SUPPLEMENTAL MATERIALS

First-in-Human, Phase I Study of PF-06753512, a Vaccine-Based Immunotherapy Regimen (VBIR), in Non-Metastatic Hormone-Sensitive Biochemical Recurrence and Metastatic Castration-Resistant Prostate Cancer (mCRPC)

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Contents

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Methods

Sampling for pharmacokinetics and immunogenicity assessments

For Cohort 3A-mCRPC (the first cohort with tremelimumab as part of the study treatment), blood samples for determination of tremelimumab drug concentrations were collected at pre-dose (within 6 h prior to tremelimumab dose) on Day 1, any time between Day 3 to Day 6 (48 to 120 h), Day 8 (168 h ± 24 h), Day 15 (336 h ± 48 h), and Day 22 (504 h ± 48 h) after the Day 1 tremelimumab dose; and additionally at pre-dose (within 6 h prior to tremelimumab dose) on Days 29, 57, and 85 of Cycle 1; at pre-dose (within 6 h prior to tremelimumab dose) on Days 1 and 29 of Cycle 2; at the end of treatment (EOT) visit; and at 2, 4, and 6 months after EOT during the follow-up period. For Cohorts 6A-mCRPC, 7A-mCRPC, 9A-mCRPC, 1B-BCR, 3B-mCRPC, and 5B-BCR, blood samples for determination of tremelimumab drug concentrations were collected on Days 1, 29, 57, and 85 of Cycle 1; Days 1 and 29 of Cycle 2; at EOT; and at 2, 4, and 6 months after EOT during the follow-up period. For all patients who received tremelimumab, blood samples for detection of anti-drug antibodies (ADA) against tremelimumab were collected on Days 1, 29, and 85 of Cycle 1; Day 29 of Cycle 2; at EOT; and at 2, 4, and 6 months after EOT during the follow-up period. Samples collected on dosing days were obtained within 6 h prior to tremelimumab dosing.

Sasanlimab was part of the study treatment for Cohorts 6A-mCRPC, 7A-mCRPC, 9A-mCRPC, 3B-mCRPC, and 5B-BCR. For Cohorts 6A-mCRPC, 7A-mCRPC, and 9A-mCRPC, blood samples for determination of sasanlimab drug concentrations were collected at pre-dose (within 6 h prior to sasanlimab dose) on Day 1, any time between Day 3 to Day 6 (48 to 120 h), Day 8 (168 h ± 24 h), Day 15 (336 h ± 48 h), and Day 22 (504 h ± 48 h) after the Day 1 sasanlimab dose; and additionally at pre-dose (within 6 h prior to sasanlimab dose) on Days 29, 57, and 85 of Cycle 1; at pre-dose (within 6 h prior to sasanlimab dose) on Days 1 and 29 of Cycle 2; at EOT; and at 2, 4 and

6 months after EOT during the follow-up period. For Cohorts 3B-mCRPC and 5B-BCR, blood samples for determination of sasanlimab drug concentrations were collected on Days 1, 29, 57, and 85 of Cycle 1; Days 1 and 29 of Cycle 2; at EOT; and at 2, 4, and 6 months after EOT during the follow-up period. Blood samples for detection of ADA against sasanlimab were collected from all patients who received sasanlimab on Days 1, 29, and 85 of Cycle 1; Day 29 of Cycle 2; at EOT; and at 2, 4, and 6 months after EOT during the follow-up period. Samples collected on dosing days were obtained within 6 h prior to sasanlimab dosing. Samples collected on dosing days were obtained within 6 h prior to sasanlimab dosing.

Sample size

The exact sample size of the 3+3 designs in the dose-escalation portion could not be pre-specified because of the dynamic feature of the design; however, it was estimated to be 24-48 patients and depended upon the observed safety profile. At least 3 patients were treated at each regimen dose level. Dose cohorts with an acceptable safety profile (0/3 or 1/6 patients with dose-limiting toxicities [DLTs]) could have been expanded up to N=15.

In the dose-expansion portion, the sample sizes for each cohort were determined clinically rather than statistically. In Cohort 1B-BCR, 20 patients were to be enrolled. For Cohort 3B-mCRPC, 18 patients were to be enrolled to assess objective response rate (ORR). A Bayesian approach with a non-informative Jeffery's prior beta (0.5,0.5) was used to calculate the posterior probability the ORR of the study treatment exceeded various ORR thresholds. For example, if 6 responders were observed in the 18 patients, the posterior probability that the true ORR was greater than 25% would be about 80%. For Cohort 5B-BCR 15 patients were to be enrolled.

Analysis populations

The following populations were analyzed in this study. The safety analysis set included all enrolled patients who received ≥1 dose of one of the components of the PrCa VBIR regimen. The full analysis set included all enrolled patients. The per protocol analysis set included all enrolled patients (for each indication) who received ≥1 dose of all assigned regimen components administered on Cycle 1 Day 1 and who did not have major protocol deviations during the first 28 days post-vaccination. The primary pharmacodynamic (PD) analysis population was based on a modified intention-to-treat (mITT) analysis set, which was defined as all enrolled patients who received ≥1 dose of all assigned regimen components administered on Cycle 1 Day 1 of treatment. However, because all the PD analyses was based on data as observed and no explicit imputation was applied, to be included in the mITT population, a patient must have ≥1 valid and determinate assay result related to the proposed analysis. Patients who had no valid and determinate assay result related to any proposed analysis will be excluded from the mITT analysis set. Supportive analysis populations might be based on the pharmacodynamic evaluable populations, which consisted of all patients in the mITT population who had been dosed through Day 57, Day 85, or Day 113. Additionally, tumor response and PD analyses might be repeated in patients (in the mITT population) who had no major protocol deviations during the first and second treatment cycle. The pharmacokinetic (PK) parameter analysis population was defined as all enrolled patients treated who had sufficient information to estimate ≥1 of the PK parameters of interest and who had no major protocol deviations influencing the PK assessment. The immunogenicity analysis set includes all enrolled patients who received ≥1 dose of one of the components of the regimen.

Dose-limiting toxicities

Any of the following adverse events that occurred in the first 28 days following the first AdC68 vaccination (not considered related to disease/progression) were classified as DLTs. For patients in

Cohorts 1A-mCRPC -3A-mCRPC and 6A-mCRPC –9A-mCRPC only: Grade 3 neutropenia lasting >7 days; febrile neutropenia; Grade ≥3 neutropenic infection or thrombocytopenia; Grade ≥3 anemia lasting >7 days; and Grade ≥3 lymphopenia lasting >14 days. For all cohorts: Grade ≥3 laboratory abnormalities either associated with symptoms or associated with worsening of an existing condition or that suggests a new disease process or that requires additional active management; Grade ≥3 toxicities, including toxicities of the major organs which include heart, kidney, liver, lung, colon, pancreas, brain and adrenal, hypophysis and thyroid glands; Grade 3 flu-like symptoms lasting >3 days with adequate treatment; and fever of >40.0ºC, or 104.0ºF, lasting for >3 days with adequate treatment.

Supplemental Table S1: Summary of the best overall response and duration of response in patients with mCRPC

	Cohort 7A- mCRPC **(N=14))**	**Cohort 3B-** mCRPC **(N=18)**	**All mCRPC (N=54)**
Per RECIST			
Confirmed CR, n (%)	0	1 (5.6)	1 (1.9)
Confirmed PR, n (%)	1 (7.1)	1 (5.6)	2 (3.7)
Stable disease, n (%)	1 (7.1)	4 (22.2)	8 (14.8)
Non-CR/Non-PD	1 (7.1)	1 (5.6)	5 (9.3)
Progressive disease, n (%)	7 (50.0)	2 (11.1)	11 (20.4)
Not evaluable, n (%)	4 (28.6)	9 (50.0)	27 (50.0)
ORR, % (95% CI)	7.1 (0.2–33.9)	11.1 (1.4–34.7)	5.6 (1.2–15.4)
DCR, n (%)	3 (21.4)	7 (38.9)	16 (29.6)
Median (range) duration of response, months	7.4 (7.4–7.4)	4.7 (3.9–5.6)	5.6 (3.9–7.4)
Median (range) duration of stable disease, months	9.6 (8.1–11.1)	5.4 (4.9–7.3)	5.4 (1.4–13.3)
Per irRECIST			
Confirmed irCR, n (%)	0	1 (5.6)	1 (1.9)
Confirmed irPR, n (%)	1 (7.1)	1 (5.6)	2 (3.7)
irStable disease, n (%)	2 (14.3)	6 (33.3)	15 (27.8)
irProgressive disease, n (%)	6 (42.9)	1 (5.6)	8 (14.8)
Not evaluable, n (%)	5 (35.7)	9 (50.0)	28 (51.9)
ORR, % (95% CI)	7.1 (0.2–33.9)	11.1 (1.4–34.7)	5.6 (1.2–15.4)
DCR, n (%)	3 (21.4)	8 (44.4)	18 (33.3)
Median (range) duration of response, months	7.4 (7.4–7.4)	4.7 (3.9–5.6)	5.6 (3.9–7.4)
Median (range) duration of stable disease, months	9.6 (8.1–11.1)	5.4 (2.8–7.3)	5.4 (1.4–13.3)

mITT population. Patients with mCRPC were in Cohorts 1A-mCRPC -9A-mCRPC and 3B-mCRPC. Treatment included AdC68 vector 4×10^{11} VP (Cohort 1A-mCRPC only) or 6×10^{11} VP (all other cohorts), plasmid DNA boosts 5 mg (all cohorts), tremelimumab 40 mg (Cohort 9A-mCRPC only) or 80 mg (all other cohorts), and sasanlimab 130 mg (Cohorts 6A-mCRPC and 9A-mCRPC) or 300 mg (Cohorts 7A-mCRPC and 3B-mCRPC).

*Cohort 7A-mCRPC enrolled 15 patients with mCRPC, one patient was accidentally dosed at the Cohort 6A-mCRPC regimen. This table accounts for the 14 patients that were dosed at Cohort 7A-mCRPC regimen.

BCR, biochemical recurrence; CI, confidence interval; CR, complete response; DCR, disease control rate; ir, immune-related; mCRPC, metastatic castration-resistant prostate cancer; mITT, modified intent-to-treat; N/A, not available; ORR, objective response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; VP, viral particle.

Supplemental Table S2: Antigen-specific T-cell response*

	Antigens					
	PSMA		PSA		PSCA	
	n/N	Rate, %	n/N	Rate, %	n/N	Rate, %
Cohort 5B-BCR	12/14	85.7	13/14	92.9	10/14	71.4
Cohorts 7A-mCRPC and 3B-mCRPC	10/11	90.9	8/11	72.7	10/11	90.9
Total	22/25	88.0	21/25	84.0	20/25	80.0

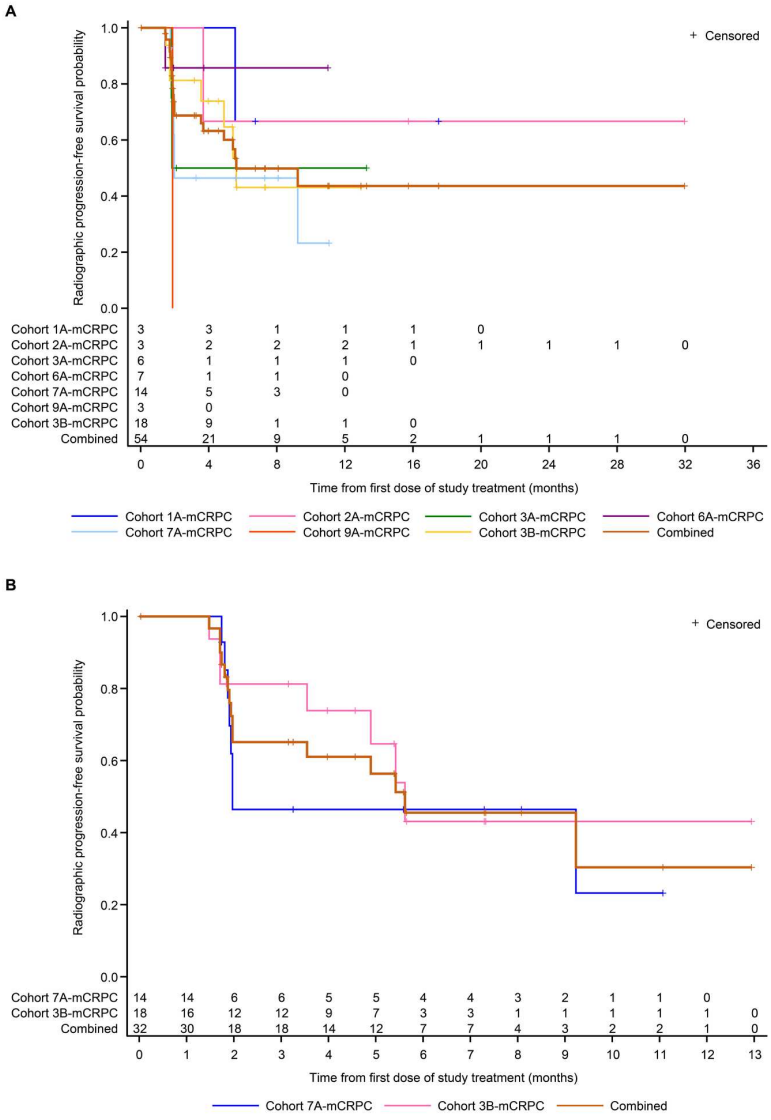
*Antigen-specific T-cell response was defined as at least 2-fold increase of interferon gamma secreting spot-forming cells at any timepoint post-vaccination compared with baseline.

Patients with mCRPC were in Cohorts 7A-mCRPC and 3B-mCRPC were treated at the RP2D, which included AdC68 vector 6×10^{11} VP, plasmid DNA boosts 5 mg, tremelimumab 80 mg, and sasanlimab 300 mg. Patients with BCR in Cohort 5B-BCR were treated with AdC68 vector 6×10^{11} VP, plasmid DNA boosts 5 mg, tremelimumab 80 mg, and sasanlimab 130 mg.

BCR, biochemical recurrence; mCRPC, metastatic castration-resistant prostate cancer; n, number of patients with antigen-specific T-cell response; N, number of patients in the population; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; VP, viral particle.

Supplemental Figure S1: Radiographic progression free survival in patients with mCRPC (mITT population)

(A) Kaplan-Meier plot for all patients with mCRPC. (B) Kaplan-Meier plot for patients with mCRPC in Cohorts 7A-mCRPC and 3B-mCRPC.

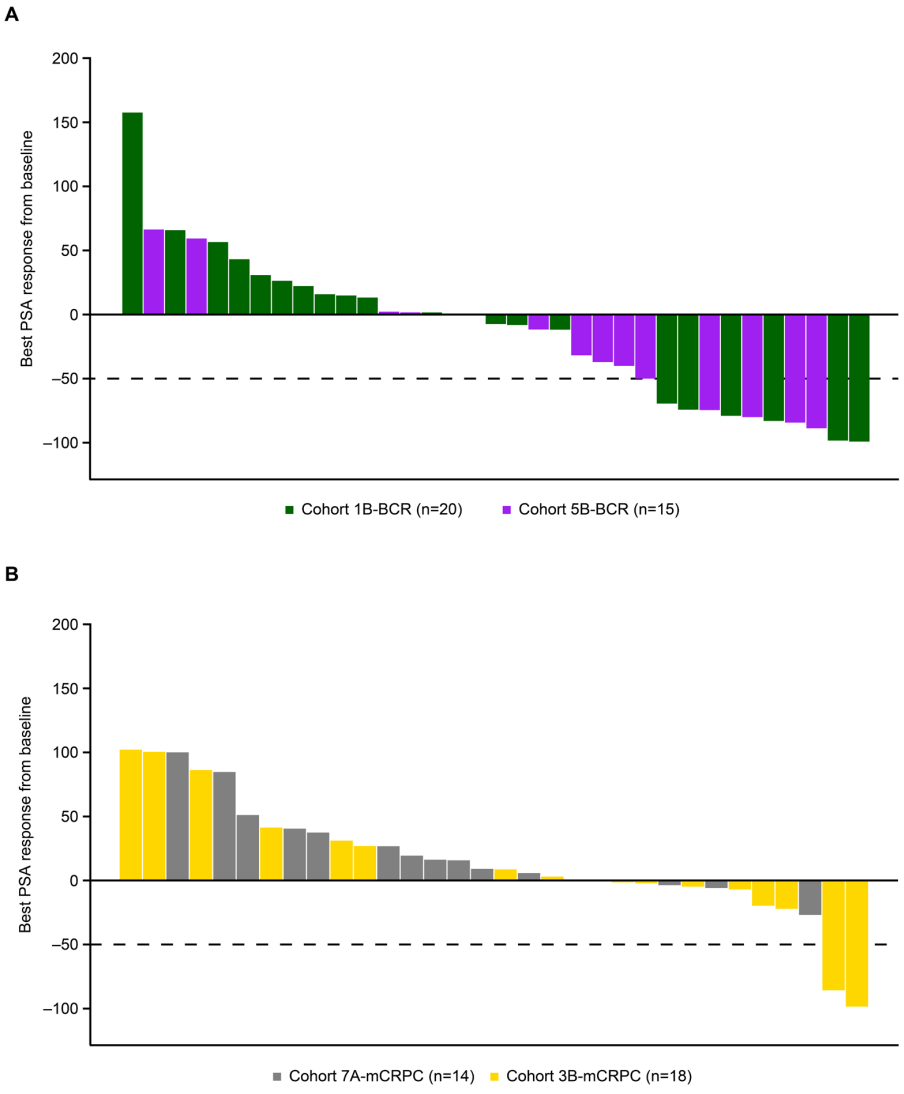


Treatment of each cohort see the footnote of Supplementary Table 1.

mCRPC, metastatic castrate resistant prostate cancer; mITT, modified intention-to-treat.

Supplemental Figure S2: Waterfall plot of the best PSA response*

(A) Patients with BCR in Cohorts 1B-BCR and 5B-BCR. (B) Patients with mCRPC in Cohorts 7A-mCRPC and 3B-mCRPC †

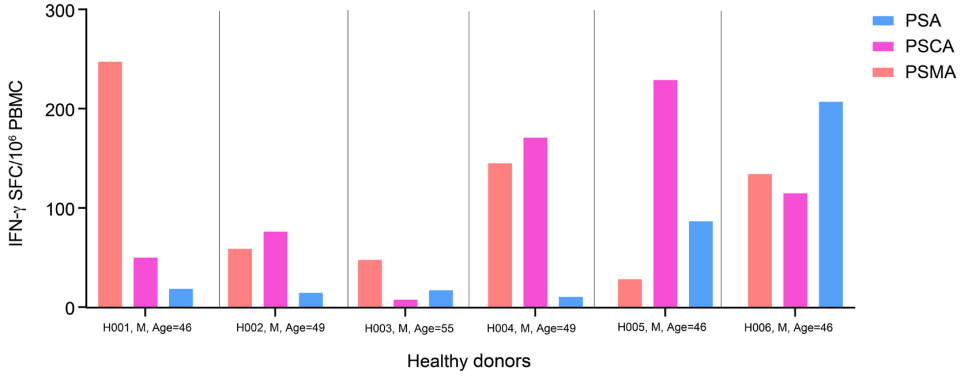


*Pharmacodynamic population. PSA assessments performed after the start of any new anticancer treatment have been excluded from the analysis. PSA assessment was performed at the study center's local laboratory.

†Patients with mCRPC were treated at the RP2D (AdC68 vector 6×10^{11} VP, plasmid DNA boosts 5 mg, tremelimumab 80 mg, sasanlimab 300 mg). Patients with BCR in Cohort 1B-BCR were treated with AdC68 vector 6×10^{11} VP, plasmid DNA boosts 5 mg, and tremelimumab 80 mg. Patients with BCR in Cohort 5B-BCR were treated with AdC68 vector 6×10^{11} VP, plasmid DNA boosts 5 mg, tremelimumab 80 mg, and sasanlimab 130 mg.

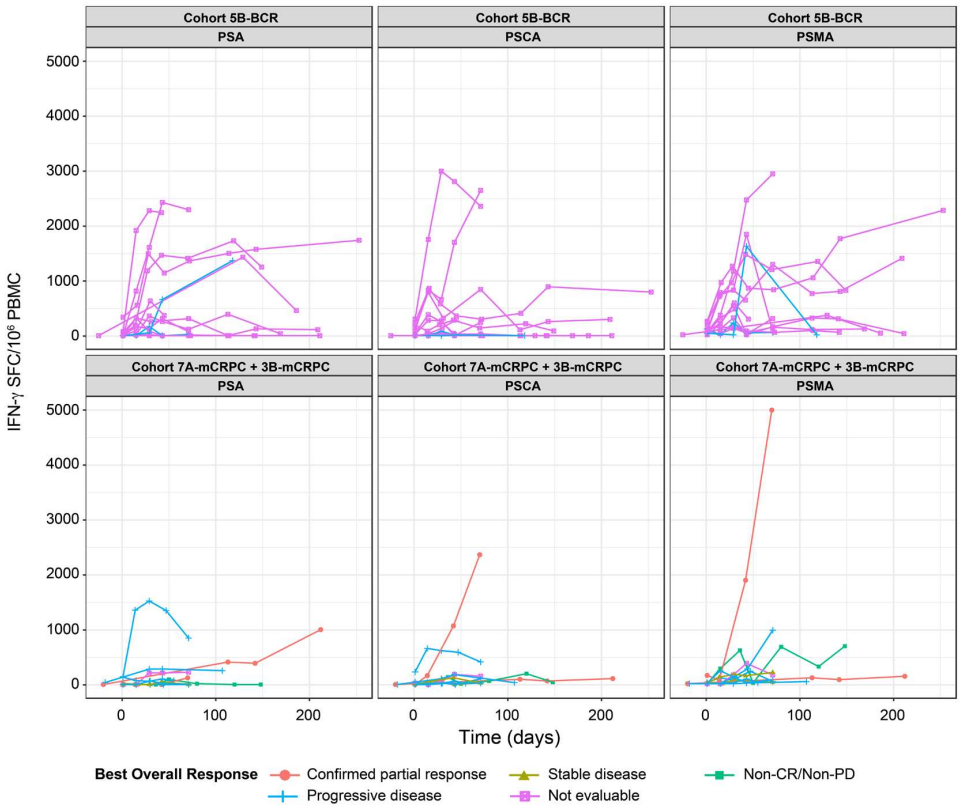
BCR, biochemical recurrence; mCRPC, metastatic castration-resistant prostate cancer; PSA, prostate-specific antigen; RP2D, recommended Phase 2 dose; VP, viral particle.

Supplemental Figure S3: T-cell immune response of healthy donors



IFN, interferon; M, male; PBMC, peripheral blood mononuclear cell; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; SFC, spot-forming cells.

Supplemental Figure S4: T-cell immune response as presented by measurement of each antigen



Patients with mCRPC in Cohorts 7A-mCRPC and 3B-mCRPC were treated at the RP2D, which was AdC68 6×10^{11} VP, the DNA booster vaccine, tremelimumab 80 mg, and sasanlimab 300 mg. For patients with BCR in Cohort 5B-BCR, the treatment was AdC68 6×10^{11} VP, the DNA booster vaccine, tremelimumab 80 mg, and sasanlimab 130 mg.

BCR, biochemical recurrence; CR, complete response; IFN, interferon; mCRPC, metastatic castration-resistant prostate cancer; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; RP2D, recommended Phase 2 dose; SFC, spot-forming cells; VP, viral particle.