ORIGINAL ARTICLE

Safety and Immunogenicity of the PRAME Cancer Immunotherapeutic in Patients with Resected Non–Small Cell Lung Cancer: A Phase I Dose Escalation Study

Jean-Louis Pujol, MD,[a,*] Tommaso De Pas, MD,[b] Achim Rittmeyer, MD, PhD,[c] Eric Vallières, MD,[d] Bartosz Kubisa, MD, PhD,[e] Eugeny Levchenko, MD,[f] Sebastian Wiesemann, MD,[g] Gregory A. Masters, MD,[h] Robert Shen, MD,[i] Sergei A. Tjulandin, MD, PhD,[j] Hans-Stefan Hofmann, MD,[k] Nicolas Vanhoutte, PhD,[l] Bruno Salaun, PhD,[l] Muriel Debois, SSc,[l] Silvija Jarnjak, MD, PhD,[l] Pedro Miguel De Sousa Alves, PhD,[l,m] Jamila Louahed, PhD,[l] Vincent G. Brichard, MD, PhD,[l,n] Frédéric F. Lehmann, MD, PhD[l,m]

[a]*Arnaud de Villeneuve Hospital, Respiratory Diseases, Thoracic Oncology Unit, Montpellier, France*
[b]*Medical Oncology Unit of Respiratory Tract and Sarcomas, Thoracic Oncology Division, European Institute of Oncology, Milan, Italy*
[c]*Immenhausen Lung Clinic, Department of Thoracic Oncology, Immenhausen, Germany*
[d]*Swedish Cancer Institute, Division of Thoracic Surgery, Seattle, Washington*
[e]*Pomeranian Medical University of Szczecin, Thoracic Surgery and Transplantation Department, Szczecin-Zdunowo, Poland*
[f]*N.N. Petrov Institute of Oncology, Department of Tumor Biology, St. Petersburg, Russia*
[g]*Freiburg University Clinic, Department of Thoracic Surgery, Freiburg, Germany*
[h]*Christiana Care Health Services, Helen F Graham Cancer Center, Newark, Delaware*
[i]*Mayo Clinic College of Medicine, Department of Surgery, Division of General Thoracic Surgery, Rochester, Minnesota*
[j]*Russian Cancer Research Center, Department of Clinical Pharmacology and Chemotherapy, Moscow, Russian Federation*
[k]*Merciful Brothers Hospital, Department of Thoracic Surgery, Regensburg, Germany*
[l]*GSK Vaccines, Rixensart, Belgium*
[m]*Celyad, Mont-Saint-Guibert, Belgium*
[n]*Vionova-Biosciences, Lasne, Belgium*

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ABSTRACT

Introduction: Adjuvant platinum-based chemotherapy is standard treatment for surgically resected stage II to IIIA NSCLC, but the relapse rate is high. The preferentially expressed antigen of melanoma (PRAME) tumor antigen is expressed in two-thirds of NSCLC and offers an attractive target for antigen-specific immunization. A phase I dose escalation study assessed the safety and immunogenicity of a PRAME immunotherapeutic consisting of recombinant PRAME plus proprietary immunostimulant AS15 in patients with surgically resected NSCLC (NCT01159964).

Methods: Patients with PRAME-positive resected stage IB to IIIA NSCLC were enrolled in three consecutive cohorts to receive up to 13 injections of PRAME immunotherapeutic (recombinant PRAME protein dose of 20 μg, 100 μg, or 500 μg, with a fixed dose of AS15). Adverse events, predefined dose-limiting toxicity, and the anti-PRAME humoral response (measured by enzyme-linked immunosorbent assay) were

*Corresponding author.

Prof. Pujol and Dr. De Pas equally contributed to this work.

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Address for correspondence: Jean-Louis Pujol, Hôpital Arnaud de Villeneuve, Maladies Respiratoires-Unité Oncologie Thoracique, Avenue du Doyen Giraud, 34295 Montpellier, France. E-mail: jl-pujol@chu-montpellier.fr

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coprimary end points. Anti-PRAME cellular responses were assessed.

Results: A total of 60 patients were treated (18 received 20 μg of PRAME, 18 received 100 μg of PRAME, and 24 received 500 μg of PRAME). No dose-limiting toxicity was reported. Adverse events considered by the investigator to be causally related to treatment were grade 1 or 2, and most were injection site reactions or fever. All patients had detectable anti-PRAME antibodies after four immunizations. The percentages of patients with PRAME-specific CD4-positive T cells were higher at the dose of 500 μg compared with lower doses. No predefined CD8-positive T-cell responses were detected.

Conclusion: The PRAME immunotherapeutic had an acceptable safety profile. All patients had anti-PRAME humoral responses that were not dose related, and 80% of those treated at the highest dose showed a cellular immune response. The dose of 500 μg was selected. However, further development was stopped after negative results with a similar immunotherapeutic in patients with NSCLC.

Keywords: Adjuvant setting; NSCLC; Immunotherapy; PRAME antigen; Safety

Introduction

Lung cancer is the leading cause of cancer deaths worldwide, with most cases being of the NSCLC histological tumor type.[1] Complete surgical resection of early-stage NSCLC offers the best chance of cure, but the 5-year relative survival rates are 58.2% and 29.8% for patients with localized and regional disease, respectively.[2] Adjuvant platinum-based chemotherapy is now a standard treatment option for surgically resected stage II to IIIA disease.[3,4] The moderate benefits of adjuvant chemotherapy in NSCLC suggest that alternative strategies to improve the outcome of these patients are needed.[5–8]

Cancer immunotherapy strategies aim to induce or boost immune-mediated tumor cell destruction (active immunotherapy) or to counteract the mechanisms by which tumor cells evade or suppress immune-mediated destruction (passive immunotherapy).[9–13] Several strategies, including immune-checkpoint inhibitors[14–16] and active immunotherapies,[17–22] have entered late-stage clinical development. Immune checkpoint inhibitors include the anti–cytotoxic T-lymphocyte associated protein 4, anti–programmed cell death 1, and anti–programmed death ligand 1 compounds that target tumor-mediated inhibition of cytotoxic T-cell activity. Preliminary evidence suggests promising activity with manageable toxicity when these compounds are administered as monotherapies or in combination with chemotherapy or targeted agents.[23,24] Currently, there are no immunotherapeutic agents approved for use as adjuvant therapy for surgically resected NSCLC.[3] The human tumor antigen preferentially expressed antigen of melanoma (PRAME) was originally identified by using a cytolytic T-lymphocyte clone derived from a patient with melanoma, and its messenger RNA is expressed in low levels in normal ovary, endometrium, kidney, and adrenal medulla.[25,26] PRAME has been identified as a BC-box subunit of cullin 2–based E3 ubiquitin ligase with interactions with nuclear transcription factor Y.[27,28] Functionally, PRAME may be associated with suppression of retinoic acid receptor signaling and cell death regulation,[29] and it may be directly involved in oncogenesis.[29,30] Expression of PRAME has been documented in a variety of cancers, including NSCLC (adenocarcinoma [46%] and squamous cell carcinoma [78%]),[25] metastatic melanoma (95%),[25] breast carcinomas (27%),[25,31] and neuroblastoma (>90%).[32] For some solid tumors such as neuroblastoma and breast cancer, PRAME expression has been linked to an unfavorable prognosis.[32–35]

We investigated the safety and immunogenicity of escalating doses of a recombinant PRAME protein (recPRAME, GSK, Rixensart, Belgium) administered with a fixed dose of the proprietary immunostimulant AS15 (referred to as the PRAME immunotherapeutic) in patients with PRAME-positive pathological stage IB to IIIA NSCLC after complete surgical resection. Here we report safety and immunogenicity data measured 3 weeks after the fourth administration of the PRAME immunotherapeutic that led to dose selection according to predefined rules.

Methods

The open-label, phase I study was conducted in 22 centers in Germany, France, Italy, Poland, the Russian Federation, and the United States (ClinicalTrials.gov identifier NCT01159964). The study protocol was approved by institutional review boards at each participating center. Written informed consent was obtained from each patient before the performance of any study-specific procedures.

Coprimary objectives were to document and characterize for each dose of the PRAME immunotherapeutic tested the potential dose-limiting toxicities (DLTs) and the anti-PRAME humoral immune response. Secondary objectives included evaluation of the overall safety profile and cell-mediated antigen-specific immune (CMI) responses.

Patients

Patients were 18 years of age or older with pathological stage IB to IIIA NSCLC after complete (R0) surgical

resection. The resection had to be at least a lobectomy. Patients were allowed to receive adjuvant platinum-based chemotherapy before enrollment. The tumor had to be PRAME-positive as determined on the basis of formalin-fixed paraffin-embedded tissue samples by quantitative reverse-transcriptase polymerase chain reaction at a central laboratory (Response Genetics, Inc., Los Angeles, CA).[36] Protocol-defined inclusion and exclusion criteria are provided in the Supplementary Methods.

Treatment Regimen

The composition of the PRAME immunotherapeutic (recPRAME plus AS15) is provided in the Supplementary Methods. The PRAME immunotherapeutic was administered intramuscularly into the deltoid muscle or thigh, alternating sides for each dose.

Escalating doses of recPRAME (20 μg, 100 μg, and 500 μg) combined with a fixed dose of AS15 were evaluated in three consecutive cohorts. A maximum of 13 injections of the PRAME immunotherapeutic were to be administered according to the following schedule: the first five injections were given at 3-week intervals followed by eight injections at 12-week intervals. Patients were actively followed for an additional year for safety and clinical outcomes. This regimen has been used for the investigation of other immunotherapeutics, including the melanoma-associated antigen A3 (MAGE-A3) immunotherapeutic in patients with NSCLC.[37]

Escalation to each dose level occurred when all 15 planned patients had initiated treatment with the lower dose and when at least three patients had received at least four injections. Dose escalation procedures are provided in the Supplementary Methods.

Assessment of Safety

All adverse events (AEs) occurring throughout the study until 30 days after the last administration of the study product were recorded. Serious adverse events (SAEs) related to study treatment, DLTs, onset of autoimmune disease, and pregnancy outcomes were recorded for 1 year after administration of the last study treatment. Disease recurrences and deaths due to disease were not considered SAEs. AE severity was graded according to the Common Terminology Criteria for Adverse Events, version 4.0.[38] AEs were coded to the preferred term level using the Medical Dictionary for Regulatory Activities.[39]

A DLT was defined as any of the following AEs considered related or possibly related to administration of the PRAME immunotherapeutic: (1) a grade 3 or higher AE (myalgia, arthralgia, headache, fever, rigors/chills, or fatigue persisting had to have persisted for 48 hours despite therapy to be considered a DLT), (2) a grade 2 or higher allergic reaction occurring within 24 hours after injection of PRAME immunotherapeutic, (3) any decrease in renal function with a creatinine clearance less than 40 mL/min, and (4) any symptomatic and confirmed adrenal insufficiency. Renal and adrenal AEs were included because PRAME is expressed at low levels in normal kidney and adrenal tissues.

At each visit, blood and urine samples were collected for evaluation of a variety of routine hematologic, biochemical, and coagulation parameters. Of note, measurement of serum cortisol level and renal function tests and urinalysis were performed at each visit, and an antinuclear antibody test was performed at every second visit.

A data and safety monitoring committee and internal safety review team reviewed the safety data, the clinical relevance of any DLT event, and its relationship to the study treatment.

Immunogenicity

Humoral Immunity. Anti-PRAME antibody concentrations were measured before administration of the first dose, 3 weeks after dose 2, and 3 weeks after dose 4.

Anti-PRAME immunoglobulin G (IgG) antibodies were measured by enzyme-linked immunosorbent assay as described in the Supplementary Methods. A humoral immune response was defined as a postimmunization anti-PRAME antibody concentration equal to or above the clinical cutoff value (12 EU/mL, defined from 102 healthy donors) in initially seronegative patients (seroconversion) and a twofold or greater increase in postimmunization anti-PRAME antibody concentrations in initially seropositive patients.

Cell-Mediated Immunity. CMI response was measured before the first dose and 3 weeks after dose 4, as described in the Supplementary Methods. Briefly, the presence and functionality of the PRAME-reactive T-cell response was assessed by stimulation of peripheral blood mononuclear cells in an in vitro multiple-well assay.

PRAME T-cell immunogenicity (characterized by detection and quantification of T cells producing both interferon-γ [IFN-γ] and tumor necrosis factor-α [TNF-α] in an in vitro assay) cutoff scores for a positive response were defined from a panel of healthy donors ($n = 23$, cutoff of 2.68 for CD4-positive T-cell analysis and 1.15 for CD8-positive T-cell analysis). A patient was considered a CD4-positive or CD8-positive T-cell responder if the ratio of immunogenicity scores between a positive postimmunization sample and its corresponding baseline was 4 or greater. Frequencies of PRAME-specific T cells were estimated on the basis of quantification of defined positive wells in the in vitro assay. Frequency cutoffs were defined from healthy donors as 6.32×10^{-6} for CD4 positivity and 1.9×10^{-6} for CD8 positivity.

Dose Selection Criteria

The dose was selected on the basis of safety and immunogenicity data. A specific dose was considered adequate if no more than two cases of DLT were reported at any time among the 15 patients in each cohort and if the dose showed anti-PRAME antibody responses of 70% or more (≥11 of 15 patients) after four immunizations. If more than one dose level satisfied both the safety and humoral immune response criteria, selection of the dose would also take into account CMI responses. If the best immunological dose could not be determined by applying these criteria, the highest dose with acceptable safety and immunogenicity was to be selected.

Statistical Analysis

All statistical analyses were performed using SAS software version 9.2 (SAS, Inc., Cary, NC). The study was descriptive and no comparative tests were performed. The total treated cohort included all patients enrolled into the study who had received at least one injection of PRAME immunotherapeutic. The according-to-protocol cohort for immunogenicity included all patients who met the eligibility criteria, complied with the protocol-defined procedures, had received at least the first four PRAME immunotherapeutic doses, and had completed the study visit 3 weeks after the fourth immunization. Geometric mean antibody concentrations were calculated for anti-PRAME IgG antibodies.

Results

From July 12, 2010, to October 17, 2011, 357 patients were screened. Of the 342 patients with a valid test result, 198 (57.9%) had a *PRAME*-positive tumor and 60 were enrolled into the study (Fig. 1). The data lock point (DLP) for dose selection was on 28 February 2012. The overall mean age of patients in the total treated population was 62.8 years. Squamous cell carcinoma and adenocarcinoma were the most frequent histological tumor types in each cohort (Table 1). Approximately 40% of patients in each cohort had received prior platinum-based adjuvant chemotherapy.

At the time of the DLP, 365 doses of PRAME immunotherapeutic were administered across the three

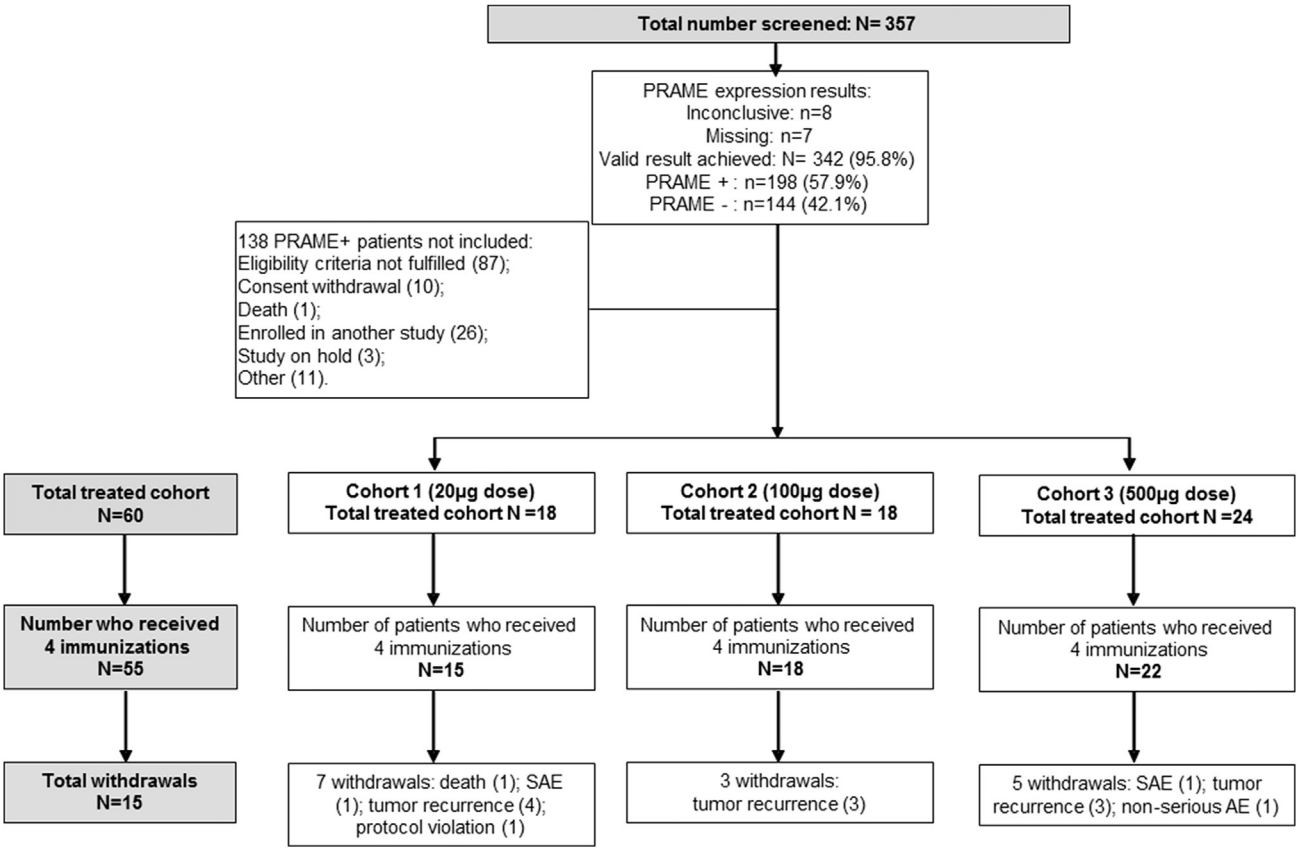

Figure 1. Patient flow through the study until 3 weeks after the fourth immunization. *Missing* means no tumor sample received by the laboratory. *Inconclusive* means invalid test result or quantity not sufficient for testing. Numbers of patients who completed the study until 3 weeks after the fourth immunization: 15 in cohort 1, 17 in cohort 2, and 22 in cohort 3. PRAME, preferentially expressed antigen of melanoma; SAE, serious adverse event; AE, adverse event.

Table 1. Demographic and Disease Characteristics (Total Treated Cohort)

Characteristics	Cohort 1 (20 μg) n = 18	Cohort 2 (100 μg) n = 18	Cohort 3 (500 μg) n = 24
Age at screening, y			
Mean ± SD	61.3 ± 7.55	62.3 ± 8.12	64.1 ± 8.16
Median	61.0	62.0	64.5
Range	46-71	50-76	48-78
Sex			
Female	4	2	10
Male	14	16	14
ECOG PS			
0	13	11	13
1	5	7	11
T category			
T1A	0	1	1
T1B	0	1	1
T2A	8	11	12
T2B	2	2	6
T3	8	3	4
N category			
N0	12	8	16
N1	5	7	7
N2	1	3	1
Stage			
IB	7	6	10
IIA	1	7	7
IIB	7	0	4
IIIA	3	5	3
Histopathological type			
Adenocarcinoma	5	5	10
Adenocarcinoma, bronchioloalveolar	2	0	0
Adenosquamous carcinoma	1	0	1
Large cell carcinoma	1	1	1
Squamous cell carcinoma	8	11	12
Other	1	1	0
Platinum-based adjuvant chemotherapy			
Yes (no)	8 (10)	7 (11)	9 (15)

ECOG PS, Eastern Cooperative Oncology Group performance status.

cohorts, all patients had received at least one dose, 45 of 60 patients continued to receive treatment, and the highest number of doses administered to any patient was 10.

After the DLP, issues impacting the electronic data capture system used in this study (the e-N@ble Web system) were detected between April and May 2013. The issues related to incorrect signatory display and the audit trail. There was no impact on data integrity or on the analyzed data. The technical root cause was identified and corrective actions were taken. There was no impact on subject safety. Submission of this paper was delayed until all issues were fully resolved.

Safety

DLT and Study Withdrawals. No case of DLT was reported.

Five patients were withdrawn from study treatment before the fifth immunization (see Fig. 1), including three who were withdrawn for safety reasons. One patient in cohort 1 was withdrawn on account of a related SAE. In this 69-year-old male with underlying myopericarditis after pneumonectomy, hypertension, atrial fibrillation, and dyspnea, acute pulmonary edema (grade 2) developed on the day after dose 2 and 7 days after reduction of the furosemide dose and lasted for 1 day. Two patients in cohort 3 were withdrawn: one owing to an SAE (transitional cell carcinoma considered unrelated to treatment) and one because of a nonserious AE.

AEs. Between dose 1 and the DLP, at least one AE (related or unrelated) was reported by 78% of patients (14 of 18) in cohort 1, by 83% (15 of 18) in cohort 2, and by 100% (24 of 24) in cohort 3. All but three of the reported AEs were grade 1 or grade 2. There were 46 patients (12 each in cohorts 1 and 2 and 22 in cohort 3) who reported treatment-related AEs, all of which were grade 1 or 2 (Table 2). The most frequently reported

Table 2. Summary of Treatment-Related Adverse Events Reported at Least Twice in Any Group (Any Grade) from Dose 1 until the Data Lock Point, by Maximum Grade (Total Treated Cohort)

	Cohort 1 n = 18[a]		Cohort 2 n = 18[a]		Cohort 3 n = 24[a]	
Adverse Event	Grade 1 n[b]	Grade 2 n[b]	Grade 1 n[b]	Grade 2 n[b]	Grade 1 n[b]	Grade 2 n[b]
Injection site reaction	4	5	8	3	13	6
Pyrexia	4	3	6	2	7	1
Fatigue	1	–	2	1	4	1
Chills	2	1	3	–	1	1
Asthenia	1	–	2	–	–	3
Headache	–	–	3	–	2	–
Arthralgia	–	1	1	–	2	–
Influenza-like illness	–	1	–	–	2	–
Myalgia	–	–	–	–	3	–
Pain in extremity	–	–	–	–	2	–

Note: See Supplementary Table 1 for all treatment-related adverse events from dose 1 until the data lock point, by maximum grade (total treated cohort).
[a]No. patients with at least one administered dose.
[b]No. patients reporting the adverse event at least once.

related AEs were injection site reactions including pain, erythema, and edema. Other frequent related AEs included pyrexia, fatigue, chills, and asthenia. No potential immune-mediated disease was reported.

Two deaths occurred during the study, both on account of progression of NSCLC. One of these patients died while still undergoing the study treatment, whereas the other died after having been withdrawn from the study treatment because of disease recurrence. Five SAEs were reported, including one (acute pulmonary edema) that was considered possibly related to the treatment and led to withdrawal of the patient from the study (see earlier and Supplementary Table 1).

Abnormal Laboratory Test Results. There were three cases of grade 3 abnormal laboratory parameters, none of which were reported as AEs: hyponatremia (an increase from a sodium level of 131 mmol/L at screening to 127 mmol/L at the time of the sixth immunization [ongoing at the DLP] but no hyperkalemia) developed in one patient (in cohort 2), and two patients (in cohort 3) had increased γ-glutamyl transferase levels present at screening that remained unchanged during the study.

The abnormal laboratory test results reported as AEs were one case of grade 1 decreased creatinine clearance (in cohort 2) that developed 21 days after dose 2, resolved after 35 days, and was considered unrelated to treatment; one case of grade 1 decreased blood cortisol level (in cohort 3) that developed on the day of dose 3, was considered unrelated to treatment, and resolved after 11 days; and one case of grade 1 increased creatinine clearance (in cohort 3) that developed on the day of dose 2, was considered treatment related, and had an unknown duration.

Immunogenicity

Two patients (one in cohort 1 and one in cohort 2) were seropositive for anti-PRAME IgG antibodies at baseline. Neither had received prior chemotherapy. All patients, except one in cohort 1 and two in cohort 3 (two of these patients had not received prior chemotherapy), were seropositive after two doses, and all were seropositive and had a humoral response (see "Methods") at the post–dose 4 assessment (Fig. 2). Anti-PRAME antibodies were higher after dose 4 than after dose 2 in all cohorts. Seropositivity rates and geometric mean antibody concentrations after four immunizations were similar in patients who had or had not received prior platinum-based chemotherapy (Fig. 2).

After four doses, the numbers of patients with PRAME-specific CD4-positive T cells (TNF-α–positive/IFN-γ–positive) immunogenicity scores equal to or above the cutoff were five of 10 in cohort 1, seven of 10 in cohort 2, and 14 of 15 in cohort 3 (Table 3). There was a tendency toward a better induction of anti-PRAME CD4+ T cells with increasing recPRAME dose, with the highest estimated frequencies of PRAME-specific CD4-positive cells observed in cohort 3. Similar immunogenicity scores among individuals who had or had not received prior chemotherapy were observed (Fig. 3). Taking baseline immunogenicity scores into account, after four immunizations the percentages of patients with a PRAME-specific CD4-positive T-cell response were 33% in cohort 1, 60% in cohort 2, and 80% in cohort 3 (see Table 3).

After four doses, the numbers of patients with PRAME-specific CD8-positive T cells (TNF-α–positive/IFN-γ–positive) immunogenicity scores equal to or above the cutoff were one of 14 in cohort 1, zero of 13 in

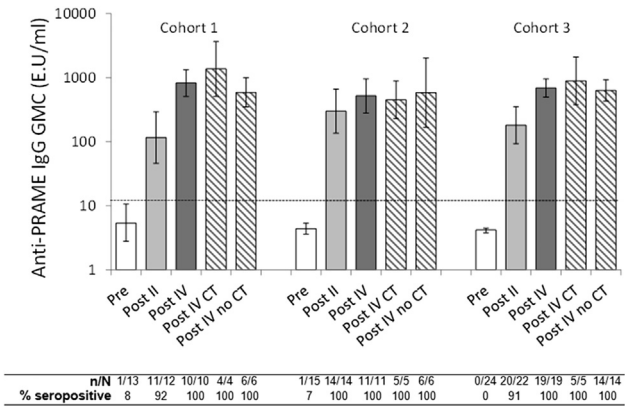

Figure 2. Seropositivity rates and geometric mean antibody concentrations (GMCs) for preferentially expressed antigen of melanoma (PRAME) immunoglobulin G (IgG) antibodies (according-to-protocol cohort for immunogenicity). N is number of patients with available results, n/% is number/percentage of patients with concentrations above the cutoff, vertical lines indicate 95% confidence intervals, dotted line shows assay cutoff (12 EU/mL). Abbreviations: Pre, before dose 1; Post II, 3 weeks after the second dose; Post IV, 3 weeks after the fourth dose; Post IV CT, patients who had received platinum-based chemotherapy before study enrollment; Post IV no CT, patients who had not received prior chemotherapy.

cohort 2, and one of 14 in cohort 3. No CD8-positive T-cell responder could be identified (see Table 3).

Dose Selection

All doses fulfilled the predefined criteria for dose selection in terms of safety and humoral immunogenicity. There was a tendency toward better induction of anti-PRAME CD4+ T cells with increasing antigen dose without a major increase in AE frequency. Thus, as defined per protocol, the dose of 500 μg was selected for further investigation.

Discussion

This phase I study was designed to select a recPRAME dose for further development on the basis of safety and immunogenicity criteria. We observed no cases of DLT, and the safety profile was consistent with that in a parallel study of the PRAME immunotherapeutic in patients with melanoma.[40]

In all patients, the PRAME immunotherapeutic induced a humoral response that appeared to be independent of the dose level. Pretreatment anti-PRAME antibodies were observed in only two patients, although our sample size of 60 patients was too small to provide definitive estimates of baseline anti-PRAME antibody production in patients with resected PRAME-positive NSCLC tumors. Spontaneous humoral immune responses against PRAME have not been described.[41] As in our experience with the PRAME immunotherapeutic, few patients with NSCLC and melanoma expressing MAGE-A3 had baseline anti–MAGE-A3 antibodies.[42,43] By contrast, baseline antibodies to other tumor antigens such as NY-ESO-1 are more frequently detected (up to 23% for NY-ESO-1 baseline antibodies in NSCLC[44]). The ability of some patients to mount an immune response to tumor antigens may reflect differences in tumor biology, individual immune competence, and previous or ongoing natural responses against the tumor.

Table 3. PRAME-Specific CD4-Positive and CD8-Positive T Cells (TNF-α-Positive/IFN-γ-Positive) Immunogenicity Score, Cellular Response, and Frequency before Treatment and after Dose 4 (According-to-Protocol Cohort)

	Immunogenicity Score[a] (≥Cutoff)		Response Rates to PRAME[b] (≥Cutoff and 4× Baseline)	Frequency above the Cutoff[c]	
	Baseline n/N (%)	After Dose 4 n/N (%)	n/N (%)	Baseline n/N (%)	After Dose 4 n/N (%)
CD4 cells TNF-α (+), IFN-γ (+) (cutoff = 2.68)					
Cohort 1	0 of 11 (0%)	5 of 10 (50%)	3 of 9 (33%)	0 of 11 (0%)	6 of 10 (60%)
Cohort 2	2 of 11 (18%)	7 of 10 (70%)	6 of 10 (60%)	1 of 11 (9%)	7 of 10 (70%)
Cohort 3	5 of 16 (31%)	14 of 15 (93%)	12 of 15 (80%)	2 of 16 (13%)	14 of 15 (93%)
CD8 cells TNF-α (+), IFN-γ (+) (cutoff = 1.15)					
Cohort 1	2 of 11 (18%)	1 of 9 (11%)	0 of 8 (0%)	0 of 11 (0%)	0 of 9 (0%)
Cohort 2	0 of 11 (0%)	0 of 10 (0%)	0 of 10 (0%)	0 of 11 (0%)	0 of 10 (0%)
Cohort 3	1 of 16 (6%)	1 of 13 (8%)	0 of 13 (0%)	0 of 16 (0%)	1 of 13 (8%)

See the Supplementary Data for details of the methods of assessment of cell-mediated immunity responses. N is number of patients in the according-to-protocol cohort at each visit (for response rates, N is the number of patients with preimmunization and postimmunization results), and n is the number of responders (column 4) or number with immunogenicity score (column 5) or frequency (column 6) above the defined cutoff.

[a]See Supplemental Data for definition of immunogenicity score.

[b]Response rate means that the ratio of immunogenicity scores positive postimmunization sample over baseline) is 4 or greater.

[c]Frequencies of PRAME-specific T cells were estimated on the basis of quantification of defined positive wells in the in vitro assay (cutoff = 6.32×10^{-6} for CD4-positive cells and 1.9×10^{-6} for CD8-positive cells).

PRAME, preferentially expressed antigen of melanoma; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ.

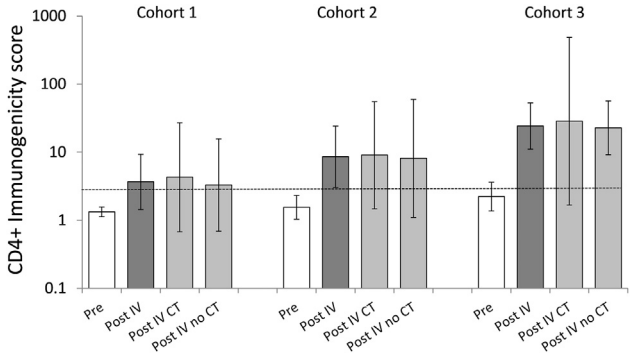

Figure 3. Preferentially expressed antigen of melanoma (PRAME)-specific CD-positive T-cell (tumor necrosis factor-α-positive/interferon-γ-positive) immunogenicity scores before treatment and after dose 4 (according-to-protocol cohort for immunogenicity). Vertical lines indicate 95% confidence intervals, dotted line shows cutoff (2.68). See Supplementary data for details of the derivation of cutoffs and methods. Pre, before dose 1; post IV, 3 weeks after the fourth dose; Post IV CT, patients who had received platinum-based chemotherapy before study enrollment; Post IV no CT, patients who had not received prior chemotherapy.

The percentage of patients showing PRAME-specific CD4-positive responses appeared to be dose dependent and was observed to be highest in cohort 3. In a parallel study in patients with metastatic melanoma, the PRAME immunotherapeutic also induced humoral immune responses in all patients and a CD4-positive response in most patients.[40]

In our study, signs of CD8-positive T-cell immunogenicity were detectable in a few patients and protocol-defined CD8-positive T cells responses were absent. By contrast, PRAME-specific CD8-positive T-cell immunogenicity was reported ex vivo in patients with leukemia.[45] Immunization of patients with acute myeloid leukemia with dendritic cells generated from autologous leukemic blasts induced specific CD8-positive T-cell responses (using enzyme-linked immunospot assay) against PRAME.[46]

CD8-positive cells are considered the main effector cells involved in direct killing of malignant cells, and adoptive cell transfer of tumor-infiltrating CD8-positive cells to patients with melanoma can mediate tumor regression.[47] However, recent evidence points to an important and previously underestimated role of CD4-positive cells in facilitating CD8-positive cell activity and in direct killing of tumor cells.[48,49] In addition to influencing development of CD8-positive cell memory, the anticancer activities of CD4-positive cells include directly and indirectly activating cytotoxic killing and production of cytokines that impact tumor cell–aging mechanisms.[48] Adoptive transfer of CD4-positive T cells along with CD8-positive T cells in mice appeared to improve and prolong therapeutic efficacy.[50] Thus, strong CD4-positive responses induced by the PRAME immunotherapeutic may point to the presence of enhanced antitumor activity. Furthermore, recent results of large trials evaluating immune checkpoint inhibitors and another cancer immunotherapeutic suggest that the induction of tumor-specific immune responses may be insufficient for improving clinical outcomes in the absence of releasing the immune blockade (Vansteenkiste et al., in press).[4–16,23,24,37]

As observed in the parallel study in metastatic melanoma, no patient had a CD8-positive T-cell response according to the predefined response criteria, although signs of T-cell immunogenicity were detected. Very low levels of antigen-specific CD8-positive cells induced by vaccination with a tumor antigen have been reported previously.[51] Furthermore, active immunotherapy with recombinant proteins has not been associated with substantial CD8-positive T-cell responses.[52] Thus, although the sensitivity and specificity of T-cell assays will directly affect detection rates, it is likely that the weak T-cell responses we observed are due to low induction of antigen-specific CD8-positive responses by the recombinant protein. It is worth noting that at the time of the study, the role of CD8-positive T cells and checkpoint inhibition in anticancer responses was less well recognized than today. Our data raise the question whether a combination of the PRAME immunotherapeutic and a checkpoint inhibitor would have enhanced effects on cytotoxic T cells.

CMI responses should be included as end points for future studies of cancer immunotherapeutics.

An exploratory analysis showed no negative impact of prior chemotherapy on humoral or CMI responses.

In conclusion, consistent with a parallel phase I dose escalation study in patients with metastatic melanoma, in this study conducted in patients with resected NSCLC, the PRAME immunotherapeutic dose of 500 μg was immunogenic in most patients with NSCLC with a clinically acceptable safety profile. This dose was selected for further evaluation in a randomized phase II study (NCT01853878). However, the study was stopped early when results of a large phase III study of another similar immunotherapeutic showed no benefit of treatment compared with placebo in patients with NSCLC.[37]

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.08.120.

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