Effective and ineffective personalized therapy based on serum HLA from a 30-year odyssey

Kyoji Ogoshi\textsuperscript{1, 2}, on behalf of the HLA Study Group of The Japanese Society of Strategies for Cancer Research and Therapy\textsuperscript{3}.

\textsuperscript{1}Department of Gastroenterological Surgery, Tokai University, \textsuperscript{2}Japanese Society of Strategies for Cancer Research and Therapy

\textbf{Funding}

This work was supported by grants from The Japanese Society of Strategies for Cancer Research and Therapy.

\textbf{Abstract}

\textbf{Background:} This article is a summary of clinical research results from a study beginning approximately 30 years ago. We have focused on human leukocyte antigens (HLA) as one of several important genetic factors for the formulation of effective personalized therapy for cancer patients that can be applied in clinical settings. HLA were also studied on the basis of the original study hypothesis that pathological states and pharmacokinetics are similar among patients with similar genetic information.

\textbf{Methods:} HLA antigens were serologically tested using the National Institutes of Health standard microlymphocytotoxicity method for HLA-A, -B, -C, -DR and -DQ from Aug. 1977 to Aug. 2005 (n=1753) and also tested for the reproducibility and validation over the period from Sept. 2005 to Feb. 2010 (n=209). In this study, it was shown that individuals could be identified and grouped according to pair-matched parameters generated from serum HLA profiles at the protein level to represent the genetic information.

\textbf{Results:} In patients with similar HLA profiles, the effective and ineffective personalized therapies were also similar, leading to a good ability for successful prognosis. With existing cancer therapies, 60.9% of patients experienced a positive outcome. However, 10.2% of patients received ineffective personalized therapy. If effective personalized therapy is not given, oral administration of polysaccharide Kureha and/or fluoropyrimidines regimen will be needed for at least 2 years.

\textbf{Conclusion:} Our hypothesis that effective and ineffective personalized therapies are similar in patients with similar genetic information, resulting in accurate therapeutic prognosis, has been established.

\textbf{Key Words:} HLA, personal HLA score, pair-matched parameter, effective personalized therapy, ineffective personalized therapy.

\textit{(Received October 1, 2011; Accepted October 21, 2011)}

\textbf{Introduction}

The Human Genome Project commenced at The Alta Summit workshop in December 1984\textsuperscript{4} and it has been anticipated that if scientific elucidation of individual genomic information was possible, it could lead to striking developments in medical and biotechnological research. It is conceivable that if all of the human genomic information could be sequenced, more effective personalized drugs and treatments could be discovered, finer elucidation of the pathogenesis of diseases could be given and more accurate predictions of the risks of treatment could be made, before the effects or side effects of therapies set in. After sequencing of the entire human genome was completed in 2004\textsuperscript{5}, it was estimated that there are approximately 20,000–25,000 genes present in the human genome. However, at present, it is not yet possible for the medical world to use this knowledge for the benefit of patients in a clinical setting. There is a massive cost involved in examining, reading, analyzing and decoding the entire human DNA, and for such tasks to be feasible, the development of suitable analytical software and access to high-performance super computers is required.

This article is a summary of the results of a clinical study that started in 1977, 34 years ago, when few thought of how to take advantage of genetic information, let alone the significance of specific genes or HLA for clinical applications. However, even in those days, I considered that HLA must be one of the important factors involved in the formulation of effective personalized therapy for cancer patients. Our original hypothesis, that pathological states and pharmacokinetics should be similar among patients with similar genetic information, was
also thought to be important for the formulation of effective personalized therapy for cancer patients. At present, it unfortunately appears that these views are still in the minority.

Meanwhile, subsequent studies on human major histocompatibility gene complex (MHC) HLA were remarkable. These glycoproteins (also referred to as major histocompatibility antigens, MHC antigens or MHC molecules) are encoded in MHC and are involved in the elimination of infectious pathogens such as bacteria and viruses, rejection of cancer cells and the rejection reaction of organ grafts. They accomplish this by presenting the antigen to play very important roles in immunity, with the human MHC (HLA) present on a short arm of chromosome 6. The entire nucleotide sequence of human MHC and its genetic map were sequenced in 1999. Therefore, HLA are encoded by genes that are indispensable for existing as humans. The significance and importance of HLA in cancer therapies has been enhanced by the discovery of cancer peptides. Thus, the past 34 years have provided excellent support for verifying our hypothesis.

The primary aims of this study are to verify the hypothesis that effective and ineffective personalized therapy is similar among patients having similar genetic information, to determine whether successful prognosis (good or bad) can be given based on personalized therapy in patient groups, and to prove its reproducibility. Once the patient group to which the effective personalized therapy had been given was identified, the optimal treatment period of oral administration of the drug was also verified.

Materials and methods

Data Pool

TNPP refers to the total national patient pool of 1753 patients throughout Japan from member hospitals of the Japanese Society of Strategies for Cancer Research and Therapy, including the Tokai University patient pool (TUPP), over the period from Aug. 1977 to Aug. 2005. The median age was 60 y (range: 22–93 y). There were 1245 men (median age 61 y, range: 22–92 y) and 508 women (median age 58 y, range: 23–93 y) included in the pool. Five-year survivors and 5-year follow-up cases numbered 1100 (62.7%) and 1596 (91%) as of Sep. 2010, respectively. Ten-year survivors and 10-year follow-up cases numbered 492 (35.4%) and 1026 (73.8%), respectively.

TU Data Set 2007 (n=1391) refers to Tokai University’s set of patient data as of Aug. 2007. Five-year survivors and 5-year follow-up patients as of Aug. 2007 numbered 842 (60.5%) and 1255 (91.2%), respectively. Ten-year survivors and 10-year follow-up cases numbered 492 (35.4%) and 1026 (73.8%), respectively.

TU Data Set 2010 (n=1391) refers to Tokai University’s set of patient data as of Sept. 2010. Five-year survivors and 5-year follow-up patients as of Sept. 2010 numbered 903 (64.9%) and 1322 (95.0%), respectively. Ten-year survivors and 10-year follow-up cases numbered 665 (47.8%) and 1215 (87.3%), respectively.

WN Data Set 2010 (n=362) refers to the set of patient data for the whole nation of Japan, excluding Tokai University (TU), from May 1987 to March 2003. Five-year survivors and 5-year follow-up patients as of Sept. 2010 numbered 197 (54.4%) and 274 (75.9%), respectively. Ten-year survivors and 10-year follow-up cases numbered 47 (13.0%) and 139 (38.4%), respectively.

New TNPP Data Set 2010 (n=209) refers to the set of data of the whole nation of Japan (n=3), including Tokai University (n=206), between Sept. 2005 and Feb. 2010. Initial therapy of New TNPP Data Set refers to an initial treatment for gastric cancer and oral administration of anti-cancer drugs (F and PSK) after gastrectomy for a period of >6 months. Three-year survivors and 3-year follow-up patients as of Sept. 2010 numbered 14 (56.0%) and 24 (96.0%), respectively. Treatments included no therapy, PSK therapy, F therapy, or FPSK therapy. Because MMC or MMC combination therapy is no longer considered common in clinical settings in Japan, many patients did not desire these therapies.

Therapy

Six hundred seven patients from TNPP received no anti-cancer drug, 574 received several types of gastrectomy, 13 received endoscopic mucosal resections, and 20 received no gastrectomy and no anti-cancer drug (treatment refusal). One hundred twenty three patients received PSK, 136 received F, and 93 received MMC as a monotherapy. One hundred sixty eight patients received F+PSK (FPSK), 354 received MMC+F (MF), and 272 received MMC+F+PSK (MFPSK) as combined polytherapy. These therapies were designated as familial therapies for gastric cancers under the social insurance system in Japan.
Medication after gastrectomy

PSK therapy consisted of oral administration of 3.0 g/day after gastrectomy for a period of over 1 year.10,11 F therapy consisted of oral administration of fluoropyrimidines after gastrectomy for over 1 year: 5-fluorouracil 150 mg/day, Tegafur 600 mg/day, UFT 600 mg/day, or TS-1 40–80 mg/day, which all had a final active substance of 5-FU.12,13 MMC therapy consisted of intravenous injection of MMC 20 mg intra-operatively and/or 10 mg on post-operative day 1.14 FPSK therapy consisted of F+PSK therapy. MF therapy consisted of MMC+F therapy. MFPSK therapy consisted of MMC+F+PSK therapy.

Blood samples and HLA examination

Blood samples were collected in Tokai University Hospital from Aug. 1977 to Mar. 1987, as well as throughout Japan at member hospitals of the Japanese Society of Strategies for Cancer Research and Therapy and the Mitsubishi Chemical Medicine Corporation (Tokyo) from May 1987 to Aug. 2005. A total of 209 patients (New TNPP) was collected for the reproducibility and validation over the period from Sept. 2005 to Feb. 2010. HLA antigens were serologically tested using the NIH standard microlymphocytotoxicity method for HLA-A, -B, -C, -DR and -DQ in Mitsubishi Chemical Medicine Corporation. Patient informed consent from all participating hospitals was obtained from the outset of this study.

HLA scoring (HAS) procedure

HLA score was defined as the number of significant samples per 100 random samples derived from statistical examinations [Kaplan-Meier method, Cox-multivariate analysis (factors: age and gender) and \( \chi^2 \)-test, using a 5-year data set] of the random 80% patient samples. This was repeated 100 times. Positive scores indicated significantly better survival between antigen (+) and (−), and negative scores vice versa. \( TU \ Data \ Set \ 2010 \ (n=1391) \) and \( WN \ Data \ Set \ 2010 \ (n=362) \) were compared using HLA score (number of significant samples per 100 random samples).

New HLA scoring (nHAS) procedure

From \( TU \ Data \ Set \ 2007 \ (n=1391) \), approximately 1000 and 800 patients (80% and 60% patient samples, respectively) were randomly selected and divided into two groups for each sample set. The first group composed of 800 and 600 patients per sample set, arbitrarily selected. The second group consisted of 200 and 400 patients per sample set, also arbitrarily selected. Both groups were administered the same medication for treatment of gastric cancer and these patients samples were used to determine the nHAS based on 3/4 and 4/4 rule (see Glossary).

Statistical Analysis

All statistical analyses were carried out using SPSS software, version 18 (SPSS Inc., Chicago, IL, USA.). Mean values were compared using Student's-test. A \( \chi^2 \)-square test was used to compare the prevalence of HLA and the prognoses of patients. Results were considered significant when \( p<0.05 \). The survival period of cancer patients was defined as the interval from initial treatment to death (overall survival rate after treatment), with data regarding survivors censored at the last follow-up (Sept. 2010). Survival curves were calculated using the Kaplan-Meier product-limit estimate and differences in survival were assessed by the log-rank test and Cox univariate and multivariate analysis with relative risk and 95% CI.

Reproducibility and validation

1. HLA score (HAS) for patient treatment

HLA score was defined as the number of significant samples per 100 random samples derived from statistical examinations [Kaplan-Meier method, Cox-multivariate analysis (factors: age and gender) and \( \chi^2 \)-test, using a 5-year data set] of the random 80% patient samples. This was repeated 100 times. Positive scores indicated significantly better survival between antigen (+) and (−), and negative scores vice versa. \( TU \ Data \ Set \ 2010 \ (n=1391) \) and \( WN \ Data \ Set \ 2010 \ (n=362) \) were compared using HLA score (number of significant samples per 100 random samples).

2. New HLA score (nHAS) for patient treatment

From \( TU \ Data \ Set \ 2007 \), approximately 1000 patients [80% patient sample A (PSA)] were randomly selected. Patients in PSA were randomly divided into two groups. The first group consisted of 800 patients and the second group consisted of 200 patients. The division was arbitrary. Again using \( TU \ Data \ Set \ 2007 \), another 800 patients [60% patient sample B (PSB)] were randomly selected and arbitrarily divided into two groups. Using PSA and PSB, four data sets were then constructed: nHAS1 from the 4/4 rule of statistical significance of PSA patients; nHAS2 from the 3/4 rule of statistical significance of PSA patients; nHAS3 from the 4/4 rule of statistical significance of PSB patients; and nHAS4 from the 3/4 rule of statistical significance of PSB patients. Table S1 shows nHAS 1, 2, 3, and 4 of each HLA antigen according to the therapies in \( TU \ Data \ Set \ 2007 \).

3. Personal HLA score (PHAS) for patient treatment

PHAS of a patient was calculated as the sum of their nHASs according to the therapy they received.

4. Subgroups of PHAS according to patients’ therapy in TNPP

Patient PHAS were divided into four subgroups: A, B, C, and D, according to the following protocol: subgroup A included PHAS scores (PHAS of SGA) for patients that stayed alive during the study period; subgroup B PHAS scores (PHAS of SGB) included all patients that died during the study; subgroup C PHAS scores (PHAS of SGC) included those patients with scores not belonging to either SCA or SGB; and subgroup D PHAS scores (PHAS of SGD) included patients with no HAS (Table S2).
5. Pair-matched parameters (PMPs) selected by subgroup according to patient treatment

Eight PMPs were selected: (1) PMP1: From Tokai University’s 60% random sample (60% TU Data Set 2007) using the 4/4 rule of nHASl. (2) PMP2: From Tokai University’s 60% random sample (60% TU Data Set 2007) using the 3/4 rule of nHAS1. (3) PMP3: From Tokai University’s 60% random sample (60% TU Data Set 2007) using the 4/4 rule of nHAS2. (4) PMP4: From Tokai University’s 60% random sample (60% TU Data Set 2007) using the 3/4 rule of nHAS2. (5) PMP5: From Tokai University’s 80% random sample (80% TU Data Set 2007) using the 4/4 rule of nHAS3. (6) PMP6: From Tokai University’s 80% random sample (80% TU Data Set 2007) using the 3/4 rule of nHAS3. (7) PMP7: From Tokai University’s 80% random sample (80% TU Data Set 2007) using the 4/4 rule of nHAS4. (8) PMP8: From Tokai University’s 80% random sample (80% TU Data Set 2007) using the 3/4 rule of nHAS4.

We used 4/4 rule which refers to all statistical methods [Kaplan-Meier method, Cox-multivariate analysis (factors: age and gender), χ²-test, using 5-year and 10-year data sets], with p<0.05 in four arbitrary groups, and 3/4 rule which refers to all statistical methods with p<0.05 in 3 out of 4 arbitrary groups. In the same way, the rest of PMPs represented from New HLA Score 2, 3, and 4.

If in TU Data Sets 2007 a patient matched a specific therapy by using all PMPs (PMP1-8), this therapy or these therapies were finely defined as his or her effective therapy in this study (see Glossary).

6. Optimal treatment period of oral administration of F and PSK regimen

The administration period of the F regimen group consisted of patients receiving F, FPSK, MF and MFPSK therapy and PSK regimen group of those receiving PSK, FPSK, MFPSK therapy. The administration period of these groups were divided to two groups; less than 2 years and over than 2 years.

Results

Table S3 shows the list of HLA examined in TUPP and WNPP. There was no significance difference in HLA frequency between the two data pools. There were no antigens of HLA-A23 and B8 in 1753 Japanese patients, and A25, A28, A29, A32, A34, A36, A66, A68, A69, A74, B14, B18, B4, B6, B41, B42, B45, B47, B49, B50, B53, B63, B73, B76, B77, and Cw2 were lacking in 1406 Japanese patients. Fig. S1 shows the distribution of data samples from throughout Japan.

HASs from the number of significant samples per 100 random samples

Table S4 shows the number of significant samples per 100 random samples from three statistical examinations of TU Data Set 2007: χ²-test at 5 years after treatment (A); the Kaplan–Meier method (B); and Cox multivariate analysis (C). Positive scores indicated significantly better survival between antigen (+) and (−), and negative scores vice versa.

Survival analysis by using HAS in the treatment of patients

There was significantly worse survival in patients with HLA-A31 (+) that were treated by MF therapy. A significant difference between antigen (+) and (−) was only reproducible in patients with HLA-A31 who received MF therapy [TU Data Set 2010 (A) and WN Data Set 2010 (B)] with p=0.021 and RR of 1.597 (1.075–2.373), and p=0.027, 2.333 (1.103–4.934), respectively. Among HLA antigens tested in this study, in patients with such as HLA-DQ7 who received F therapy, significant differences between antigen (+) and (−) was shown in TU Data Set 2010 with p=0.009, 14.505 (1.956-107.538), but not in WN Data Set 2010 (Fig. S2).

Using new HLA score (nHAS) in the treatment of patients

By using each of 8 pair-matched parameters (PMP), 1753 patients from the TNPP were classified: 102 subgroups of patients which fit PMP1; 102 subgroups of patients which fit PMP2; 186 subgroups of patients which fit PMP3; 186 subgroups of patients which fit PMP4; 206 subgroups of patients which fit PMP5; 366 subgroups of patients which fit PMP6; 182 subgroups of patients which fit PMP7; and 190 subgroups of patients which fit PMP8.

PMPs 1–4 from the 60% TU Data Set 2007 identified the patients who received effective personalized therapy and had survival over 5 years less than 2%. A random selection of 50–80% of patients were chosen and split into two groups and the parameters were examined 5–20 times. Among PMPs 5–8 from the 80% TU Data Set 2007, PMP 6, that is, if a random selection of 60% of patients were chosen and split into two groups and the parameters were examined 6 times, identified the 14.1% TNPP patients who received effective personalized therapy and had survival over 5 years (Fig. S3).

Table 1 shows the results of the patients who were selected to receive effective or ineffective therapy or were unclassified in TNPP. Patients were classified using Navicanna software (developed in-house). TNPP identified 1068 patients (60.9%) as having effective therapies, 178 (10.2%) with ineffective therapies and 507 (28.9%) as unclassified by using PMPs 1–8. By using this method the effective personalized therapy of No, PSK, F, FPSK, MMC, MF, or MFPSK could be detected from 54.5% to 66.7% of patients.

By using PMPs 1–8 patients were classified according to the following classifications: patients who received
effective therapy; those who received neither effective nor ineffective therapy; those who received ineffective therapy; and unclassified patients. Significant differences were observed between patients (TNPP Data Set 2010) with effective therapy (5-, 10-, 15-, and 20-year survival rate was 99.2%, 97.4%, 94.1%, and 89.3%, respectively) vs. those with neither effective nor ineffective therapy (67.8%, 56.4%, 45.8%, and 35.8%, respectively), those with ineffective therapy (18.5%, 11.1%, 4.9%, and 2.5%, respectively), and unclassified patients (71.3%, 57.4%, 49.8%, and 38.2%, respectively), with p<0.001 and relative risks (RR) of 12.777 (95% CI, 7.352–22.207), 56.131 (30.242–104.207), and 11.838 (6.785–20.653), respectively (Fig. 1).

Figure 2 shows that in TNPP Data Set 2010 (n=1440) without hepatic and peritoneal metastasis (A), there were significant differences between patients with effective therapy (n=240) vs. those with neither effective nor ineffective therapy (n=665), patients with ineffective therapy (n=49), and unclassified (n=486), with p<0.001 and RR of 10.860 (5.939–19.858), 53.210 (26.709–106.005), and 9.232 (5.014–16.999), respectively, and significant differences between 313 patients with hepatic and/or peritoneal metastasis (B). There were significant differences between patients who received effective therapy (blue line), ineffective therapy (pink line), neither effective nor ineffective therapy (orange line), and unclassified (purple line) (log rank test, p<0.0001). There were significant differences between patients who received effective therapy (blue line), ineffective therapy (pink line), neither effective nor ineffective therapy (orange line), and unclassified (purple line) (p<0.0001).

Table 1. Outcomes of patients who were selected to receive effective or ineffective therapy or were unclassified in Total National Patient Pool (TNPP)

|                | Effective therapy | Ineffective therapy | Unclassified | Total |
|----------------|-------------------|---------------------|--------------|-------|
|                | Received          | Not received        | Total        | Received | Not received | Total | Unclassified | Total |
| No             | 251               | 817                 | 1068         | 606     | 97           | 518   | 178          | 507   |
| PSK            | 78                | 306                 | 384          | 8       | 33           | 41    | 6.8%         | 182   |
|                | 12.9%             | 50.4%               | 63.3%        | 1.3%    | 5.4%         | 6.8%  | 30.0%        | 607   |
| F              | 37                | 37                  | 74           | 2       | 10           | 12    | 9.8%         | 123   |
|                | 30.1%             | 30.1%               | 60.2%        | 1.6%    | 8.1%         | 9.8%  | 30.1%        | 123   |
| F+PSK          | 24                | 57                  | 81           | 9       | 6            | 16    | 11.8%        | 39    |
|                | 17.6%             | 41.9%               | 59.6%        | 6.6%    | 5.1%         | 11.8% | 28.7%        | 136   |
| M              | 50                | 62                  | 112          | 2       | 12           | 13    | 7.7%         | 43    |
|                | 29.8%             | 36.9%               | 66.7%        | 1.2%    | 6.5%         | 7.7%  | 25.6%        | 168   |
| M+PSK          | 20                | 41                  | 61           | 10      | 4            | 14    | 15.1%        | 93    |
|                | 21.5%             | 44.1%               | 65.6%        | 10.8%   | 4.3%         | 15.1% | 19.4%        | 93    |
| M+F            | 22                | 171                 | 193          | 31      | 8            | 27    | 16.4%        | 103   |
|                | 6.2%              | 48.3%               | 54.5%        | 8.8%    | 7.6%         | 16.4% | 29.1%        | 354   |
| M+F+PSK        | 20                | 143                 | 163          | 19      | 7            | 19    | 8.8%         | 85    |
|                | 7.4%              | 52.6%               | 59.9%        | 7.0%    | 1.8%         | 8.8%  | 31.3%        | 272   |

PSK = Polysaccharide Kureha; F = fluoropyrimidines; F+PSK = fluoropyrimidines + Polysaccharide Kureha; M = mitomycin C; M+F = mitomycin C + fluoropyrimidines; M+F+PSK = mitomycin C + fluoropyrimidines + Polysaccharide Kureha.

Fig. 1 Survival curves of TNPP Data Set 2010.
Patients who received effective therapy (blue line); those who received neither effective nor ineffective therapy (orange line); those who received ineffective therapy (pink line); and unclassified patients (purple line). Patients who received effective therapy showed the best survival rate than other groups (log rank test, p<0.0001).

Fig. 2 Survival curves of patients in TNPP Data Set 2010.
Patients without hepatic and peritoneal metastasis (A). Patients with hepatic and/or peritoneal metastasis (B). There were significant differences between patients who received effective therapy (blue line), ineffective therapy (pink line), neither effective nor ineffective therapy (orange line), and unclassified (purple line) (log rank test, p<0.0001). There were significant differences between patients who received effective therapy (blue line), ineffective therapy (pink line), neither effective nor ineffective therapy (orange line), and unclassified (purple line) (p<0.0001).
peritoneal metastasis (B) with effective therapy (n=11) vs. those with neither effective nor ineffective therapy (n=153), those with ineffective therapy (n=32), and unclassified (n=117) \( p=0.001, \text{RR} \ 11.459 (2.827–46.447); p=0.001, \text{RR} \ 34.928 (4.664–261.597); \) and \( p<0.001, \text{RR} \ 13.792 (3.382–56.247) \), respectively.

Survival curves for TU Data Set 2010 (Fig. 3A), WN Data Set 2010 (Fig. 3B) and New TNPP Data Set 2010 (Fig. 3C) in patients without hepatic and peritoneal metastasis (Aa, Ba and Ca) and in those with hepatic and/or peritoneal metastasis (Ab, Bb and Cb) were compared.

In Fig. 3Aa, significant differences in survival are shown between patients with effective therapy (n=183) vs. those with neither effective nor ineffective therapy (n=524), those with ineffective therapy (n=40), and unclassified (n=375) \( p<0.001, \text{RR} \ 9.378 (5.119–17.179), p<0.001, \text{RR} \ 41.711 (20.629–84.335) \) and \( p<0.001, \text{RR} \ 8.411 (4.559–15.520) \). In Fig. 3Ab, a significant difference in survival is shown between patients with effective therapy (n=9) and those with neither effective nor ineffective therapy (n=130) \( p=0.021, \text{RR} \ 38.026 (1.712–844.783) \).

In Figure 3Ba, significant differences in survival are shown between patients with effective therapy (n=57) vs. those with neither effective nor ineffective therapy (n=141), those with ineffective therapy (n=9), and unclassified (n=111) \( p=0.003, \text{RR} \ 20.322 (2.830–145.933), p=0.007, \text{RR} \ 84.598 (3.347–2138.445) \), and \( p=0.002, \text{RR} \ 24.239 (3.356–175.042) \). In Fig. 3Bb, no significant differences are shown.

As shown in Fig. 3Ca, there was no significant difference between groups by Cox analysis; however, a significant difference was seen between patients without hepatic and/or peritoneal metastasis with effective therapy and those with neither effective nor ineffective therapy by log rank test \( p=0.043 \). Figure 3Cb also shows a significant difference between patients with hepatic and/or peritoneal metastasis with effective therapy and those with neither effective nor ineffective therapy \( p=0.028, \text{RR} \ 6.972 (1.237–39.285) \).

Oral administration duration in TNPP

The administration period of the F regimen in TNPP Data Set 2010 receiving F, FPSK, MF and MFPSK therapy and as well as no adjuvant group and MMC therapy group as controls, and that identified with an effective personalized therapy (A), neither effective nor ineffective therapy (B), ineffective therapy (C) and unclassified patients (D) was determined (Fig. 4). There was no significant difference in survival among patients receiving ef-
fective therapy for less than 2 years (n=53) and over than 2 years (n=54). However, there was a significant difference in survival between patients that received therapy for less than 2 years (n=136) and over than 2 years (n=106) in patients who received neither effective nor ineffective therapy, \( p<0.001, \text{RR} \ 4.371 \ (3.186–5.997) \), in those (n=11 and n=12, respectively) who received ineffective therapy \( p=0.001, \text{RR} \ 2.513 \ (1.426–4.426) \) and in those (n=121 and n=70, respectively) unclassified \( p<0.001, \text{RR} \ 2.371 \ (1.662–3.382) \).

The administration period of the PSK regimen in TNPP Data Set 2010 receiving PSK, FPSK and MFPSK therapy and as well as no adjuvant group and MMC therapy group as controls, and that identified with an effective personalized therapy (A), neither effective nor ineffective therapy (B), ineffective therapy (C) and unclassified patients (D) was determined (Fig. 5). There was no significant difference among patients receiving effective therapy for less than 2 years (n=60) and over than 2 years (n=56); however, a significant difference was seen in patients who received neither ineffective nor effective F regimen therapy for over than 2 years and <2 years (log rank test, \( p<0.0001 \)). There was a significant difference between patients who received ineffective F regimen therapy for over than 2 years and <2 years (log rank test, \( p=0.001 \)). In unclassified patients, there was a significant difference between patients who received PSK for over than 2 years and <2 years (\( p<0.0001 \)).
and in those unclassified (n=218 and n=102) [p<0.001, RR 2.707 (1.703–4.304)].

**Discussion**

There are no previous reports that personalized medicine has succeeded in taking advantage of the HLA information provided by the Human Genome Project until this study. Reasons for this might be owing to Japanese characteristics of HLA, i.e. the highest frequency of homogeneity in the world\(^5\), only three types [PSK, F, MMC (M)] of clinically administered drugs being commonly used in Japan, and more than 1000 cases of 5-year survival and 1000 cases of successfully determined 10-year prognosis. There are also no previous reports about the relation between optimal gene(s) and personalized medicine for cancer patients in the same institute by the same doctor who could refer to the decision making of treatment for a long time.

This study was able to elucidate several important findings related to HLA and personalized medicine. The effective or ineffective personalized therapies in patients with similar HLA profiles was found to be similar and, therefore, if an effective personalized therapy is given to the patients, the prognosis of the patients is good, and vice versa. Using existing therapies, if effective personalized therapy is given, there was a 60.9% positive response in patients. However, ineffective personalized therapy was given to 10.2% of the patients, thus it is necessary to be aware of relevant factors in therapy selection. If the effective personalized therapy is not given, it is necessary to administer PSK and F regimen for at least 2 years. The personalized therapy that was effective or ineffective for a patient was identified for 71.1% of existing therapies, but could not be classified for 28.9% of existing therapies. Those therapies that could not be classified might potentially include personalized therapies with therapeutic drugs that could not be examined in this study or with new drugs. It will be necessary to examine these therapies in the future; however, the current findings are clinically applicable.

The possibility that patients having a similar pharmacological metabolism would have a similar pathological state of cancer (e.g. carcinogenesis mechanism, metastasis mechanism, intracellular drug metabolism) is considered a clinically relevant area of investigation. The concept of molecular-targeted therapy research on the right therapy for the right tumor in recent years is thought to offer numerous contributions to human health care while also supporting the basic clinical notion of patient-centered therapy. Molecular-targeted anticancer drugs are specific to their target molecules and action (e.g. trastuzumab is an antibody, whereas imatinib and gefinitib are kinase inhibitors)\(^{16,18}\), however, while they have a strong anticancer effect at their target location, they can also produce severe side effects such as interstitial pneumonia. It has been subsequently reported that the effect of gefinitib is strong in female, Asian and non-smoking patients having mutated EGFR, which is the target molecule\(^9\). The difference in racial response was noticed for the first time in the effect of gefinitib. Likewise, for cetuximab and panitumumab, which are used in the treatment of bowel cancer, positive treatment response has been observed in patients exhibiting the wild-type KRAS gene\(^20,21\). However, no satisfactory results have been obtained in their prediction of therapeutic effects and side effects. Unfortunately, increased understanding of the complexity of tumors has added more challenges to clinical treatment and, as a result, such gene information is still not sufficient for clinicians, and patient groups with the possibility for long-term survival have still not been identified.

As such, a simple method that can identify an individual by the genetic information of a normal cell, i.e. the human genome, is desired; therefore, the concept of the right therapy for the right patient appears more suitable for patient-centered therapy in the clinical setting than right therapy for the right tumor. In this study, it was shown that individuals could be identified and grouped according to pair-matched parameters generated from serum HLA profiles at the protein level to represent the genetic information. Using quantification theory 3, we determined that patients could be classified into four groups based on homogenous properties of Japanese HLA\(^22,23\). Within each group, a subgroup using PSK as the effective therapy was compared with a subgroup without PSK. The patient group to which the effective therapy of PSK had been given had good prognosis; however, it was impossible to identify the therapy and the patient group with the potential for survival over 5–10 years by this methodology. Moreover, positive responsiveness to FPSK therapy was observed in patients with HLA-B54 in this and our previous studies\(^24\). Given the low frequency of HLA-B54 in Europeans and Americans, the results from the study group tested with PSK may be specific to the Japanese or Asians in general. Thus, although the results in this study may be specific to the Japanese, results that are common to non-Japanese would also be of value. We believe that the difference in drug sensitivity (effectiveness and side effects) in individuals based on racial and genetic information may be elucidated by performing double-blind controlled studies multilaterally using the methodology in this study, and that this methodology may serve to enhance personalized medical care in a systematic and internationally applicable manner.

The drug metabolism and the drug action mechanism in patient groups can now be estimated and may become an important clue for future drug discovery because it can be applied to effective therapy using existing drugs. It is expected that the methodology in this study may
lead to the identification of patient groups from which pathological analysis of disease and therapeutic effects can be obtained by genomic analysis, as well as narrowing down of the pathological prognosis of the cancer; e.g. a cancer-prone patient group, a cancer metastasis-prone patient group, a drug side-effect-prone patient group. Clinically relevant genome analysis searches and a decrease in the time for genomic analysis would be additional benefits.

We propose that, in the future, when a randomized controlled study of a new drug is performed, the HLA profile of patients should be determined so that effective personalized therapy can be identified for the study drug. Thus, if randomized controlled studies are repeatedly planned and performed in this way, personalized therapy in the true sense can be established for the individual patient (the treatment can be tailored for the individual patient with individual disease). Moreover, if this study is continued at a national level and the cases are accumulated, people prone to developing a disease can be predicted and further enhancement of accuracy can be produced for disease-specific genes. Quality of life may also be enhanced by this technique through the reduction of medical expenses, where “efficient and minimum” treatment is tailored to the specific constitutional predisposition of patients, and further the international economic effects may be observed though the development of novel biological technology.

In conclusion, it was not possible to identify an effective personalized therapy and confirm its reproducibility by serum HLA alone. However, similar patient groups could be identified along with an effective personalized therapy, and its reproducibility could be examined by examining further parameters of the HLA profile at the protein level. Effective personalized therapy was identified for ~70% of patients and reproducibility was satisfactory. Therefore, we believe that the hypothesis that effective and ineffective personalized therapies are similar in patients with similar genetic information, resulting in accurate therapeutic prognosis, has been established.

Acknowledgments
We thank all participating patients, K. Isono, the former President of The Japanese Society of Strategies for Cancer Research and Therapy, members of HLA Study Group of The Japanese Society of Strategies for Cancer Research and Therapy (former Chairman Y. Koyanagi and present Chairman S. Takenoshita), collaborators of the members of The Japanese Society of Strategies for Cancer Research and Therapy, and K. Iwata for help with the analysis of the data. This study was supported by grants from The Japanese Society of Strategies for Cancer Research and Therapy.

Supplementary Material
List of Abbreviations
Glossary terms
Fig. S1
Fig. S2

References
1) Robert, C. D. (1989) The Alta Summit, December 1984. Genomics 5: 661–663.
2) International Human Genome Sequencing Consortium. (2004) Finishing the euchromatic sequence of the human genome. Nature 431: 931–945.
3) Benacerraf, B., McDevitt, H. O. (1972) Histocompatibility-linked immune response genes. Science 175: 273–279.
4) Zinkernagel, R. M., Doherty, P. C. (1974) Restriction of intr vito T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature 248: 701–702.
5) Benacerraf, B. (1981) Role of MHC gene products in immune regulation. Science 212: 1229–1238.
6) Soejigand, A., Ryder, L. P. (1976) Interaction of HLA molecules with non-immunological ligands as an explanation of HLA and disease association. Lancet 2: 547–549.
7) Eddin, M. (1988) Function by association? MHC antigens and membrane receptor complexes. Immunology Today 9: 218–219.
8) The MHC sequencing consortium. (1999) Complete sequence and gene map of a human major histocompatibility complex. Nature 401: 921–923.
9) Nakazato, H., Koike, A., Saji, S., Ogawa, N., Sakamoto, J. (1994) Study Group of Immunochemotherapy with PSK for Gastric Cancer, Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Lancet 343: 1122–1126.
10) Ogoshi, K., Kondoh, Y., Tajima, T., Mitomi, T. (1992) Glycosidically bound sialic acid levels as a predictive marker of postoperative adjuvant therapy in gastric cancer. Cancer Immunol. Immunother. 35: 175–180.
11) Lu, H., Yang, Y., Gud, E., Wenner, C. A., Chang, A., Larson, E. R., Dang, Y., Martzen, M., Standish, L. J., Disis, M. L. (2011) Polysaccharide krestin is a novel TLR2 agonist that mediates inhibition of tumor growth via stimulation of CD8 T cells and NK cells. Clin. Cancer Res. 17: 67–76.
12) Aykan, N. F., Ileleivich, E. (2008) The role of UFT in advanced gastric cancer. Ann. Oncol. 19: 1045–1052.
13) Sakuramoto, S., Sasako, M., Yamaguchi, T., Kinoshita, T., Fujii, M., Nashimoto, A., Furukawa, H., Nakajima, T., Ohashi, Y., Imamura, H., Higashino, M., Yamamura, Y., Kurita, A., Arai, K., ACTS-GC Group. (2007) Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N. Engl. J. Med. 357: 1810–1820.
14) Nakajima, T., Nashimoto, A., Kitamura, M., Kito, T., Iwanaga, T., Okabayashi, K., Sasaki, M., Goto, M. (1999) Gastric Cancer Surgical Study Group, Adjuvant mitomycin and fluorouracil followed by oral uracil plus tegafur in serosa-negative gastric cancer: a randomised trial. Lancet 24: 273–277.
15) Ogoshi, K., Isono, K. (2001) Incidence of heterozygotes and homozygotes of major histocompatibility complex in Japanese compared to non-Japanese. Ann. Cancer Res. Ther. 9: 87–98.
16) Piccart-Gebhart, M. J., Proctor, M., Leyland-Jones, B., Goldhirsh, A., Untch, M., Smith, I., Gianni, L., Baselga, J., Bell, R., Jackisch, C., Cameron, D., Dowsett, M., Barrios, C. H., Steger, G., Huang, C. S., Andersson, M., Inbar, M., Lichinitser, M., Läng, I., Nitz, U., Iwata, H., Thomsen, C., Lohrisch, C., Suter, T. M., Rüschhoff, J., Suto, T., Greatorex, V., Ward, C., Straehle, C., McFadden, E., Dolci, M. S., Gelber, R. D., Herceptin Adjuvant (HERA) Trial Study Team. (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N. Engl. J. Med. 353: 1659–1672.
17) Druker, B. J., Lydon, N. B. (2000) Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J. Clin Invest. 105: 3–7.
18) Sordella, R., Bell, D. W., Haber, D. A., Settleman, J. (2004) Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305: 1163–1167.
19) Tsao, M. S., Sakurada, A., Cutz, J. C., Zhu, C. Q., Kamel-Reid, S., Squire, J., Lorimer, I., Zhang, T., Liu, N., Daneshmand, M., Marrano, P., Santos, G. C., Lagarde, A., Richardson, F., Seymour, L., Whitehead, M., Ding, K., Pater, J., Shepherd, F. A. (2005) Erlotinib in lung cancer — Molecular and clinical predictors of outcome. N Engl. J. Med. 353:133–144.

20) Yen, L. C., Uen, Y. H., Wu, D. C., Lu, C. Y., Yu, F. J., Wu, I. C., Lin, S. R., Wang, J. Y. (2010) Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. Ann. Surg. 251:254–260.

21) Doi, T., Tahara, M., Yoshino, T., Yamazaki, K., Tamura, T., Yamada, Y., Yang, B. B., Oliner, K. S., Otani, S., Asahi, D. (2011) Tumor KRAS status predicts responsiveness to panitumumab in Japanese patients with metastatic colorectal cancer. Jpn. J. Clin. Oncol. 41:210–216.

22) Hayashi, F., Hayashi, C., Ogoshi, K. (1994) Classification of gastric cancer patients based on HLA antigen expression using quantification method III. Ann. Cancer Res. Ther. 3:117–120.

23) Ogoshi, K., Koyanagi, Y., Tsuji, K., Isono, K. (2008) Outcome of HLA-oriented therapy for gastric cancer in retrospective and prospective study. Ann. Cancer Res. Ther. 8:36–43.

24) Ogoshi, K., Isono, K. (2009) HLA-B54 is a candidate of response to Fluoropyrimidine plus PSK therapy in gastric cancer. Ann. Cancer Res. Ther. 17:40–44.