試験開始後も、GCP に基づくモニタリングおよび監査が実施される。

C．研究結果

1．臨床試験全体の研究結果については総括研究報告書に記載

2．当該分担での研究結果は現時点では以下の如くである。

IRB 審査

早期第Ⅱ相臨床試験：未定

被験者への同意説明：0 名

同意取得後、本登録に至った症例：0 名

治験薬投与を行った症例：0 名

D．考察

申請時研究計画では、平成24年3月に第I相医師主導治験を開始して10月までに15症例の去勢抵抗性前立腺がん患者を対象とした第I相臨床試験を終了し、推奨された至適用量にて平成24年度後半よりドセタキセル投与を行う20症例を対象に早期第II相臨床試験を行う。早期第II相臨床試験の登録期間は平成25年2月より平成H26年1月までとして、追跡期間は最終症例の登録後12ヶ月として全体の試験期間は24ヶ月を予定し、試験期間内に中間評価を行う計画であった。しかし、PMDAよりGLPに準拠した本剤の非臨床試験として反復投与毒性試験が必要との助言を受け、平成24年2月より反復投与毒性試験を開始し、5月の中間報告を以て、5月下旬に治験計画届出を予定しており、当初の予定より数ヶ月遅延している。この数ヶ月の遅延については、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

E．結論

申請時研究計画に沿って概ね順調に経過していると自己評価している。PMDA助言に従った非臨床試験として反復投与毒性試験の実施のため治験開始が数ヶ月遅延しているが、今後、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

F．研究発表

1．論文発表

なし

2．学会発表

なし

G．知的財産権の出願・登録状況

（予定を含む。）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

厚生労働科学研究費補助金
（難病・がん等の疾患分野の医療の実用化研究（がん関係研究分野）研究事業）
分担研究報告書

去勢抵抗性前立腺がんに対する新規がんペプチドワクチン療法開発のための第Ⅰ相・第Ⅱ相（前半）臨床試験に関する研究

研究分担者　江藤 正俊　　熊本大学 教授

研究要旨
本研究では、去勢抵抗性前立腺がん患者を対象として、新規ペプチドワクチンの安全性、至適投与量を推定する第Ⅰ相臨床試験、並びに推奨された投与量での標準治療であるドセタキセルを用いた化学療法併用における早期第Ⅱ相臨床試験を実施することにより、併用療法の有効性、安全性を確認し、早期のproof of concept（POC）を得る。その後、製薬企業に技術移転し、併用療法における新規ペプチドワクチンの実用化を目指す。
従来のペプチドワクチンは、HLA型別に異なるワクチンを必要とする上、久留米大学で開発中の患者毎に異なるペプチドを選択投与するテーラーメイド型においては、治療薬としての汎用性に欠けるという短所を有していたが、当該ペプチドワクチンはその短所の克服のみならず、ペプチドワクチンの長所（がんのCTLエピトープのみ）とテーラーメイド型の長所（2次免疫賦活）および蛋白ワクチンの長所（HLA非拘束）を有しており、世界初の研究と言える。

A．研究目的

本研究の目的は、去勢抵抗性前立腺がんに対する新規ペプチドワクチンの開発及び標準治療であるドセタキセルを用いた化学療法との併用療法の確立である。具体的には、去勢抵抗性前立腺がん患者を対象とした新規ペプチドワクチンの安全性および至適投与量を推定する第Ⅰ相臨床試験、ならびに推奨された投与量とドセタキセルを用いた化学療法との併用における有効性および安全性を探索する早期第Ⅱ相臨床試験を実施し、早期のproof of concept（POC）を得た後に、製薬企業へ技術移転して、併用療法における新規ペプチドワクチンの実用化を目指す。

B．研究方法

本研究では、まずは、去勢抵抗性前立腺がん患者15症例を対象として、前立腺がん患者用に開発された、HLA-A2、A24、A3スーパータイプ、A26拘束性の20種類のがんペプチドから構成される20種混合ペプチドワクチン（以下KRM-20と記載）の最小免疫反応量を推定するために投与量を3群設定し、無作為割付により各群（各用量5症例）に割り付ける。無作為割付に従い、ペプチド乳化製剤を割付用量に調整し、毎週1回、合計6回の皮下投与を行い、その安全性及び至適投与量の推定、並びに特異的免疫能変化及び血清PSA値の探索的検討を行う多施設共同無作為割付第Ⅰ相臨床試験を実施する。本試験の主要評価項目は安全性（全有害事象）であり、集積期間は4ヶ月、試験期間は8ヶ月である。

次に、去勢抵抗性前立腺がん患者20症例を対象として、第Ⅰ相臨床試験で推奨された投与量と標準治療であるドセタキセルを用いた化学療法との併用療法とKRM-20単独療法との比較試験を実施し、その有効性及び安全性を探索する多施設共同無作為割付早期第Ⅱ相比較臨床試験を実施する。本試験の主要評価項目は無増悪生存期間である。

（倫理面への配慮）

臨床試験（治験）に先立ち、安全性を担保するための安全性薬理試験並びに必要不可欠なラットを用いた各種毒性試験をGLP基準に基づき実施し、ヒトにおける臨床試験の実施に問題は無いと判断された。

本研究は、患者を対象とした介入試験であり、薬事法下の医師主導治験である。「ヘルシンキ宣言」ならびに「医薬品の臨床試験の実施の基準に関する省令（GCP）」を遵守して実施される。

治験実施計画書及び患者同意説明文書は、医薬品医療機器総合機構（PMDA）による治験相談を実施済みであり、今後、各実施医療機関のIRBにおいても科学的及び倫理的な面からの審査・承認を経た後、治験届出後に治験を開始する予定である。さらに公的登録サイト（UMIN、JAPIC）に登録して行う。

被験者からの同意取得にあたっては、同意説明文書を用いて試験の内容、予想される不利益・危険性、同意撤回の自由等を説明する。被験者が説明内容を十分に理解したことを確認した上で、本試験への参加について被験者本人の自由意思による同意を文書により取得する（インフォームドコンセント）。また、HLA-A血清対応型タイピング結果が判明した後に、検査結果を被験者に報告、説明し、文書による同意の確認を得る。

試験開始後も、GCPに基づくモニタリングおよび監査が実施される。

C．研究結果

1．臨床試験全体の研究結果については総括研究報告書に記載

2．当該分担での研究結果は現時点では以下の如くである。

IRB審査

早期第Ⅱ相臨床試験：未定

被験者への同意説明：0名

同意取得後、本登録に至った症例：0名

治験薬投与を行った症例：0名

D．考察

申請時研究計画では、平成24年3月に第I相医師主導治験を開始して10月までに15症例の去勢抵抗性前立腺がん患者を対象とした第I相臨床試験を終了し、推奨された至適用量にて平成24年度後半よりドセタキセル投与を行う20症例を対象に早期第II相臨床試験を行う。早期第II相臨床試験の登録期間は平成25年2月より平成H26年1月までとして、追跡期間は最終症例の登録後12ヶ月として全体の試験期間は24ヶ月を予定し、試験期間内に中間評価を行う計画であった。しかし、PMDAよりGLPに準拠した本剤の非臨床試験として反復投与毒性試験が必要との助言を受け、平成24年2月より反復投与毒性試験を開始し、5月の中間報告を以て、5月下旬に治験計画届出を予定しており、当初の予定より数ヶ月遅延している。この数ヶ月の遅延については、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

E．結論

申請時研究計画に沿って概ね順調に経過していると自己評価している。PMDA助言に従った非臨床試験として反復投与毒性試験の実施のため治験開始が数ヶ月遅延しているが、今後、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

F．研究発表

1．論文発表

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G．知的財産権の出願・登録状況

（予定を含む。）

1．特許取得

なし

2．実用新案登録

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なし

厚生労働科学研究費補助金
（難病・がん等の疾患分野の医療の実用化研究（がん関係研究分野）研究事業）
分担研究報告書

去勢抵抗性前立腺がんに対する新規がんペプチドワクチン療法開発のための第Ⅰ相・第Ⅱ相（前半）臨床試験に関する研究

研究分担者　角間 辰之　　久留米大学 教授

研究要旨
本研究では、去勢抵抗性前立腺がん患者を対象として、新規ペプチドワクチンの安全性、至適投与量を推定する第Ⅰ相臨床試験、並びに推奨された投与量での標準治療であるドセタキセルを用いた化学療法併用における早期第Ⅱ相臨床試験を実施することにより、併用療法の有効性、安全性を確認し、早期のproof of concept（POC）を得る。その後、製薬企業に技術移転し、併用療法における新規ペプチドワクチンの実用化を目指す。
従来のペプチドワクチンは、HLA型別に異なるワクチンを必要とする上、久留米大学で開発中の患者毎に異なるペプチドを選択投与するテーラーメイド型においては、治療薬としての汎用性に欠けるという短所を有していたが、当該ペプチドワクチンはその短所の克服のみならず、ペプチドワクチンの長所（がんのCTLエピトープのみ）とテーラーメイド型の長所（2次免疫賦活）および蛋白ワクチンの長所（HLA非拘束）を有しており、世界初の研究と言える。

A．研究目的

本研究の目的は、去勢抵抗性前立腺がんに対する新規ペプチドワクチンの開発及び標準治療であるドセタキセルを用いた化学療法との併用療法の確立である。具体的には、去勢抵抗性前立腺がん患者を対象とした新規ペプチドワクチンの安全性および至適投与量を推定する第Ⅰ相臨床試験、ならびに推奨された投与量とドセタキセルを用いた化学療法との併用における有効性および安全性を探索する早期第Ⅱ相臨床試験を実施し、早期のproof of concept（POC）を得た後に、製薬企業へ技術移転して、併用療法における新規ペプチドワクチンの実用化を目指す。

B．研究方法

本研究では、まずは、去勢抵抗性前立腺がん患者15症例を対象として、前立腺がん患者用に関発された、HLA-A2、A24、A3スーパータイプ、A26拘束性の20種類のがんペプチドから構成される20種混合ペプチドワクチン（以下KRM-20と記載）の最小免疫反応量を推定するために投与量を3群設定し、無作為割付により各群（各用量5症例）に割り付ける。無作為割付に従い、ペプチド乳化製剤を割付用量に調整し、毎週1回、合計6回の皮下投与を行い、その安全性及び至適投与量の推定、並びに特異的免疫能変化及び血清PSA値の探索的検討を行う多施設共同無作為割付第Ⅰ相臨床試験を実施する。本試験の主要評価項目は安全性（全有害事象）であり、集積期間は4ヶ月、試験期間は8ヶ月である。

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（倫理面への配慮）

臨床試験（治験）に先立ち、安全性を担保するための安全性薬理試験並びに必要不可欠なラットを用いた各種毒性試験をGLP基準に基づき実施し、ヒトにおける臨床試験の実施に問題は無いと判断された。

本研究は、患者を対象とした介入試験であり、薬事法下の医師主導治験である。「ヘルシンキ宣言」ならびに「医薬品の臨床試験の実施の基準に関する省令（GCP）」を遵守して実施される。

治験実施計画書及び患者同意説明文書は、医薬品医療機器総合機構（PMDA）による治験相談を実施済みであり、今後、各実施医療機関のIRBにおいても科学的及び倫理的な面からの審査・承認を経た後、治験届出後に治験を開始する予定である。さらに公的登録サイト（UMIN、JAPIC）に登録して行う。

被験者からの同意取得にあたっては、同意説明文書を用いて試験の内容、予想される不利益・危険性、同意撤回の自由等を説明する。被験者が説明内容を十分に理解したことを確認した上で、本試験への参加について被験者本人の自由意思による同意を文書により取得する（インフォームドコンセント）。また、HLA-A血清対応型タイピング結果が判明した後に、検査結果を被験者に報告、説明し、文書による同意の確認を得る。

試験開始後も、GCP に基づくモニタリングおよび監査が実施される。

C．研究結果

1．臨床試験全体の研究結果については総括研究報告書に記載

2．当該分担での研究結果は現時点では以下の如くである。

統計学的研究成果：(1) 最小免疫反応量を推定するために投与量を 3 群設定し、無作為割付により各群（各用量 5 症例）に割り付ける症例数設計を行った。(2)動的割付のプログラミングを作成した。これらの研究成果の詳細は治験実施計画書及び統計解析計画書に記述した。

D．考察

申請時研究計画では、平成24年3月に第I相医師主導治験を開始して10月までに15症例の去勢抵抗性前立腺がん患者を対象とした第I相臨床試験を終了し、推奨された至適用量にて平成24年度後半よりドセタキセル投与を行う20症例を対象に早期第II相臨床試験を行う。早期第II相臨床試験の登録期間は平成25年2月より平成H26年1月までとして、追跡期間は最終症例の登録後12ヶ月として全体の試験期間は24ヶ月を予定し、試験期間内に中間評価を行う計画であった。しかし、PMDAよりGLPに準拠した本剤の非臨床試験として反復投与毒性試験が必要との助言を受け、平成24年2月より反復投与毒性試験を開始し、5月の中間報告を以て、5月下旬に治験計画届出を予定しており、当初の予定より数ヶ月遅延している。この数ヶ月の遅延については、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

E．結論

申請時研究計画に沿って概ね順調に経過していると自己評価している。PMDA助言に従った非臨床試験として反復投与毒性試験の実施のため治験開始が数ヶ月遅延しているが、今後、症例登録の促進を図り、研究期間終了までに解消すべく努力する。

F．研究発表

1．論文発表

なし

2．学会発表

なし

G．知的財産権の出願・登録状況

（予定を含む。）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

Ⅲ．研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Noguchi M, Uemura H, Naito S, Akaza H, Yamada A, Itoh K	A phase I study of personalized peptide vaccination using 14 kinds of vaccine in combination with low-dose estramustine in LA-A24-positive patients with castration-resistant prostate cancer.	Prostate	71	470-479	2011
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Yoshiyama K, Terazaki Y, Matsueda S, Shichijo S, Noguchi M, Yamada A, Mine T, Ioji T, Itoh K, Shirouzu K, Sasada T and Takamori S	Personalized peptide vaccination in patients with refractory non-small cell lung cancer.	Int J Oncol	40	1492-1500	2012
Yoshitomi M, Yutani S, Matsueda S, Ioji T, Komatsu N, Shichijo S, Yamada A, Itoh K, Sasada T, Kinoshita H	Personalized peptide vaccination for advanced biliary tract cancer: IL-6, nutritional status, and pre-existing antigen-specific immunity as possible biomarkers for patient prognosis.	Exp Ther Med	3	463-469	2012
Terazaki Y, Yoshiyama K, Matsueda S, Watanabe N, Yamada A, Mine T, Terasaki M, Itoh K, Takamori S, Sasada T	Immunological evaluation of personalized peptide vaccination in refractory small cell lung cancer.	Cancer Science	103(4)	638-44	2012

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Ⅳ. 研究成果の刊行物・別冊

The Prostate 71:470 – 479 (2011)

A Phase I Study of Personalized Peptide Vaccination Using 14 Kinds of Vaccine in Combination With Low-Dose Estramustine in HLA-A24-Positive Patients With Castration-Resistant Prostate Cancer

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BACKGROUND. To evaluate the safety, tolerability, immune response, and antitumor activity of a combination of personalized peptide vaccination (PPV) and estramustine phosphate (EMP) in patients with castration-resistant prostate cancer (CRPC).

METHODS. In a phase I dose-escalation study, four peptides showing the highest levels of peptide-specific immunoglobulin G (IgG) to 14 vaccine candidates (ITK-1) were subcutaneously injected every week in three different dose settings (1, 3, and 5 mg per peptide) for 6 weeks with a low dose of EMP, and the patients were followed by maximum 2 years extension study either weekly or bi-weekly six times PPV as one course with a low dose of EMP.

RESULTS. Fifteen patients were enrolled in the phase I study. No serious treatment-related adverse events were observed. The most common adverse events were grade 2 skin reactions at the injection sites. The maximum acceptable dose of ITK-1 was 8.643 mg. There were no treatment-related systemic adverse events of grade 3 or more, and maximum tolerated dose could not be determined. Cytotoxic T lymphocyte responses measured by interferon-γ release assay were boosted in 10 of 15 (67%) patients, and IgG responses were boosted in 7 of 15 (47%) patients. Twelve patients proceeded to the extension study, and the median survival time was 23.8 months during a median follow-up of 23.8 months.

CONCLUSIONS. PPV treatment for HLA-A24 positive patients with CRPC could be recommended for further stages of clinical trials because of its safety and the higher frequency of boosting immune responses. *Prostate 71: 470–479, 2011.* © 2010 Wiley-Liss, Inc.

KEY WORDS: **personalized peptide vaccine; immunotherapy; phase I study; estramustine phosphate**

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Grant sponsor: Green Peptide Co., Ltd.

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INTRODUCTION

In the initial trials, peptide-based vaccine treatment of cancer patients rarely induced clinical responses and the levels of immune responses was low, indicating that the classical type of peptide vaccines did not have a promising future in the treatment of advanced cancer [1,2]. However, there have been slow but substantial advances in peptide vaccines and dendritic cell (DC)-based vaccines with regard to both clinical responses and immunological markers [3–12].

We previously reported that repeated multiple peptide vaccine regimen planned according to the pre-existing immunity (personalized peptide vaccine: PPV) could prolong the overall survival of patients with advanced cancer, and IgG specific to each peptide can frequently be detected in pre- and post-vaccination plasma [13]. In the previous trial, PPV was administered in 113 patients with advanced cancer, and the levels of peptide-specific cytotoxic T lymphocyte (CTL) precursors were measured by the interferon (IFN)-γ release assay and those of anti-peptide immunoglobulin (IgG) were estimated by enzyme-linked immunosorbent assay (ELISA). The level of anti-peptide IgG was a laboratory marker that predicted clinical responses to the PPV with a positive relationship to overall survival. Further, we showed that 58 patients with castration-resistant prostate cancer (CRPC) treated with a combination therapy of PPV and a low dose of estramustine phosphate (EMP) survived for a relatively long period of 17 months, which was comparable with the results of chemotherapy with docetaxel, and serious adverse events occurred less frequently in the study [4].

ITK-1 is a peptide set consisting of 14 kinds of peptide discovered as a HLA class I epitope, which being developed by Green Peptide Co., Ltd. All the 14 peptide candidates can induce CTLs, and each of them can induce HLA-A24-restricted and tumor-specific CTL activity in peripheral blood mononuclear cells (PBMCs) of cancer patients [14–18]. We have conducted a phase I study on PPV and low-dose EMP in HLA-A24-positive patients with CRPC in order to define the safety, tolerability, and immune and prostate-specific antigen (PSA) responses of this drug combination.

PATIENTS AND METHODS

Patients

This was a multi-center study and approved by each institutional review board (IRB) that evaluated it from the viewpoint of the science and ethics in all four hospitals in Japan before the initiation of the study. Patients who had a histological diagnosis of prostate adenocarcinoma (PC) and progressive disease (PD) by diagnostic imaging (computerized tomography; CT, magnetic resonance imaging; MRI or bone scintigraphy) or PSA after both androgen deprivation therapy either by castration or with luteinizing hormone-releasing hormone (LHRH) agonists and anti-androgen therapy, as well as oral EMP treatment were eligible. PSA progression was defined as at least three consecutive rises in serum PSA taken over 2 weeks apart, in the setting of castration levels of testosterone. Patients were required a washout period of at least 4 weeks before the first vaccination after the completion of prior hormone therapy, hormone-chemotherapy, chemotherapy, or immune therapy. Anti-androgen therapy was discontinued for at least 4 weeks before the first vaccination for patients receiving flutamide and 6 weeks for those receiving bicalutamide. All patients had an Eastern Cooperative Oncology Group performance status of 0 or 1, HLA-A24-positive type, and serum testosterone level $\leq$50 ng/dl, and were maintained on LHRH agonist therapy or castration. Adequate organ functions were required and were defined as white blood cell count $\geq$3,000/mm^3, lymphocyte count $\geq$1,200/mm^3, hemoglobin $\geq$9 g/dl, platelets $\geq$100,000/mm^3, total bilirubin $\leq$1.5 mg/dl, AST and ALT $\leq$2$\times$ (upper normal limit), and serum creatinine $\leq$1.4 mg/dl. Patients with comorbidities including serious cardiovascular, hepatic, nephritic, and hematological diseases $\geq$grade 3 of Common Terminology Criteria for Adverse Events (CTCAE), serious gastric ulcers, and infectious diseases with antibiotic treatment, were excluded. Radiation therapy or immunosuppressive treatment using a systematic steroid within the last 1 year was not permitted. All patients gave written informed consent approved by each IRB.

Study Design

This was a phase I open-labeled dose-escalation study. After a pre-vaccination measurement of peptide-specific IgG in the plasma of patients reactive to 14 kinds of vaccine candidate peptides (ITK-1) with the ability to induce CTLs, patients were treated with 6 weekly subcutaneous administration of the top four peptides showing the strongest antibody responses at three different dose settings (1, 3, and 5 mg/peptide), with daily oral EMP 313.4 mg in the phase I study. This was followed by a maximum of 2 years in an extension study of six PPVs either weekly or bi-weekly as one course. All patients were treated at the hospital during the first 1 week followed by outpatient clinic visits. ITK-1 consists of 14 kinds of peptides: $SART2_{93-101}$, $SART3_{109-118}$, $Lck_{208-216}$, $PAP_{213-221}$, $PSA_{248-257}$, $EGFR_{800-809}$, $MRP3_{503-511}$, $MRP3_{1293-1302}$, $SART2_{161-169}$,

$Lck_{486-494}$, $Lck_{488-497}$, $PSMA_{624-632}$, $EZH2_{735-743}$, and $PTHrP_{102-111}$. All peptides were prepared under Good Manufacturing Practice (GMP) compliance by American Peptide Company (San Diego, CA) and by PolyPeptide Laboratories (San Diego, CA), and were supplied in lyophilized vials; 4 mg, including inactive ingredients, under GMP compliance. Selected peptides were dissolved in 1 ml distilled water and emulsified with 1 ml of incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France), under GMP compliance. Each of four peptides in 0.5 ml emulsion at a dose level of 1 mg/peptide (4 mg/2 ml), 1.5 ml emulsion at a dose level of 3 mg/peptide, and 2.5 mL emulsion at a dose level of 5 mg/peptide were injected subcutaneously into the thigh, the hip or the lower part of trunk area. Each peptide was independently injected nearby. EMP was administered orally as a 156.7 mg capsule, one capsule twice daily, for a total daily dose of 313.4 mg, half of the standard dose of EMP (626.8 mg/day) to avoid immunosuppression as reported in our previous study [19]. From the starting dose of 1 mg/peptide, subsequent dose levels were increased after the evaluation of the safety data by the Data and Safety Monitoring Committee (DSMC) according to the dose escalation design of the protocol. The initial cohort included six patients. If the DSMC recommended proceeding to the next level as a result of the safety evaluation of the prior level, new six patients were enrolled. The highest dose level enrolled three patients at first and was evaluated the safety data by the DSMC to include additional three patients. The maximum acceptable dose (MAD) was defined as the lowest dose level at which at least two-thirds of patients experienced grade 2 or greater injection site reactions after the sixth treatment. The maximum tolerated dose (MTD) was defined as the lowest dose level at which more than one-third of patients experienced grade 3 or greater systemic adverse events caused by ITK-1 after the sixth treatment. Adverse events were graded according to the CTCAE version 3.0 and were coded using MedDRA/J (Medical Dictionary for Regulatory Activities Terminology/Japanese) version 12.0. Patients who experienced no significant (≥CTCAE grade3) adverse events and no disease progression, and signed informed consent were eligible to extend treatment until disease progression or unacceptable adverse events occurred, or the patient met other withdrawal criteria.

Pretreatment and Follow-Up Studies

A complete history, physical examination, and routine laboratory studies, including complete blood counts, biochemical tests, ECG, relevant radiologic studies, PSA, and urinalysis were performed before treatment and repeated after every six injections.

Immune Responses

For evaluation of immune responses, peptide-specific CTL precursors in PBMCs and peptide-specific IgG levels in plasma were measured as described previously [13]. Also, peptide-specific IgG levels were measured using patient's plasma of the screening examination to select the best peptides. Briefly, 30 ml of peripheral blood samples were obtained from each patient to measure peptide specific CTL and IgG prior to vaccination, at the fourth and after the sixth vaccinations, and after every sixth vaccination in the extension study, and then the PBMCs and plasma were isolated by Ficoll–Conray density gradient centrifugation. We reported that the IgG specific to each peptide measured by Luminex system as the fluorescence intensity unit (FIU) could frequently be detected in pre- and post-vaccination plasma, and the level of peptide-specific IgG is a laboratory marker that predicts clinical responses to the PPV with a good relationship to overall survival [13,20]. Therefore, peptides were chosen on the basis of evaluation of peptide-specific IgG levels in plasma. Peptide-specific CTL precursors in PBMCs were detected using a previously reported culture method [21]. Briefly, PBMCs (1×10^5 cells/well) were incubated with 10 μM of each peptide in U-bottom-type 96-well microculture plates (Nunc, Roskilde, Denmark) in 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640 medium, 45% AIM-V® medium (Invitrogen Corp., Carlsbad, CA), 10% FCS, 20 U/ml of interleukin-2 (IL-2), and 0.1 mM MEM nonessential amino acid solution (Invitrogen Corp.), 36 mg/L gentamicin sulfate (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Half of the medium was removed and replaced with new medium containing a corresponding peptide (20 μM) every 3 days for up to 12 days. On the 12th day of the culture, 24 hr after the last stimulation, these cells were harvested, washed three times, and then tested for their ability to produce IFN-γ in response to C1R-A2402 cells preloaded with either a corresponding peptide or HIV peptide (RYLRQQLLGI) as a negative control in HLA-A24. The target cells (C1R-A2402, 1×10^4/well) were pulsed with each peptide (10 μM) for 2 hr, and then effector cells (1×10^5/well) were added to each well with a final volume of 200 μl. After incubation for 18 hr, the supernatants (100 μl) were collected, and the amounts of IFN-γ were measured using an ELISA (limit of sensitivity: 10 pg/ml). All experiments were performed in quadruplicate assay.

Definition of Treatment Outcomes

Outcomes were assessed by post-therapy changes in serum PSA and immune responses. A post-therapy

TABLE I. Baseline Demographics

Characteristics	No. of patients (%)
No. of patients	15
Age, years	
Median	73
Range	63–78
ECOG PS	
0	14 (93)
1	1 (7)
Gleason score	
7	3 (20)
8	5 (33)
9	4 (27)
10	1 (7)
Unknown	2 (13)
PSA (ng/mL)	
Median	39.6
Range	0.2–354.4
Site(s) of metastasis	
None	4 (27)
Lymph node	2 (13)
Bone	6 (40)
Lymph node + bone	1 (7)
Other	2 (13)
Local therapy	
Prostatectomy	4 (27)
EBRT	3 (20)
No definitive local therapy	8 (53)
Hormone therapy	
Primary therapy only	1 (7)
≥2 therapies	14 (93)
Chemotherapy	
EMP	15 (100)
Other	2 (13)

ECOG PS, Eastern Cooperative Oncology Group performance status; PSA, prostate-specific antigen; EBRT, external-beam radiation therapy; EMP, estramustine phosphate.

decrease of PSA to a normal range was defined as a complete response (CR) and a decrease in PSA of ≥50% from baseline was defined as a partial response (PR) in the phase I study. Also, a post-therapy PSA decrease of <50% or an increase >25% from baseline were interpreted as no change (NC) [22] and PSA above 125% of the baseline PSA value was defined as PD. Positive immune responses were defined as post-IgG levels/pre-IgG levels ≥3, post-IFN-γ levels/pre- IFN-γ levels ≥3, respectively. All patients were followed up every 3 months for life. Data, except the survival data, were analyzed by November 2009 using SAS (Statistical Analysis System) software version 9.1.3. The Student's *t*-test and the chi-square test were used to compare quantitative and categorical variables, respectively. Overall survival was calculated from the study registration date to the date of the last follow-up or the death from any cause. The Kaplan–Meier method was used to estimate product-limit estimate curves with the survival data obtained in March 2010. Tests results were considered significant at a two-sided significance level of 5%. The analysis was performed by intent to treat.

RESULTS

Patient Characteristics

Fifteen patients were recruited to the study between April 2006 and September 2007. Patient characteristics are listed in Table I. All patients were HLA-A24-positive, and had hormone and EMP refractory prostate cancer. In addition, all 15 patients were evaluated for the safety and the efficacy of the PPV treatment.

Dose Escalation

The dose-escalation scheme is presented in Table II. Maximum dose escalation preplanned for each peptide of 5 mg/2.5 mL (4 peptides, 20 mg/10 mL) was achieved. There were no treatment-related grade 3 or 4 adverse events or deaths in this study. Grade 2 injection site reactions were observed in two of six patients in the first dose level of 1 mg/peptide, and five of six patients in the second dose level of 3 mg/peptide after the sixth treatment. At the 5 mg/peptide dose

TABLE II. The Results of Dose-Escalation in Phase I Study

Peptides dose level (mg/peptide)	No. of patients		No. of patients	
	Enroll	Discontinued or skipped[a]	MAD (≥grade 2 injection site reaction)	MTD (≥grade 3 systemic treatment-related AE)
1	6	0/6	2/6	0/6
3	6	0/6	5/6	0/6
5	3	3/3	3/3	0/3
Total	15	3/15	10/15	0/15

MAD, maximum acceptable dose; MTD, maximum tolerated dose; AE, adverse event.
[a]Patients were discontinued or skipped the treatment because both widespread grade 2 injection site reactions and patients' own requests.

level, three patients were treated, but the vaccination was skipped or discontinued in all three patients considering the ethical viewpoint because of patients' own requests and physical burden, caused by widespread grade 2 injection site reactions. After these treatment-related adverse events, two of three 5 mg/peptide dose level patients were entered in the extension study and then the dose level was reduced to 3 mg/peptide during treatment. The DSMC reviewed the results and recommended stopping the additional three enrollments for the dose level of 5 mg/peptide. Subsequently, the MAD for PPV was calculated to be 8.643 mg/4 peptide (2.161 mg/peptide) based on the logistic regression model.

Adverse Events

There were no treatment-related serious adverse events and no grade 3 or greater adverse events in the phase I study. In contrast, a grade 3 injection site reaction and a grade 3 pyrexia occurred in one patient each during the extension study. All treatment-related adverse events observed in whole study (phase I and extension study) are listed in Table III. The primary nonhematologic treatment-related adverse events were injection site reaction (93.3%), malaise (33.3%), edema peripheral (33.3%), and fatigue (20.0%). These adverse events were manageable with routine intervention. Hematologic adverse events were, grade 1 white blood cell count increased and grade 1–2 lymphocyte count decreased occurred in 4 of 15 (26.7%) and 3 of 15 (20.0%) patients, respectively. One patient at a dose level of 5 mg/peptide had a grade 1 blood fibrinogen increased, and another patient at a dose level of 3 mg/peptide had grade 1 blood triglycerides increased during the first course, and these changes returned to normal levels on the next course.

Immune Response

The best peptides for each patient were selected based on peptide-specific IgG levels for each peptide at the screening examination (data not shown). The results of the immune response in the first course are given in Table IV. After the sixth vaccination, IgG responses were increased in one of six patients with 1 mg/peptide, four of six patients with 3 mg/peptide, and two of three patients with 5 mg/peptide tested. CTL responses measured by IFN-γ release assay were increased in four of six patients with 1 mg/peptide, six of six patients with 3 mg/peptide, and zero of three patients with 5 mg/peptide tested.

Clinical Response

PSA response after the sixth vaccination was CR in one patient (6.7%) receiving 3 mg/peptide, PR in one patient (6.7%) receiving 1 mg/peptide, and PD in two patients (13.3%) receiving 5 mg/peptide. At the time of data analysis, nine patients had died and all deaths were attributed to prostate cancer or metastases. The median follow-up time for all patients was 23.8 months, ranging from 3.0 to 38.3 months. None of the patients was lost to follow-up during this analysis. The median overall survival was 23.8 months for all 15 patients (95% CI, lower limit was 15.6 months, upper limit was not estimated; Fig. 1).

DISCUSSION

We performed a multicenter, open-label, phase I trial to evaluate the safety, tolerability, immune response, and PSA response of a combination of escalating doses of PPV and low-dose EMP. All patients had hormone and EMP-refractory prostate cancer. The treatment regime was well tolerated at all dose levels, except the injection site reaction at the highest dose level of 5 mg/peptide observed in all three patients enrolled, and no MTD was established in this trial. The most common adverse event was injection site reaction. The concept of dose escalation in a phase I trial to identify an MTD may not be applicable to most therapeutic cancer vaccines [23]. Peptide vaccines based on non-mutated melanoma antigens such as MART-1/Melan A and gp100 were initially evaluated in a phase I setting, at doses ranging from 0.1 to 10 mg [24,25]. However, no toxicity was observed even at the highest doses, and in vitro analysis did not reveal any correlation between the peptide dose and the generation of specific T-cell reactivity from the PBMCs of the vaccinated patients. Neither the safety nor efficacy of the vaccine can be assessed in patients with a blunted immune response since both safety and efficacy depend on the immune response. In contrast, our initial trial for colorectal cancer patients with 0.3, 1, and 3 mg/injections of SART3 peptide showed that a dose of 3 mg/injection was better than that of 0.3 and 1 mg/injection based on the induction of cellular immune responses to both tumor cells and peptides [26]. The current phase I study also showed that a dose of 3 mg/injection was better than those of 1 and 5 mg/injection based on the induction of cellular immune responses to peptides, although total doses of four peptides were 4 mg/2 mL, 12 mg/6 mL, and 20 mg/10 mL. Under these conditions, there were no serious adverse events caused by ITK-1; however, grade 2 injection site reactions were observed in two of six patients receiving 1 mg/0.5 mL/peptide, five of six patients receiving 3 mg/1.5 mL/peptide, and three of three patients receiving 5 mg/2.5 mL/peptide in the phase I study. The vaccination was skipped or discontinued in three of three patients receiving 5 mg/2.5 mL/peptide

TABLE III. Treatment-Related Adverse Events for Castration-Resistant Prostate Cancer

	No. of patients experienced treatment-related adverse events during phase I study/whole study[a] by grade									Total (15 patients)	
	1 mg/peptide group (6 patients)			3 mg/peptide group (6 patients)			5 mg/peptide group (3 patients)			All grade	
MedDRA/J ver12.0 symptom: preferred Trem(PT)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	P I	Whole
Vomiting	1/1									1 (6.7%)	1 (6.7%)
Ventricular extrasystoles	0/1										1 (6.7%)
Fatigue	0/1	0/1		1/0	0/1					1 (6.7%)	3 (20.0%)
Injection site reaction	2/2	2/3		1/1	5/4	0/1		3/3		13 (86.7%)	14 (93.3%)
Malaise	1/2			0/1	0/1		0/1			1 (6.7%)	5 (33.3%)
Oedema peripheral	1/2	0/1			0/1		0/1			1 (6.7%)	5 (33.3%)
Pyrexia						0/1					1 (6.7%)
Aspartate aminotransferase increased	0/1										1 (6.7%)
Blood fibrinogen increased							1/1			1 (6.7%)	1 (6.7%)
Blood triglycerides increased				1/1						1 (6.7%)	1 (6.7%)
Crystal urine present	0/1										1 (6.7%)
Blood urine present				0/1							1 (6.7%)
Lymphocyte count decreased	1/1	1/1			1/1					3 (20.0%)	3 (20.0%)
Neutrophil count increased	0/1										1 (6.7%)
Urinary casts	0/1										1 (6.7%)
White blood cell count increased	0/1			1/2			1/1			2 (13.3%)	4 (26.7%)
White blood cells urine positive	0/1			0/1							2 (13.3%)
Bacteria urine identified				0/1							1 (6.7%)
Dizziness				0/1							1 (6.7%)
Dizziness postural				0/1							1 (6.7%)
Headache				1/0	0/1					1 (6.7%)	1 (6.7%)
Insomnia		0/1									1 (6.7%)
Cough	0/1										1 (6.7%)
Rash generalized					0/1						1 (6.7%)

[a]Whole study means phase I and extension study.

TABLE IV. Immunorogical Responses During the Personalized Peptide Vaccination

Dose of peptide	Pts No.	Peptide	Anti-peptide IgG response (FIU)[a]				Anti-peptide cellular response (pg/ml)[b]			
			Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)	Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)
1 mg	1	Lck-486	94	90	81	—	ND	ND	ND	—
		PSMA-624	<5	<5	<5	—	ND	ND	ND	—
		PTHrP-102	42	30	23	—	113	ND	ND	—
		SART3-109	31	24	21	—	ND	ND	ND	—
	2	Lck-486	310	206	976	Positive	667	ND	204	—
		MRP3-1293	38	21	28	—	ND	ND	186	Positive
		SART2-93	20	11	9	—	ND	ND	656	Positive
		SART3-109	27	13	18	—	899	ND	ND	—
	3	Lck-486	102	102	114	—	ND	78	ND	—
		Lck-488	45	46	52	—	462	ND	ND	—
		MRP3-1293	52	45	50	—	ND	ND	ND	—
		PAP-213	252	210	215	—	ND	ND	ND	—
	4	Lck-486	200	199	247	—	ND	ND	1,393	Positive
		Lck-488	<5	<5	<5	—	ND	ND	472	Positive
		PSA-248	117	99	109	—	ND	ND	ND	—
		PTHrP-102	171	138	142	—	564	ND	ND	—
	5	Lck-486	575	364	396	—	ND	117	57	—
		Lck-488	144	102	92	—	ND	ND	439	Positive
		MRP3-1293	91	64	51	—	133	160	ND	—
		PAP-213	90	70	77	—	3,764	ND	114	—
	6	MRP3-1293	779	586	411	—	ND	477	ND	—
		PSA-248	804	756	1,825	—	ND	ND	ND	—
		PTHrP-102	502	414	310	—	ND	93	753	Positive
		SART3-109	142	152	83	—	ND	ND	3,276	Positive
3 mg	7	Lck-486	202	216	9,028	Positive	ND	1,636	ND	—
		MRP3-1293	29	21	22	—	ND	ND	ND	—
		PAP-213	<5	<5	5	—	274	ND	1,494	Positive
		PSA-248	11	12	1,902	Positive	173	ND	ND	—
	8	Lck-486	298	261	287	—	2,543	ND	ND	—
		Lck-488	10	9	11	—	ND	ND	598	Positive
		MRP3-1293	23	21	23	—	ND	ND	ND	—
		PAP-213	8	5	9	—	ND	ND	2,613	Positive
	9	Lck-486	329	290	308	—	ND	ND	72	—
		Lck-488	128	103	106	—	ND	119	627	Positive
		MRP3-1293	53	36	40	—	ND	1,706	ND	—
		PAP-213	<5	<5	10,992	Positive	ND	683	ND	—

(*Continued*)

TABLE IV. (*Continued*)

Dose of peptide	Pts No.	Peptide	Anti-peptide IgG response (FIU)[a] Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)	Anti-peptide cellular response (pg/ml)[b] Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)
	10	Lck-486	826	1,632	16,376	Positive	127	ND	7,014	Positive
		Lck-488	21	22	48	—	117	227	115	—
		MRP3-1,293	21	22	24	—	ND	109	ND	—
		PAP-213	15	15	60	Positive	189	ND	285	—
	11	Lck-208	19	18	21	—	211	54	ND	—
		Lck-486	434	349	105	—	ND	ND	ND	—
		Lck-488	12	12	12	—	ND	ND	5,258	Positive
		PTHrP-102	102	99	135	—	ND	2,991	2,934	Positive
	12	Lck-486	392	549	348	—	ND	ND	1,136	Positive
		Lck-488	87	96	64	—	ND	ND	ND	—
		PSA-248	157	2,653	18,163	Positive	ND	ND	ND	—
		SART3-109	76	87	58	—	ND	ND	794	Positive
5 mg	13	Lck-486	183	231	861	Positive	184	103	104	—
		PAP-213	39	35	8,490	Positive	232	ND	ND	—
		SART2-93	56	49	51	—	59	215	ND	—
		SART3-109	31	31	38	—	391	ND	165	—
	14	Lck-486	162	120	2,950	Positive	185	348	126	—
		MRP3-1293	29	27	149	Positive	97	104	ND	—
		SART2-161	16	17	27	—	178	200	263	—
		SART3-109	23	20	108	Positive	1,285	117	1,024	—
	15	Lck-486	809	837	916	—	1,339	ND	ND	—
		MRP3-1293	710	543	550	—	251	ND	ND	—
		SART2-161	72	46	57	—	ND	ND	55	—
		SART3-109	311	248	236	—	100	ND	110	—

[a]Values indicate fluorescence intensity unit (FIU) of IgG antibodies reactive to each peptide.
[b]Values indicate the mean of specific interferon-γ production in positive wells reactive to each peptide.

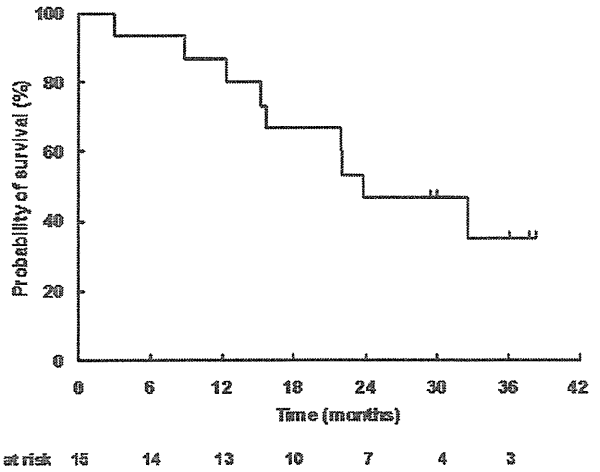

Fig. 1. Kaplan–Meier estimates of overall survival for 15 patients treated by personalized peptide vaccination with low-dose estramustine. Median overall survival is 23.8 months.

because of both widespread grade 2 skin reactions and patients' own requests. Subsequently, we calculated MAD as 8.643 mg/4 peptides in this study. Therefore, considering the adverse events, tolerability, and immune responses, the 3 mg/1.5 mL/peptide dose of PPV will be recommended for further clinical trials.

In the present study, CTL responses measured by IFN-γ release assay and IgG responses were enhanced in 10/15 (66.7%) and 7/15 (46.7%) of the examined patients, respectively, and in the PSA response, CR and PR was one patient each (6.7%) and PD was two patients (13.3%) after the sixth vaccination. In addition, the long-term (23.8 months) median survival time after combination therapy with PPV and low-dose EMP observed in the extension study indicated that this treatment suppresses tumor growth. However, the exact mechanism of this interaction is unclear and further studies are needed.

In conclusion, the results of safety, immune responses, and improved overall survival without MTD, as well as the consistency between these results and the data from our previous trials [4,19,27], could lead to us to the next phase of randomized clinical trial wherein we can confirm the survival benefit of such personalized immunotherapy in HLA-A24 positive patients with CRPC.

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