## Supplementary Table 1. Name, source TAA, position, amino acid sequence, and HLA type in the mixed 20-peptide vaccine (KRM-20)

| Peptide name | Source TAA | Position of peptide | Amino acid sequence | HLA type       |
|--------------|------------|---------------------|---------------------|----------------|
| SART3-109    | SART3      | 109-118             | VYDYNCHVDL          | A24, A3 family, A26 |
| Lck-208      | p56 lck    | 208-216             | HYTNASDGL           | A24            |
| PAP-213      | PAP        | 213-221             | LYCESVHNF           | A24            |
| PSA-248      | PSA        | 248-257             | HYRKWIKDTI          | A24            |
| EGFR-800     | EGF-R      | 800-809             | DYVREHKDNI          | A24            |
| MRP3-1293    | MRP3       | 1293-1302           | NYSVRYRPGL          | A24            |
| Lck-486      | p56 lck    | 486-494             | TFDYLRVSL           | A24            |
| Lck-488      | p56 lck    | 488-497             | DYLRSVLEDF          | A24            |
| PSMA-624     | PSMA       | 624-632             | TYSVSFD8L           | A24            |
| PTHrP-102    | PTHrP      | 102-111             | RYLTQETNKV          | A24            |
| CypB-129     | Cyclophilin B | 129-138        | KLKHYGPGWV          | A2, A3 family* |
| Lck-246      | p56 lck    | 246-254             | KVERLGA             | A2             |
| WHSC2-103    | WHSC2      | 103-111             | ASLSDPWV            | A2, A3 family* |
| UBE-43       | UBE2V      | 43-51               | RLQEWCSVI           | A2             |
| WHSC2-141    | WHSC2      | 141-149             | ILGELREKV           | A2             |
| HNRPL-140    | HNRPL      | 140-148             | ALVEFEDVL           | A2, A3 family, A26 |
| SART3-302    | SART3      | 302-310             | LLQAEAPRL           | A2             |
| SART3-734    | SART3      | 734-742             | QIRPSFSNR           | A3 family*     |
| Lck-90       | p56 lck    | 90-99               | ILEQSGEWWK          | A3 family*     |
| Lck-449      | p56 lck    | 449-458             | VIQNLERGyr          | A3 family*     |

* A3 family, HLA-A3, A11, A31, and A33

Abbreviations: CypB, cyclophilin B; EGFR, epidermal growth factor-receptor; HLA, human leukocyte antigen; HNRPL, heterogeneous nuclear ribonucleoprotein L; Lck, p56[^d]; MRP3, multidrug resistance-associated protein 3; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PTHrP, parathyroid hormone-related peptide; SART3, squamous cell carcinoma antigens 3; TAA, tumor-associated antigen; UBE2V, ubiquitin-conjugated enzyme variant Kua; WHSC2, Wolf-Hirshhorn syndrome critical region 2.
**Supplementary Table 2.** HLA-matched peptide-specific IgG and CTL responses in the KRM-20 arm

| Pts. No. | HLA type   | No. of HLA-matched peptides | IgG response (No. of positive peptides) | CTL response (No. of positive peptides) |
|----------|------------|-----------------------------|----------------------------------------|----------------------------------------|
| 20201    | A24/A11    | 16                          | negative (0)                           | positive (2)                           |
| 20203    | A24/A11    | 16                          | negative (0)                           | negative (0)                           |
| 20206    | A24/A33    | 16                          | negative (0)                           | positive (1)                           |
| 20301    | A33/A26    | 8                           | positive (8)                            | negative (0)                           |
| 20302    | A2/A24     | 17                          | negative (0)                           | negative (0)                           |
| 20303    | A2/A24     | 17                          | positive (4)                            | negative (0)                           |
| 20305    | A24        | 10                          | negative (0)                           | negative (0)                           |
| 20401    | A2/A26     | 9                           | negative (0)                            | negative (0)                           |
| 20405    | A24/A26    | 12                          | positive (1)                            | negative (0)                           |
| 20406    | A2/A24     | 17                          | positive (1)                            | negative (0)                           |
| 20407    | A24/A11    | 16                          | negative (0)                            | negative (0)                           |
| 20502    | A2/A24     | 17                          | positive (2)                            | negative (0)                           |
| 20601    | A11/A26    | 8                           | negative (0)                            | negative (0)                           |
| 20602    | A2/A33     | 13                          | negative (0)                            | negative (0)                           |
| 20603    | A24        | 10                          | positive (10)                           | positive (3)                           |
| 20702    | A2/A26     | 9                           | negative (0)                            | positive (1)                           |
| 20703    | A24/A31    | 16                          | negative (0)                            | negative (0)                           |
| 20801    | A24        | 10                          | negative (0)                            | negative (0)                           |
| 20904    | A24/A31    | 16                          | negative (0)                            | negative (0)                           |
| 20906    | A24/A11    | 16                          | negative (0)                            | negative (0)                           |
| 21002    | A2/A26     | 9                           | positive (2)                            | negative (0)                           |
| 21005    | A24/A11    | 16                          | negative (0)                            | negative (0)                           |
| 21007    | A2/A11     | 13                          | positive (4)                            | positive (2)                           |

Abbreviations: CTL, cytotoxic T lymphocytes; HLA, human leukocyte antigen; IgG, immunoglobulin G
### Supplementary Table 3. Summary of in-study adverse events

|                           | KRM-20 arm (n = 23) | Placebo arm (n = 26) |
|---------------------------|---------------------|----------------------|
|                           | Any grade | Grade 3 | Grade 4 | Grade 5 | Any grade | Grade 3 | Grade 4 | Grade 5 |
| Injection site reaction   | 16 (70)   | 0       | 0       | 0       | 10 (39)   | 0       | 0       | 0       |
| Alopecia                  | 14 (61)   | 0       | 0       | 0       | 17 (65)   | 0       | 0       | 0       |
| Neutropenia               | 11 (48)   | 2 (9)   | 7 (30)  | 0       | 15 (58)   | 3 (12)  | 6 (23)  | 0       |
| Peripheral neuropathy     | 11 (48)   | 0       | 0       | 0       | 13 (50)   | 0       | 0       | 0       |
| Fatigue                   | 9 (39)    | 0       | 0       | 0       | 2 (8)     | 0       | 0       | 0       |
| Upper respiratory infection| 7 (30)    | 0       | 0       | 0       | 1 (4)     | 0       | 0       | 0       |
| Leucopenia                | 5 (22)    | 3 (13)  | 1 (4)   | 0       | 1 (4)     | 1 (4)   | 0       | 0       |
| Fever                     | 5 (22)    | 1 (4)   | 0       | 0       | 3 (12)    | 0       | 0       | 0       |
| Peripheral edema          | 5 (22)    | 0       | 0       | 0       | 2 (8)     | 0       | 0       | 0       |
| Diarrhea                  | 5 (22)    | 0       | 0       | 0       | 7 (27)    | 0       | 0       | 0       |
| Appetite loss             | 5 (22)    | 1 (4)   | 0       | 0       | 4 (15)    | 0       | 0       | 0       |
| Oral mucositis            | 4 (17)    | 0       | 0       | 0       | 5 (19)    | 0       | 0       | 0       |
| Nail discoloration        | 4 (17)    | 0       | 0       | 0       | 0         | 0       | 0       | 0       |
| Joint pain                | 3 (13)    | 0       | 0       | 0       | 4 (15)    | 0       | 0       | 0       |
| Dry skin                  | 3 (13)    | 0       | 0       | 0       | 1 (4)     | 0       | 0       | 0       |
| Anemia                    | 2 (9)     | 1 (4)   | 0       | 0       | 2 (8)     | 1 (4)   | 0       | 0       |
| Decreased lymphocyte count| 2 (9)     | 0       | 0       | 0       | 2 (8)     | 1 (4)   | 0       | 0       |
| Febrile neutropenia       | 0         | 0       | 0       | 0       | 2 (8)     | 1 (4)   | 1 (4)   | 0       |
| Pneumonia                 | 0         | 0       | 0       | 0       | 3 (11)    | 0       | 0       | 2 (8)   |
| Fracture                  | 0         | 0       | 0       | 0       | 2 (8)     | 1 (4)   | 0       | 0       |
| Decreased platelet count  | 0         | 0       | 0       | 0       | 1 (4)     | 1 (4)   | 0       | 0       |
| Depression                | 0         | 0       | 0       | 0       | 1 (4)     | 1 (4)   | 0       | 0       |

Data are number of patients (%); patients may have had more than one event. All grade 1 or 2 adverse events developing in >10% of patients are reported. All grade 3, 4, and 5 adverse events are reported.
Protocol
Protocol: Phase 2, randomized, placebo-controlled study of docetaxel in combination with a mixed 20-peptide vaccine for patients with castration-resistant prostate cancer (UMIN000011028)

Background
The prognosis of patients with castration-resistant prostate cancer remains poor. Treatments that can provide stable disease control with long-term survival benefits are needed. One such treatment is considered to be the combination therapy of a cancer vaccine with chemotherapy. However, the optimal combination therapy using a cancer vaccine with docetaxel for chemotherapy-naïve patients with castration-resistant prostate cancer remains unknown.

Aims
We aim to examine whether a novel cancer vaccine consisting of 20 mixed peptides (KRM-20) designed to induce cytotoxic T lymphocytes in combination with docetaxel and dexamethasone enhances the anti-tumor effects in patients with castration-resistant prostate cancer.

Methods
Study design and population
This is a double-blind, placebo-controlled, randomized phase 2 study. Chemotherapy-naïve patients with progressive castration-resistant prostate cancer will be enrolled from 10 medical centers in Japan.

Inclusion criteria
The subjects must satisfy the following conditions.
1. Patients must be diagnosed as prostate cancer pathologically at the initial treatment.
2. Patients who had progressive disease after androgen deprivation therapy (ADT) either by surgical castration, gonadotropin-releasing hormone or antagonist treatment. Progressive disease while receiving ADT, defined by any 1 of the following:
   1) At least two consecutive rises in serum PSA obtained at a minimum of 1-week intervals.
   2) Measurable disease with ≥50% increase in the sum of the cross products of all measurable lesions, or the development of new measurable lesions by RESIST.
   3) Non-measurable (bone) disease consisting of new areas of uptake by bone scan consistent with metastatic disease compared to previous imaging.
3. Patients have serum PSA level ≥2 ng/mL
4. Anti-androgen therapy is discontinued for at least 4 weeks before the first vaccination for patients receiving flutamide and 6 weeks for those receiving bicalutamide.
5. Patients continue to stay on medical treatment such as LHRH agonists of LHRH antagonists to maintain testosterone level of 0.5ng/mL.
6. Patients must be positive for HLA-A2, HLA-A24, HLA-A26 or HLA-A3 super type (A3, A11, A31, A33).
7. Written informed consent must be obtained from patients.
8. Patients must be more 20 year-old.
9. Patients must be at a score level of 0-1 of performance status (ECOG).
10. Patients must be expected to survive more than 6 months.
11. Patients must satisfy the followings:
    WBC ≥ 3,000/mm³, Neutrophil ≥ 2,000/mm³, Lymphocyte ≥1,000/mm³, Hb ≥ 8.0g/dl, Platelet ≥ 100,000/mm³, Serum Creatinine ≤ 2 times upper limit of normal, Total Bilirubin ≤ 1.5 times upper limit of normal, AST, ALT ≤ 2 times upper limit of normal

**Exclusion criteria**

The following patients must be excluded:
1. Patients who had received chemotherapy using docetaxel any time before the treatment.
2. Patients who had received pre-therapies including chemotherapy or immunotherapy within 28 days before the treatment.
3. Patients who had received radiotherapy or strontium-89 within the last 8 weeks before the treatment.
4. Patients with severe symptoms (active and severe infectious disease, circulatory disease, respiratory disease, kidney disease, immunodeficiency, disturbance of coagulation).
5. Patients with active multiple cancers
6. Patients with the past history of severe allergic reactions.
7. Patients who do not agree with contraception during treatment and until 70 days after treatment.
8. Patients who had enrolled in another trial within 3 months or who are treating in another trial.
9. Patients who had received any peptides consist of a mixed 20 peptides (KRM-20).
10. Patients who are difficult to participate in this trial because of psychiatric symptoms.
11. Patients who are judged inappropriate for the clinical trial by doctors.
**Randomization**

After assessment of eligibility and appropriate consent, eligible patients are randomly assigned in a 1:1 ratio to receive either KRM-20 with docetaxel and dexamethasone (study arm) or placebo with docetaxel and dexamethasone (control arm) using a minimization technique with the following stratification factors: age (<65 or ≥65 years old) and PSA (<20 or ≥20 ng/ml) at the clinical research unit of Kurume University in Kurume, Japan.

**Intervention**

Patients will receive either KRM-20 (20 mg/0.5ml) or placebo (0.5ml) mixed with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France) subcutaneously on days 1, 8, 15, 22, and 29 with oral dexamethasone (1 mg) once daily on days 1 to 36. On day 36, one hour after receiving docetaxel at 70 mg/m² intravenously, patients will receive subcutaneous KRM-20 or placebo injection. Treatment with docetaxel and the study drug is repeated every 3 weeks for up to 5 cycles, and oral dexamethasone is continued once daily until the end of the study. Dosing delay and reduction for docetaxel is permitted if toxic effects are noted. Docetaxel may be held for less than 2 weeks until recovery and reduced to 60 or 50 mg/m² in the event of neutropenia (< 2,000/mm³), platelets < 100,000/mm³, hemoglobin < 8 g/dl, total bilirubin > 1·5 x ULM, transaminase > 2 x ULM, or serum creatinine > 2 x ULM. If docetaxel is held for more than 3 weeks, patients are removed from protocol treatment. After the study, patients who received protocol treatment will be followed up for 3 years for survival analyses.

**Endpoints**

The primary endpoint in this study was the comparison of each treatment arm for the rate of > 50% PSA decline from baseline. Based on the previous report, the assumed rate of > 50% PSA decline was 65% in the KRM-20 arm and 25% in the placebo arm. The target sample size was 50 assuming an ineligibility rate of approximately 10%. Sample size computation based on the large sample test was performed with the following assumptions: type I error rate = 0.05, power 80% and the ratio of the two groups as 1:1. Secondary endpoints are safety, immune responses, progression-free survival (PFS), and overall survival (OS). The Student’s t-test and chi-square test will be used to compare quantitative and categorical variables among safety profiles and immune responses to the treatment, respectively. PFS and OS data for each arm will be
analyzed using the Kaplan-Meier method. The log-rank test will be used to compare the survival curves, and Cox proportional hazard analysis to estimate hazard ratios (HR). The confidence intervals (CI) reported are 95%. Statistical analyses are performed using SAS software version 9·1 (SAS Institute, Cary, NC) with a two-sided significance level of 5%. All analyses will be on an intention-to-treat basis.

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