test was used to analyze the statistical differences between the two groups. The Cox proportional hazards model was assessed through univariate analysis with hazard ratios (HRs) and 95% confidence intervals (CIs). Chi-squared tests were used for the discrete vari-
ables. Mean values were represented as mean ± standard error of the mean and were compared using t-tests and the Mann-Whitney U test for the independent sample and paired two-sample t-tests and the Wilcoxon signed-rank test for two related samples. P values of ≤ .05 indicated statistical significance. All statistical analyses were performed using SPSS software version 24 (SPSS Inc., Chicago, IL, USA).

RESULTS

Follow-up and Outcomes in Patients Administered with the Effective or Ineffective Therapies

The routine follow-up data cutoff date was April 2015, and the median follow-up duration was 6.9 years (interquartile range, 10 days to 37.2 years). The survivors’ results were censored at the last follow-up. Only cohort 4 included 12 patients who died within 30 days. Characteristics of 2,049 patients with primary adenocarcinoma of the stomach are shown in Appendix Table A6. As shown in Fig. 1, patients who received effective therapies benefitted more than patients who received neither effective nor ineffective therapies, unclassified patients, and those who received ineffective therapies, in both the training (Fig. 1A) and the validation sets (Fig. 1B). In the validation set, patients who received effective therapies survived throughout the follow-up period. In the training set, the first day a female patient died (Fig. 1C) was 5159 days after gastrectomy, and the first day a male patient died was 3861 days after gastrectomy (Fig. 1D).

Induction of CTL via HLA-Restricted HERV-Derived Peptides in Therapies and the Outcomes of CTL (+) or CTL (-) Patients

Among the HLAs examined, we identified 11 candidate HLAs; their OS curves are presented in Appendix Fig. A1. In the NetMHCpan - 4.1 epitope prediction search, we identified putative 1617 HLA-restricted HERV-derived epitope peptides and 973 HLA-restricted HIV-derived epitope peptides on 11 HLAs. Using the online version of Needle, 95 HERV-HIV peptides were identified. Among these peptides, we performed linkages of HLA-restricted HERV-HIV peptides with 2041 patients with the same HLAs and found new 31 HLA phenotypes (HLA-A2, -A3, -A9, -A10, -A11, -A31, -B7, -B15, -B16, -B22, -B27, -B35, -B46, -B51, -B54, -B55, -B59, -B60, -B61, -Cw1, -Cw7, -Cw8, -Cw10, -DQ1, -DQ2, -DQ3, -DQ4, -DR1, -DR4, -DR5, and -DR9). We identified the beneficial outcomes of 84 HLA-restricted HERV-HIV peptides with specific therapies by sex. Finally, we identified 155 patients whose HERV peptides might have induced CTL owing to subjection to therapy from the perspective of each HERV-HIV peptide and 177 patients from the perspective of each therapy administered. The concordance rate was 99.4%. The characteristics of the 155 and 177 patients selected from 2041 subjects are shown in Appendix Table A7 and Appendix Table A8, respectively. Among them a significant difference was observed in the frequency of sex (Chi-square test, $P = .004$ in 155 and $P < .0001$ in 177 patients), distant metastasis (Chi-square test, $P = .009$ in 155 and $P = .006$ in 177 patients), and depth of tumor ($P = .022$ in 155 and $P = .018$ in 177 patients) between CTL (+) and CTL (-) subjects.

We summarized 84 putative HLA-restricted HERV-HIV peptides in 155 CTL (+) patients and the factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced and who survived all during the follow-up period from the perspective of each HERV-HIV peptide in Table 2, 177 patients in Appendix Table A9, and similarity, identity, HLA-restricted HERV-HIV peptides, and HLA data from the Los Alamos National Laboratory HIV immunology database in Appendix Table A10. Almost all patients with these HERV-HIV peptides survived during the follow-up period in 155 patients and showed significantly better outcomes than those without HERV-HIV peptides in 177 patients (Appendix Table A11).

Fig. 2 depicts the OS curves of 155 CTL (+) and 1886 CTL (-) patients. The 20-year OS probability was 79.7% and 37.8% in 155 CTL (+) and 1886 CTL (-) patients, respectively. There was a significant difference between them (HR, 5.05; 95% CI, 3.08 to 8.28; $P < .0001$) (Fig. 2A). The 20-year OS probability was 89.9% and 30.1% in 61 CTL (+) and 535 CTL (-) females, respectively. CTL (+) females had significantly longer survival than CTL (-) subjects (HR, 8.45; 95% CI, 3.15 to 22.69; $P < .0001$) (A, upper right) and higher HR than males (the 20-year OS of 72.4% and 37.1% in 94 CTL (+) and 1359 CTL (-), respectively; HR, 3.89; 95% CI, 2.20 to 6.88; $P < .0001$) (A, lower right). There was a significant difference between the training set and validation set (the training set: the 10-year OS of 80% and 61% in 25 CTL (+) and 37.8% in 1886 CTL (-) patients, respectively. There was a significant difference between them (HR, 5.05; 95% CI, 3.08 to 8.28; $P < .0001$) (A, upper right) and higher HR than males (the 20-year OS of 72.4% and 37.1% in 94 CTL (+) and 1359 CTL (-), respectively; HR, 3.89; 95% CI, 2.20 to 6.88; $P < .0001$) (A, lower right). There was a significant difference between the training set and validation set (the training set: the 10-year OS of 80% and 61% in 25 CTL (+) and 37.8% in 1886 CTL (-) patients, respectively. There was a significant difference between them (HR, 5.05; 95% CI, 3.08 to 8.28; $P < .0001$) (A, upper right) and higher HR than males (the 20-year OS of 72.4% and 37.1% in 94 CTL (+) and 1359 CTL (-), respectively; HR, 3.89; 95% CI, 2.20 to 6.88; $P < .0001$) (A, lower right).

Appendix Fig. A2 is shown the OS curves between sex in the validation set and the training set. The validation set: the 10-year OS of 80% and 61% in 25 CTL (+) and 139 CTL (-) females (Appendix Fig. A2A), respectively; HR, 7.47; 95% CI, 1.03 to 54.4; $P = .047$ and the 20-year OS of 94.5% and 33.9% in 39 CTL (+) and 345 CTL (-) males (Appendix Fig. A2B), respectively; HR, 6.42; 95% CI, 1.58 to 26.04; $P = .009$. The training set: the 20-year OS of 90.5% and 52.5% in 36 CTL (+) and 395 CTL (-) females (Appendix Fig. A2C), respectively; HR, 8.36; 95% CI, 2.68 to 26.12; $P < .0001$ and the 20-year OS of 68.1% and 36.3% in 55 CTL (+) and 1009
CTL (-) males (Appendix Fig. A2D), respectively; HR, 3.23; 95% CI, 1.73 to 6.03; \(P < .0001\). But there was no significant difference between females and males in CTL (+) patients (log rank test \(P = .256\) and training set: \(P = .15\) and validation set: \(P = .776\)).

In 154 patients administered effective therapies in the training set, CTL (+) patients exhibited a prolonged survival time until the death of the first patient (6275 days after gastrectomy), compared to that in CTL (-) patients (3861 days) (Fig. 3A). Among patients administered effective therapies in the training set, the first day of patient death for CTL (-) females was 5159 days after gastrectomy, CTL (+) females survived throughout the follow-up period (Fig. 3A, upper right), and the first day of patient
Table 2 Putative 84 HERV-HIV peptides and factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL and who survived all during the follow-up period in 155 CTL (+) patients from the perspective of each HERV peptide

| HERV_HIV peptide | No of patients | No of patients in 155 CTL (+) patients | Factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL and who survived all during the follow-up period |
|------------------|----------------|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| GPKERVKICT_GPKKQWPL | 193 | 7 | |
| HPRNIESEPHPRISSEVHI | 193 | 2 | |
| HPRNIESEPHPRISSEVHI | 193 | 2 | |
| IPLLALGIL_IPLLGDARLVI | 193 | 2 | |
| IPLLALGIL_IPLLGDARLVI | 193 | 2 | |
| IPYNSQGQAI_IPYNPOQSQGVQG | 193 | 2 | |
| IRQKPPIF_RRIQGRILLER | 193 | 2 | |
| KPPGIFPPIPPLPSVTKLL | 193 | 2 | |
| KPPYMHV_KPPLPSVTKL | 193 | 2 | |
| ARLQDVQAQ_ARLVTTYW | 12 | 5 | |
| GRALQPVKGRAFVTIGK | 12 | 7 | |
| GRALQPVKGRAFVTIGK | 12 | 7 | |
| IPLLAGLGI_IPLGDARLVI | 12 | 2 | |
| IPLLAGLGI_IPLGDARLVI | 12 | 2 | |
| IPYNSQGQAI_IPYNPQSQGV | 12 | 2 | |
| IRQKPPIF_RRIQGLERILL | 12 | 2 | |
| KPPIFGPIF_KPPLPSVTKL | 12 | 2 | |
| KPPGMKQLK_KPPLPSVTKL | 12 | 2 | |
| NSPHEGKL_NSPTRREL | 41 | 4 | |
| NSPQTCQTE_NSPTREL | 41 | 4 | |
| NSPQMTTRL_NSPTREL | 41 | 4 | |
| KEKQMTLFL_KKGQGELGL | 931 | 24 | |
| KEKQMTLFL_KKGQGELGL | 931 | 24 | |
| SPTICQTE_RMYSPSIL | 808 | 5 | |
| SPTICQTE_RMYSPSIL | 808 | 5 | |
| SPTICQTE_RMYSPSIL | 808 | 5 | |
| EFPQTEDSIE_EFPFRDYVDRF | 808 | 22 | |
| EFPQTEDSIE_EFPFRDYVDRF | 808 | 22 | |
| FPQFFBE_FFSIPHTVP | 21 | 2 | |
| FPQFFBE_FFSIPHTVP | 21 | 2 | |
| FPQFTPLQ_FQFPQTVPLQ | 518 | 10 | |
| FPQFTPLQ_FQFPQTVPLQ | 518 | 10 | |
| FPQFTPLQ_FQFPQTVPLQ | 518 | 10 | |
| IPLLTVWDDPLTLLEAEEL | 810 | 13 | |
| IPLLTVWDDPLTLLEAEEL | 810 | 13 | |
| LEGKGPHEL_LEGKGVILVA | 9 | 2 | |
| LEGKGPHEL_LEGKGVILVA | 9 | 2 | |
| SPKEWTSIPRSTNLWAVV | 937 | 5 | |
| SPKEWTSIPRSTNLWAVV | 937 | 5 | |
| SPKEWTSIPRSTNLWAVV | 937 | 5 | |
| TQPQMIQNL_TQPVPLRPQ | 520 | 25 | |
| TQPQMIQNL_TQPVPLRPQ | 520 | 25 | |
| TQPQMIQNL_TQPVPLRPQ | 520 | 25 | |
| YPSFSQK1_YPSFTSLID | 649 | 12 | |
| YPSFSQK1_YPSFTSLID | 649 | 12 | |
| YPSFSQK1_YPSFTSLID | 649 | 12 | |
| AEIPPTWAQL_SAEVPVQL | 1162 | 24 | |
| AEWVLLDQTA_EWDRPHVPP | 619 | 29 | |
| AEAPEAMT_YCAPAGFALL | 649 | 19 | |
| CPKKEPCES_CPKVSFEPI | 247 | 5 | |
| EKYPIPHN_EEWAFAPE | 414 | 5 | |
| GEIQLVSS_GEIYKRWWI | 619 | 10 | |
| HERV_HIV peptide               | No of patients | No of patients in 155 CTL (+) patients | Factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL and who survived all during the follow-up period |
|-------------------------------|----------------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| IEEKYPINY_IEELRQHLL           | 1128           | 15 (male (n = 4))                      |                                                                                                                                  |
| IPAINNEKIPA_IFAETGQETA         | 222            | 9                                      |                                                                                                                                  |
| IPIYNSQPL_IPIHYCAPA            | 247            | 7                                      |                                                                                                                                  |
| IPYNSQGQA_IPYNPSQGQVV          | 63             | 10                                     |                                                                                                                                  |
| IVEEKVKEI_IVTDSQYAL            | 140            | 2                                      |                                                                                                                                  |
| KEGVAFLA_KEKKGLEL             | 931            | 19                                     |                                                                                                                                  |
| KETESLHCEY_KETINEEAA           | 1523           | 19                                     |                                                                                                                                  |
| KIKSKASY_JKIKPLPSV            | 808            | 22                                     |                                                                                                                                  |
| LPQGLNISP_LPQWKGSPA            | 21             | 8                                      |                                                                                                                                  |
| PPLIRAVTV_IQLPLPERL           | 808            | 4                                      |                                                                                                                                  |
| QAIIKLQTNL_QAISPRTL           | 1251           | 4                                      |                                                                                                                                  |
| QELHALTHV_QEKLNSAVSL          | 619            | 4                                      |                                                                                                                                  |
| QEVYIPTEL_DPNIPQEVVL          | 767            | 4                                      |                                                                                                                                  |
| RAIIFIIPL_RAIEAQQL            | 1581           | 19                                     |                                                                                                                                  |
| REPLENAL_REPHNEWTL            | 619            | 19                                     |                                                                                                                                  |
| REPLENALT_V_REPHNEWTL         | 619            | 19                                     |                                                                                                                                  |
| RKEKGVAFLA_KEKKGLEL           | 931            | 19                                     |                                                                                                                                  |
| RPLDRPASP_RPLVTIKI            | 376            | 19                                     |                                                                                                                                  |
| RQRPSRTGH_EYRKILRQR           | 292            | 11                                     |                                                                                                                                  |
| RSLSKGPTF_RSLYNTVATLY         | 1334           | 5                                      |                                                                                                                                  |
| SPAMIPKDW_SPAIQFSQM           | 635            | 5                                      |                                                                                                                                  |
| SPHEGKLI_SPHPRISSEVHI         | 556            | 5                                      |                                                                                                                                  |
| SRLAKDSKF_SRAKWNTTL           | 12             | 5                                      |                                                                                                                                  |
| SRLAIAK1L_SRLAFHHV            | 376            | 25                                     |                                                                                                                                  |
| TPQMLNLAL_TPOQLNTML           | 917            | 28 (female (n=15))                     |                                                                                                                                  |
| TSLTPLHFKEKYLPLTLSL           | 689            | 28 (female (n=15))                     |                                                                                                                                  |
| VERTNRTL_VERYLDQQL            | 20             | 4                                      |                                                                                                                                  |
| VPLTEQV_VPLOPLPL              | 247            | 4                                      |                                                                                                                                  |
| VRDFKSDCY_VRDLQAEHL           | 330            | 22                                     |                                                                                                                                  |
| YQWPPAEI_YQYMDLYV             | 808            | 22                                     |                                                                                                                                  |
| YYKLSQEL_QELKNSAVSL           | 619            | 12                                     |                                                                                                                                  |

Abbreviations: PSK; Krestin, F; Fluoropyrimidines, M; Mitomycin C, C; Cisplatin, FPSK, a combination of PSK and F therapy; MF, a combination of M and F therapy; MFP SK, a combination of PSK, F, and M therapy; CF, a combination of C and F therapy; CFPSK, a combination of PSK, F, and C therapy; CTL, CD8 cytotoxic T-lymphocytes,” all patients survived throughout the follow-up period.
Fig. 2 Kaplan-Meier curves for OS according to 155 CTL (+) or 1886 (-) patients. (A) CTL (+) patients whose HLA-restricted HERV-derived peptide might have induced CTL compared with CTL (-) patients whose HLA-restricted HERV-HIV peptides might not have induced CTL, (upper right) in females, (lower right) in males, (B) in the training set, and (C) in the validation set.

HR; hazard ratio with 95% confidence interval. CTL; CD8⁺ cytotoxic T-lymphocyte.
Changes in Lymphocyte Subpopulations

We summarized 84 putative HLA-restricted HERV-HIV peptides and changes in 492 patients whose CD8+ cells (%) were examined at baseline and 1 year after gastrectomy between HERV-HIV (+) and (-) peptides groups who received therapies (Appendix Table A13). Although patients who underwent gastrectomy alone did not show a significant increase in CD8+ cells (%) after gastrectomy (not shown), those who received therapy showed a significant increase in CD8+ cells (%) after gastrectomy. We summarized the changes in lymphocyte subpopulations in 155 CTL (+) patients from the perspective of each HERV-HIV peptide between baseline and 1 year after gastrectomy (Table 3). Baseline peripheral blood CD8+, CD4+, CD16+, and CD57+ cell (%) levels were not significantly different between CTL (+) and (-) groups.

The box-and-whisker plots of lymphocyte subpopulations (CD8+ cell (%) (A, left side), CD4+ cell (%) (A, right side) CD16+ cell (%) (B, left side), CD57+ cell (%) (B, left side), CD4/8 ratio (C)) between 155 CTL (+) and 1886 CTL (-) patients at the point of each baseline and 1 year after surgery are shown in Supplementary Appendix Fig. A4. There was no significant difference in base line CD8+ cell (%) levels among these groups. According to the 155 CTL (+) and 1886 (-) patients, sex, baseline and 1 year after surgery, the box-and-whisker plots of lymphocyte subpopulations (CD8+ cell (%) (A, left side), CD4+ cell (%) (A, right side) CD16+ cell (%) (B, left side), CD57+ cell (%) (B, left side), CD4/8 ratio (C)) are shown in Supplementary Appendix Fig. A5. Baseline CD8+ cells (%) levels in CTL (+) females were significantly lower levels than those in CTL (+) males and in CTL (-) females (Independent t-test; \(P < 0.008\) and \(P = 0.022\), Mann Whitney U test; \(P = 0.019\) and \(P = 0.028\), respectively).

In both 155 patients from the perspective of each HERV-HIV peptide (Table 4) and 177 from the perspective of each therapy administered (Appendix Table A14), patients who underwent gastrectomy alone did not show a significant change in CD4+ and CD8+ cells (%) afterward. Conversely, both the 155 and 177 patients who received therapies, peripheral blood CD8+, CD16+, and CD57+ cells (%) increased significantly 1 year after gastrectomy. Both the 155 and 177 CTL (+) patients showed a significant increase in CD8+ cells (%), showing a greater extent of increase than CTL (-) patients 1 year after gastrectomy. In contrast, CD4+ cells (%) decreased significantly, reducing the CD4/CD8 ratio. In both 155 and 177 CTL (+) females, CD8+, CD4+ and CD57+ cells (%) changed significantly after gastrectomy, but not in males.

The 20-year OS probability of the 492 patients from the perspective of each HERV-HIV peptide whose CD8+ cells (%) were examined at baseline and 1 year after gastrectomy was 75.9% and 42.9% in 25 CTL (+) and 467 CTL (-) patients, respectively (HR, 4.15; 95% CI, 1.54 to 11.14; \(P = 0.005\), log rank test: \(P = 0.002\)). CTL (+) females had significantly longer survival than CTL (-) subjects (the 20-year OS of 90.9% and 48.4% in 11 CTL (+) and 324 CTL (-), respectively; HR, 7.21; 95% CI, 7.21 to 52.02; \(P = 0.05\), log rank test; \(P = 0.022\)) and higher HR than males (the 20-year OS of 63.7% and 41.6% in 14 CTL (+) and 343 CTL (-), respectively; HR, 3.00; 95% CI, 9.6 to 9.38; \(P = 0.059\); log rank test, \(P = 0.048\) (Appendix Fig. A6). These results were almost consistent with those in 2041 patients: CTL (+) subjects benefitted 4 times more than CTL (-) subjects, and females benefited twice more than males.

The results of 177 CTL (+) patients from the perspective of each therapy administered are provided mainly in Appendix (Kaplan-Meier curves for the overall survival of 177 patients between sex in the validation set and the training set [Appendix Fig. A7]).
Fig. 3 Kaplan-Meier curves for OS according to CTL (+) or (-) patients. (A) OS curves of patients who received effective therapy and (upper right) in females and (lower right) in males between CTL (+) or CTL (-) patients. (B) OS curves of patients who received neither effective nor ineffective therapy and (upper right) in females and (lower right) in males between CTL (+) or CTL (-) patients. (C) OS curves of unclassified patients and (D) those of patients who received ineffective therapy. HR; hazard ratio with 95% confidence interval. CTL; CD8+ cytotoxic T-lymphocyte.
DISCUSSION

We identified patients harboring specific HLAs who showed improved survival outcomes when they were administered with adequate therapies designed using only baseline HLA data. However, there were no reports about specific HLAs derived from this analysis.

Our results highlight the crucial role of sex differences in cancer therapy. In this study, we aimed to improve the understanding of why only some cancers exhibit responses to therapies. HLA-restricted HERV-derived peptides, similar to HLA-restricted HIV-derived peptides, which were used and which might have induced CTL by triggering epigenetic stimulation via the adoption of novel treatment strategies, such as the regimens used in this study, could prolong survival in females more than in males. As there is no change in the lymphocytes subpopulations including CD8+ cell in the surgery alone group, gastrectomy might not be one of the factors of epigenetic stimulations. The mechanisms underlying the association between HLA-restricted HERV-derived peptides and CTL induction might reveal a potential central role of HERV genes in the sex bias in therapeutic responses.

HERVs are overexpressed in several human tumor types and are regulated epigenetically; additionally, their interaction with anticancer drugs or resistance to cancer therapies has been reported in chemotherapeutic regimens, as well as immune checkpoint blockade (ICB) therapy. HERVs and their products have high immunogenicity, which may activate the antitumor immune response. Conversely, the envelope proteins of HERVs display immunosuppressive properties in vivo.

Syncytin-2 (ERVFRD-1) is immunosuppressive, unlike syncytin-1 (ERVW-1). HHLA2 functions as a negative regulator of T cells in humans.

There are few reports on the sex differences in therapeutic responses. Intriguingly, ICB may be more effective in melanoma in males than in young (< 65 years) females; however, the impact of sex and age on the therapeutic responses underlying these disparities is controversial.

Chowell et al. reported that the germline HLA-I genotype and the somatic alterations in tumors are important in the clinical outcome of ICBs. Patients with the HLA-B44 supertype were associated with good outcomes; the HLA-B62 supertype demonstrated poor outcomes; however, the researchers did not mention sex disparities. More recently, Saini et al. have reported that more detailed research on T cell recognition of HERVs is important for developing immunotherapeutic strategies.

This study had several limitations. First, HLA data represent phenotypes and not alleles; thus, there are recessive data for HLAs (commonly known as a blank) as an independent parameter. Second, we could not validate all therapies because of the small number of validation sets and the unlikely probability of survivors receiving antitumor drugs used in this study. Third, computational epitope prediction analysis was used only for the identification of HLA-restricted epitope peptides. Fourth, although we evaluated the main conventional anticancer drugs (e.g., PSK, F, and M) used in Japan, we could assess the mechanism underlying the interplay among HLA, HERV, and treatment response. In the CDDP regimen group, we could not fully analysis because of small data. Finally, these pooled data have been accumulated.
| HERV_HIV peptide | Factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL, and who survived all during the follow-up period | No. of patients examined CD8+ cell | baseline Mean | I year after gastrectomy Mean | Std. error mean | Std. error mean | Paired t test Sig. (2-tailed) | Wilcoxon Signed Ranks Test Sig. |
|------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------------------|----------------|-------------------------------|----------------|----------------|-----------------------------|-----------------------------|
| GPKERVIKT_GPKVQWPL | PSK_male; A2; F_female; B54, B61; F_male; B54 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| HPRNQSEP_HPRESIEVHI | PSK_female; B35, B51, DQ4; F_female; B72, DQ1; F_male; B72 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| IPTLGILG_IPLGDLIVALVI | PSK_female; B35, B51, B61; F_male; B54 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| IPSQNSQGQAI_IPYNSGQGQV | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| KQPWFFIF_KPPLPSVTKL | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| ARLQVQAQK_ARLVTTYY | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| GRALQPVGK_GRAFVTIGK | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| GRALQPVFVP_GRAFVTIGK | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| RRGLDMTAA_GRRGWEALKY | PSK_male; A2, Cw10; F_female; B60, DR1 | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| NSPGKGL_NSPTRREL | - | - | - | - | - | - | - | - |
| NSPDQCSSF_NSFPTRREL | - | - | - | - | - | - | - | - |
| KEKGMFTFL KEKGGLEGL | PSK_male; B16, F_female; B27, F_male; B27; M_male; A31, B46, B51, Cw8, DQ3, DR9; M_male; B35, Cw8, MF_male; B7, DR1 | 122 | 25.9 | 0.67 | 27.5 | 0.65 | .001 | < .0001 |
| SPTCQTF_RMYSPTSL | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| EPFQTEEDSV_EFPRDYVDRF | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| FPQPEVE_FPSPITVP | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| FFQFFFF_FPSPITVP | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| FPVTLLEMP_FPVTQPL | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| DPVTLLEMP_DPVTQPLL | PSK_male; B16, F_female; B27, F_male; B27; M_male; B61 | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| SPREWSIESP_SPRTLNAWV | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| SPREWSIESA_SPRTLNAWV | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| TPQMLNL_TPQVPLL | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| TPQMLNLA_TPQVPLL | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| YSPTSQIK_YSPTSITLD | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| YSPTSQKIM_YSPTSITLD | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| AEPTWAIL_SAEJVPVQL | PSK_male; B16, F_female; B27, F_male; B27; PSK_female; A31; M_male; B61 | 129 | 26.4 | 0.67 | 28.6 | 0.64 | < .0001 | < .0001 |
| HERV_HIV peptide                                      | Factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL and who survived all during the follow-up period | No. of patients examined | baseline | I year after gastrectomy | Paired t test Sig. (2-tailed) | Wilcoxon Signed Ranks Test |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|----------|--------------------------|-------------------------------|----------------------------|
| AEWVLLDQT_AEDVRVHPV                                   |                                                                                                                                            | 90                       | 26.0     | 0.78                     | 0.71                          | .005                        |
| APAAEMAT_YCAPAGFAIL                                   |                                                                                                                                            | 100                      | 27.5     | 0.72                     | 0.73                          | .172                        |
| CPKEIPKES_CPKEVSFEPI                                  |                                                                                                                                            | 25                       | 26.9     | 1.80                     | 2.96                          | .033                        |
| EKYPYNYI_EKAFSPEV                                    |                                                                                                                                            | 51                       | 28.1     | 1.07                     | 2.89                          | .348                        |
| GEIQLVISS_GEYKRWII                                   |                                                                                                                                            | 90                       | 26.0     | 0.78                     | 0.71                          | .005                        |
| IEEKYynyI_IEELRQHLL                                   |                                                                                                                                            | 152                      | 25.9     | 0.60                     | 2.72                          | .060                        |
| IPAINNKIPA_IPAETGQETA                                 |                                                                                                                                            | 29                       | 26.6     | 1.61                     | 2.92                          | .023                        |
| IPYGNsPL_IPHYCAPA                                     |                                                                                                                                            | 25                       | 26.9     | 1.80                     | 2.96                          | .033                        |
| IPYNSQQA_IPYNPQSQGV                                  |                                                                                                                                            | -                        | -        | -                        | -                            | -                           |
| IVTEKVKEl_IvTDQYAL                                    |                                                                                                                                            | 18                       | 27.5     | 1.61                     | 2.84                          | .576                        |
| KEGOvAFL _KEKGGLEGl                                   |                                                                                                                                            | 122                      | 25.9     | 0.67                     | 2.75                          | .001                        |
| KETESLHCY_KETINEAEA                                   |                                                                                                                                            | 216                      | 26.1     | 0.51                     | 2.75                          | .015                        |
| KIKSKYASY_KIKPPLSV                                   |                                                                                                                                            | 112                      | 26.2     | 0.67                     | 2.85                          | .0001                       |
| LPQGMLNSP_LPQGWKGSPA                                  |                                                                                                                                            | -                        | -        | -                        | -                            | -                           |
| PPLIRAVT_PW_LQLPPLERL                                 |                                                                                                                                            | 112                      | 26.2     | 0.67                     | 2.85                          | .0001                       |
| QAIIKLQTNL_QAISPRtL                                   |                                                                                                                                            | 172                      | 26.6     | 0.57                     | 2.77                          | .015                        |
| QELHALTHY_QELKNSAVL                                   |                                                                                                                                            | 90                       | 26.0     | 0.78                     | 2.76                          | .005                        |
| QEVIPYPETL_DP NPQQEVtL                                 |                                                                                                                                            | 106                      | 25.2     | 0.72                     | 2.65                          | .035                        |
| RAHHFPL_RAIEAQQHL                                    |                                                                                                                                            | 214                      | 25.7     | 0.51                     | 2.72                          | .0001                       |
| REPILNAl_REPHNEWtL                                    |                                                                                                                                            | 90                       | 26.0     | 0.78                     | 2.76                          | .005                        |

| HERV_HIV peptide | No. of patients examined | Mean (Std. error) | Mean (Std. error) | P value | P value |
|-------------------|--------------------------|-------------------|-------------------|---------|---------|
| AEWVLLDQT_AEDVRVHPV | 90                       | 26.0 (0.78)       | 27.6 (0.71)       | .005    | .002    |
| APAAEMAT_YCAPAGFAIL  | 100                      | 27.5 (0.72)       | 28.3 (0.74)       | .172    | .095    |
| CPKEIPKES_CPKEVSFEPI | 25                       | 26.9 (1.80)       | 29.6 (1.62)       | .033    | .047    |
| EKYPYNYI_EKAFSPEV  | 51                       | 28.1 (1.07)       | 28.9 (1.09)       | .348    | .153    |
| GEIQLVISS_GEYKRWII | 90                       | 26.0 (0.78)       | 27.6 (0.71)       | .005    | .002    |
| IEEKYynyI_IEELRQHLL | 152                      | 25.9 (0.60)       | 27.2 (0.61)       | .006    | .002    |
| IPAINNKIPA_IPAETGQETA   | 29                       | 26.6 (1.61)       | 29.2 (1.52)       | .023    | .031    |
| IPYGNsPL_IPHYCAPA     | 25                       | 26.9 (1.80)       | 29.6 (1.62)       | .033    | .047    |
| IPYNSQQA_IPYNPQSQGV   | -                        | -                 | -                 | -       | -       |
| IVTEKVKEI_IvTDQYAL    | 18                       | 27.5 (1.61)       | 28.4 (1.85)       | .576    | .686    |
| KEGOvAFL _KEKGGLEGl  | 122                      | 25.9 (0.67)       | 27.5 (0.65)       | .001    | <.0001  |
| KETESLHCY_KETINEAEA  | 216                      | 26.1 (0.51)       | 27.5 (0.51)       | <.0001  | <.0001  |
| KIKSKYASY_KIKPPLSV   | 112                      | 26.2 (0.67)       | 28.5 (0.67)       | <.0001  | <.0001  |
| LPQGMLNSP_LPQGWKGSPA | -                        | -                 | -                 | -       | -       |
| PPLIRAVT_PW_LQLPPLERL | 112                     | 26.2 (0.67)       | 28.5 (0.67)       | <.0001  | <.0001  |
| QAIIKLQTNL_QAISPRtL  | 172                      | 26.6 (0.57)       | 27.7 (0.57)       | .015    | .007    |
| QELHALTHY_QELKNSAVL  | 90                       | 26.0 (0.78)       | 27.6 (0.71)       | .005    | .002    |
| QEVIPYPETL_DP NPQQEVtL | 106                     | 25.2 (0.72)       | 26.5 (0.81)       | .035    | .021    |
| RAHHFPL_RAIEAQQHL    | 214                      | 25.7 (0.51)       | 27.2 (0.53)       | <.0001  | <.0001  |
| REPILNAl_REPHNEWtL   | 90                       | 26.0 (0.78)       | 27.6 (0.71)       | .005    | .002    |
| HERV_HIV peptide                          | Factors (administered therapy, female or male and HLA) in the individual patient whose peptide might have induced CTL and who survived all during the follow-up period | No. of patients examined | baseline Mean | baseline Std. error | 1 year after gastrectomy Mean | 1 year after gastrectomy Std. error | Paired t test Sig. (2-tailed) | Wilcoxon Signed Ranks Test | P value | P value |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|--------------------------|----------------|---------------------|-------------------------------|-------------------------------|-----------------------------------|---------------------------|----------|----------|
| PSK_female_ B35, DQ4, DR5: PSK_male_ B35, CW8; F_female_ B7; F_male_ B59; FPSK_female_ B51; M_female_ A11, A31, B16, B46, CW1, DQ1, DQ3, CW8; CF_male_ DR2 | REPLENTLV_REPHNEWTL | 90 | 26.0 | 0.78 | 27.6 | 0.71 | .005 | .002 |
| PSK_male_ B16; F_female_ B27; F_male_ B27; FPSK_female_ B54; M_female_ A31, B46, B51, CW8, DQ3, DR5, DR9; M_male_ B35, CW8; MF_male_ B7, DR1 | RKEKGVAFLA_KEGGGLEL | 122 | 25.9 | 0.67 | 27.5 | 0.65 | .001 | < .0001 |
| PSK_female_ A2, DQ3, DQ4, DR4, DR5: PSK_male_ CW1, CW7; F_female_ CW4, F_male_ B16, B54; FPSK_female_ B61; M_female_ B15; M_male_ B15; MF_female_ B60; CFPFSK_female_ A9 | RPLDRPASP_RPLVTIKI | 56 | 25.4 | 1.03 | 26.8 | 1.19 | .074 | .094 |
| PSK_female_ B51; PSK_male_ B15; F_male_ B46, B61; FPSK_female_ B16; MF_female_ DR9; MFPSK_male_ A31, B60 | RQRP SergH_EYKILQR | 34 | 27.6 | 1.38 | 28.6 | 1.30 | .373 | .113 |
| PSK_female_ B51, DQ4, PSK_male_ B7; F_female_ B27; F_male_ B27; M_female_ A11, B61 | RSLSKGTPFE_RSLYNTVATLY | 184 | 26.0 | 0.54 | 27.6 | 0.56 | < .0001 | < .0001 |
| PSK_female_ B51, B61; F_female_ A11, B27 B54; F_male_ B27, B46, B54; M_female_ CW8, M_male_ CW8; MF_female_ B60; MFPFSK_male_ B59 | SPAMIPDW_SPAIFQSSM | 77 | 26.3 | 0.89 | 28.0 | 0.92 | .026 | .014 |
| PSK_female_ DQ4, DR3; PSK_male_ CW1; F_female_ B27, B54, B61; F_male_ B16, B27, B54; FPSK_female_ A2; M_female_ A11; CFPFK_female_ A9 | SPHEGKL_SPHRISSEVHI | 79 | 25.8 | 0.87 | 27.7 | 1.00 | .005 | .009 |
| F_female_ A9, B27, DQ1; F_male_ A2, A9, B27, CW1, DQ1; FPSK_female_ A31 | SRALKDSKKE_SRANKWNTTL | 2 | 34.5 | 0.50 | 46.5 | 3.50 | .156 | .18 |
| PSK_female_ A2, DQ3, DQ4, DR4, DR5; PSK_male_ CW1, CW7; F_female_ CW4, F_male_ B16, B54; FPSK_female_ B61; M_female_ B15; M_male_ B15; MF_female_ B60; CFPFSK_female_ A9 | SRLQAIKL_DSRLAFHV | 56 | 25.4 | 1.03 | 26.8 | 1.19 | .074 | .094 |
| PSK_female_ B35, DQ4; PSK_male_ B16, B35, B46; F_female_ B27, F_male_ B27; FPSK_female_ B51; M_female_ A31, CW1, CW8, DR5, DR9; M_male_ CW8; CF_male_ DR2 | TPQMLNLAL_TPQDLNTML | 122 | 26.3 | 0.70 | 27.9 | 0.65 | .002 | .001 |
| PSK_female_ B51, DQ4, DR5; PSK_male_ CW1; F_female_ B27, B54, B61; F_male_ B16, B27, B54; FPSK_female_ A2; M_female_ A11; CFPSK_female_ A9 | TSLTPLFH_KELYPLTSL | 96 | 26.2 | 0.78 | 27.9 | 0.70 | .002 | .001 |
| PSK_male_ A2; FPSK_female_ CW7; FPSK_male_ CW7 | VERTNRTL_VERYLKDOQQL | - | - | - | - | - | - | - |
| PSK_female_ DR4; PSK_male_ A2; F_female_ B61; F_male_ B51; FPSK_female_ CW10; FPSK_male_ B16 | VPLTKEQV_VPQLPPLL | 25 | 26.9 | 1.80 | 29.6 | 1.62 | .033 | .047 |
| PSK_male_ B7, CW1, CW7; F_male_ B16; FPSK_female_ A2; M_male_ B60, DQ3; CFPFSK_female_ A31 | VRDKFSDCY_VRDQAEHL | 21 | 16.2 | 2.42 | 20.9 | 2.99 | .981 | .897 |
| PSK_female_ B35, B51, DQ4; PSK_male_ CW7; F_female_ B27; F_male_ B27; M_female_ B61 | YQWWPAEL_YQYMDDLYV | 112 | 26.2 | 0.67 | 28.5 | 0.67 | < .0001 | < .0001 |
| PSK_female_ B35, DQ4, DR5; PSK_male_ B35, CW8; F_female_ B7; F_male_ B59; FPSK_female_ B51, B54; M_female_ A11, A31, B16, B46, CW1, DQ1, DQ3; CF_male_ DR2 | YYKLSQEL_QELKNSAVSL | 90 | 26.0 | 0.78 | 27.6 | 0.71 | .005 | .002 |

Abbreviations: PSK; Krestin, F; Fluoropyrimidines, M; Mitomycin C, C; Cisplatin, FPSK, a combination of PSK and F therapy; MF, a combination of M and F therapy; MFPFSK, a combination of PSK, F, and M therapy; CF, a combination of C and F therapy; CFPFSK, a combination of PSK, F, and C therapy; CTL; CD8’ cytotoxic T-lymphocytes.
Table 4 Changes in lymphocyte subpopulations between baseline and 1 year after gastrectomy in 155 CTL (+)
patients from the perspective of each HERV peptide

|                      | No. of patients |        |        |        |        | P value | P value |
|----------------------|-----------------|--------|--------|--------|--------|---------|---------|
|                      |                 | Mean   | Std. error mean | Mean | Std. error |        |         |
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | 0.98 | .991 | .927 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

CD8 (%)

|                      |                 |        |        |        | Wilcoxon signed ranks test |
|----------------------|-----------------|--------|--------|--------|---------------------------|
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | .987 | .987 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

CD4 (%)

|                      |                 |        |        |        | Wilcoxon signed ranks test |
|----------------------|-----------------|--------|--------|--------|---------------------------|
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | .987 | .987 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

CD4/8 ratio

|                      |                 |        |        |        | Wilcoxon signed ranks test |
|----------------------|-----------------|--------|--------|--------|---------------------------|
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | .987 | .987 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

CD16 (%)

|                      |                 |        |        |        | Wilcoxon signed ranks test |
|----------------------|-----------------|--------|--------|--------|---------------------------|
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | .987 | .987 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

CD5 (%)

|                      |                 |        |        |        | Wilcoxon signed ranks test |
|----------------------|-----------------|--------|--------|--------|---------------------------|
| Patients who underwent gastrectomy alone | 32 | 25.5 | 1.17 | 25.5 | .987 | .987 |
| Patients who received therapies | 460 | 26.6 | 0.33 | 27.6 | 0.32 | < .0001 | < .0001 |
| CTL (-) | 467 | 26.6 | 0.32 | 27.4 | 0.31 | .001 | < .0001 |
| CTL (+) | 25 | 25.8 | 1.47 | 28.4 | 1.79 | .037 | .047 |

Abbreviations: CTL, CD8+ cytotoxic T-lymphocyte; CTL (+), patients whose HLA-restricted peptides might have induced CTL owing to subjection to therapies; CTL (-), patients whose HLA-restricted peptides might not have induced CTL owing to subjection to therapies. Patients who underwent gastrectomy alone were considered as controls.

from approximately 1977 to 2011; however, these results partly avoid the small size of the dataset and the use of such a dataset may be considered a novel approach to cancer therapy.

We conclude that our research on HLA-restricted HIV-HERV crosstalk provides new insights into the mechanisms of treatment response to chemotherapy, immunotherapy, and virus infection therapy. We used bioinformatics approaches to assess HLA-restricted HERV and HIV gene-derived-peptides similarity and focused on the molecular mechanisms underlying sex bias in treatment response to facilitate the development of personalized medicine for cancer treatment. The expression level of HERVs in cancer tissue samples will be evaluated by quantitative PCR or immunohistochemistry in the future, and further studies are necessary to determine the biological activity of putative HLA-restricted HERV gene-driven peptides shown in this study and to assess the interplay between MHC and T cell subpopulation recognition by experimental studies.
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All authors report no conflicts of interest.

DATA AVAILABILITY:

The data that support the findings of this study are available from the Japanese Society of Strategies for Cancer Research, however, because of restrictions, they are not publicly available. The data are available from the authors upon reasonable request and with the permission of the Japanese Society of Strategies for Cancer Research.

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AUTHOR CONTRIBUTIONS:

Conception and design: All authors

Provision of study materials or patients: see Appendix Table A1

Data analysis and interpretation: Ogoshi K

Collection and assembly of data: Ogoshi K

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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