Protocol B7791001

A PHASE 1 STUDY TO EVALUATE THE SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS OF ESCALATING DOSES OF A VACCINE-BASED IMMUNOTHERAPY REGIMEN (VBIR) FOR PROSTATE CANCER (PF-06753512)

Statistical Analysis Plan
(SAP)

Version: Amendment 2

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

Amendment 2	Section 2.1	Study design texts are updated to be consistent with the protocol amendment. A new study design schema replaces the previous one.
	Section 2.2	Objectives are described separately for Part A and Part B.
	Section 4.3	Sample size languages are updated according to the protocol amendment.
	Section 6	Endpoints are described separately for Part A and Part B.
	Section 8.1.3	Efficacy analyses languages are updated to provide more clarity.
	Section 8.1.4	A PSA-50 response rate analysis is added.
	Section 8.1.5.2	Sunitinib PK section is deleted according to the protocol amendment.
	Section 10.3	A censoring rule is added for COVID-19 caused missing tumor assessments.
Amendment 1	Section 2.1	Editorial updates to match the protocol
	Section 4.3	Added from the protocol
	Section 5.3	The definition for the per-protocol population is updated to match the protocol.
	Section 6	Texts about endpoints are updated to match the protocol
	Section 8.1.2.1	Texts added to define “treatment related AE”
	Section 8.1.3	Details added about tumor response analyses and time to event analyses.
	Section 8.1.5.3	A section about PF-06801591 PK analysis is added to match the protocol
	Section 10.3	Time to event censoring rules added

2. INTRODUCTION

This document presents the statistical analysis plan (SAP) for study B7791001. The SAP is based on the protocol amendment 7 dated November 22, 2019 and protocol amendment 8 dated May 29, 2020.

Note: in this document any text taken directly from the protocol is ***italicized***.

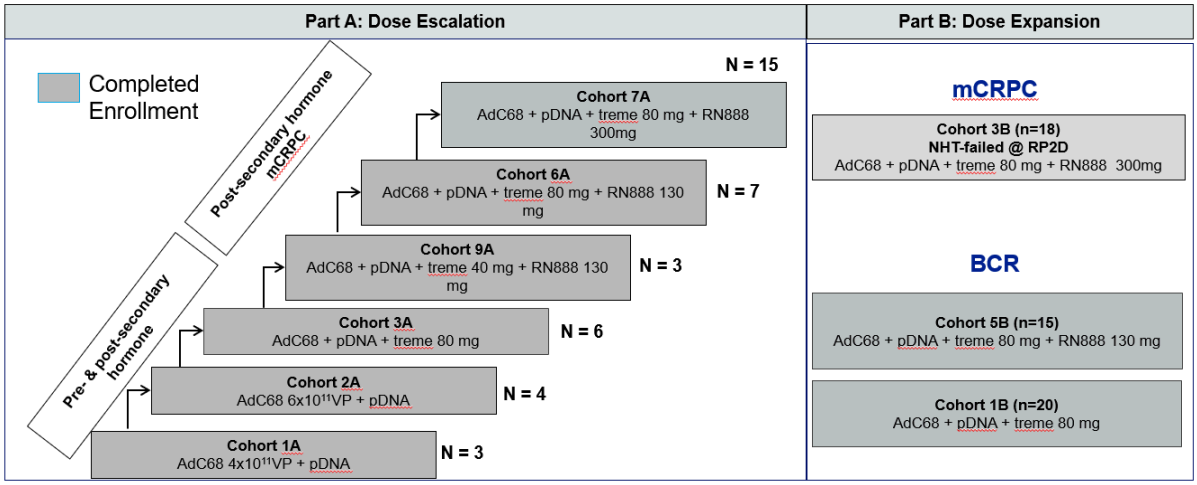
2.1. Study Design

This is a Phase 1, open label, multi-center, multiple dose, safety, PK, PD and immunogenicity study evaluating the components of a vaccine-based immunotherapy regimen for prostate cancer (PrCa VBIR). PrCa VBIR consists of the following components: Adenovirus (AdC68), pDNA and tremelimumab. In addition, cohorts will evaluate PrCa VBIR when given in combination with PF-06801591.

The study will enroll patients with the following stages of prostate cancer: patients with asymptomatic/minimally symptomatic metastatic castration-resistant prostate cancer (mCPRC) who have not received secondary hormones (M1 pre-secondary hormones), patients with rising PSA at high risk for recurrence (biochemically relapsed), and asymptomatic/minimally symptomatic mCPRC patients for whom secondary hormone treatment failed (M1 post-secondary hormones). The study is divided in to two parts, Dose Escalation (Part A) followed by Dose Expansion (Part B).

The overall study design is presented in Figure 1.

Figure 1. Overall Study Design



2.2. Study Objectives

Part A Primary Objective

- *To assess safety and tolerability of increasing dose levels of the prostate cancer vaccine-based immunotherapy regimen (PrCa VBIR) components alone and in combination with increasing doses of PF-06801591.*
- *To characterize the dose limiting toxicities (DLTs), if any are observed, and overall safety profile of escalated doses of the PrCa VBIR components alone and in combination with increasing doses of PF-06801591.*
- *To determine the Part B Expansion Dose for the PrCa VBIR components, and in combination with PF-06801591.*

Part A Secondary Objectives

- *To evaluate the immune response elicited by the PrCa VBIR to the selected prostate cancer tumor-antigens.*
- *To evaluate the overall safety profile in prostate cancer participants.*
- *To evaluate the PK of tremelimumab after subcutaneous(SC) administration.*
- *To evaluate the PK of PF-06801591 after SC administration*
- *To evaluate the anti-drug antibody (ADA) response of tremelimumab after SC administration with the other PrCa VBIR components.*
- *To evaluate the ADA response of PF-06801591 after SC administration with the other PrCa VBIR components.*

Part A Exploratory Objectives

- *To document any preliminary evidence of anti-tumor activity.*
- [redacted]
- [redacted]

Part B Primary Objective

- *To evaluate the overall safety profile of the PrCa VBIR + PF-06801591 in prostate cancer participants.*

Part B Secondary Objectives

- *For Cohort 3B: To evaluate the anti-tumor response induced by treatment in participants with mCRPC utilizing solid tumor response criteria.*
- *For Cohort 3B: To evaluate the anti-tumor response induced by treatment utilizing immune related response criteria.*
- *For Cohort 3B: to evaluate bone metastatic disease outcome in participants with mCRPC.*
- *For Cohorts 3B: To estimate the duration of radiographic Progression-Free Survival (rPFS) in participants with mCRPC.*
- *To evaluate response rate based on 50% reduction of prostate specific antigen (PSA).*
- *To evaluate PSA kinetics.*
- *To evaluate trough concentrations of tremelimumab after SC administration at selected doses.*
- *To evaluate trough concentrations of PF-06801591 after SC administration at selected doses.*
- *To evaluate the anti-drug antibody (ADA) response of tremelimumab after SC administration with the other PrCa VBIR components.*
- *To evaluate the ADA response of PF-06801591 after SC administration with the other PrCa VBIR components.*

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- [redacted]
- [redacted]
- [redacted]

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

There are no formal interim analyses planned in this study. However, this is an open label study, and therefore, the Pfizer team will review safety, immunogenicity, pharmacokinetics, pharmacodynamic, CCI [redacted] and other data throughout the study.

4. HYPOTHESES, SAMPLE SIZE AND DECISION RULES

4.1. Statistical Hypotheses

No formal testing of hypotheses will be conducted in this study.

4.2. Statistical Decision Rules

The following table shows the probability of escalating to the next dose level for a range of underlying true dose limiting toxicity *(DLT) rates. For example, for a DLT that occurs in 10% of patients, there is a greater than 90% probability of escalating. Conversely, for a DLT that occurs with a rate of 70%, the probability of escalating is 3%. It is assumed that dose escalation occurs with either 0/3 or 1/6 patients with DLTs.*

Table 1. Probability of Escalating Dose

True underlying DLT rate	10%	20%	30%	40%	50%	60%	70%	80%	90%
Probability of escalating dose	0.91	0.71	0.49	0.31	0.17	0.08	0.03	0.009	0.001

Probabilities of testing sequential doses can be calculated as the 3+3 escalation rules are predefined. For example the probability of escalating to a third dose level is 0.64 under the assumption that DLTs occur in 10% and 20% of patients respectively in the first dose and the second dose. The probability of escalating to a third dose is only 0.35 if DLTs occur in 20% and 30% of patients respectively in the first dose and the second dose tested.

4.3. Sample Size Determination

The exact sample size of the 3+3 designs in the Phase 1 Part A (Dose Escalation) cannot be pre-specified because of the dynamic feature of the design. The number of patients to be enrolled in the study will depend upon the observed safety profile, which will determine the number of patients at each dose level (eg, 3 or 6) and the number of dose levels explored.

Typically, at least 3 patients will be treated at each regimen dose level. Dose cohorts with an acceptable safety profile (0/3 or 1/6 patients with DLTs) may be expanded up to N=15 to further assess safety, immune response, pharmacokinetics, and pharmacodynamics. Decisions to enroll additional patients at dose levels already cleared for safety will be based on clinical judgment of the investigators and the sponsor considering all evaluable safety, immunoresponse, and/or pharmacodynamics data.

The estimated sample size for Part A would be 24-48 patients and will be dependent on the safety profile observed.

The sample size for Cohort 1B (AdC68 + pDNA + treme 80 mg SC for BCR patients who had no prior therapy) was determined clinically rather than statistically. Twenty patients were enrolled in that cohort to provide preliminary efficacy, safety, PK and biomarker data for this regimen.

For Cohort 3B (AdC + pDNC + treme 80 mg SC + PF-0689159 300 mg SC. for patients with mCRPC whose disease has progressed despite novel hormonal treatment), 18 patients

were enrolled. The main efficacy endpoint for this cohort will be objective response rate (ORR) based on RECIST version 1.1, the key supportive efficacy endpoint will be radiographic progression-free survival (rPFS). There are no hypothesis tests for these endpoints. Bayesian approach may be used to evaluate the efficacy data. For example, Bayesian approach with a non-informative Jeffery's prior beta (0.5,0.5) will be used to calculate the posterior probability the ORR of the study treatment exceeds various ORR thresholds of interest in the end of the study.

For Cohort 5B (AdC68 + pDNA + treme 80 mg SC + PF-0689159 130 mg SC. for BCR patients who had no prior therapy), the sample size was determined clinically rather than statistically. Fifteen patients were enrolled in that cohort to provide preliminary efficacy, safety, PK and biomarker data for the regimen.

5. ANALYSIS SETS

5.1. Safety Analysis Set

The safety analysis set includes all enrolled patients who receive at least one dose of one of the components of the regimen.

5.2. Full Analysis Set

The full analysis set includes all enrolled patients.

5.3. Per-Protocol Analysis Set

The per protocol (PP) analysis set includes all enrolled patients (for each indication) who receive at least one dose of the assigned regimen components administered on Cycle 1 Day 1 of study medication and who do not have major protocol deviations during the 28 days after the first vaccination. Major protocol deviations will be determined before data base release.

5.4. Modified Intent-to-Treat Analysis Set

The modified Intention-to-Treat (mITT) is defined as all enrolled patients who received at least one dose of all assigned regimen components administered on Cycle 1 Day 1 of treatment.

5.5. Pharmacodynamics Analysis Set

The pharmacodynamics analysis set will be based on the mITT population. A patient must have at least 1 valid and determinate assay result related to the proposed analysis. *Patients who have no valid and determinate assay result related to any proposed analysis will be excluded from the pharmacodynamics analysis set.*

5.6. Evaluable Population

As a consequence of its mechanisms of action, PrCa VBIR may require time after administration to induce a prostate cancer-specific immune response and subsequent tumor response. Therefore, tumors in patients treated with cancer vaccines may show early progression followed by subsequent response, therefore, supportive analysis populations may be based on the evaluable population (EP) *which* will *consist of all patients in the mITT*

population who have been dosed through Cycle 1 *Day 57, Day 85 or Day 113* (Day 113 is Cycle 2 Day 1). To implement this, 3 sub-populations will be created: EP C1D57, EP C1D85, and EP C2D1. EP C1D57 will include all patients who have received all assigned doses at least until C1D57. EP C1D85 and EP C2D1 are defined in the same manner.

Additionally, tumor response and PD analyses may be repeated in the mITT population in patients who have no major protocol deviations during the first and second treatment cycle.

5.7. Treatment Misallocations

For patients with errors in treatment allocation the following approach will be followed:

If a patient was:

- Enrolled but not treated, then they will be reported under their enrolled treatment group for demographic analyses only. These patients will be excluded from the immunogenicity, efficacy and safety analyses as the actual treatment is missing.

- Enrolled but took incorrect treatment, then they will be reported under their enrolled treatment group for efficacy analyses based on mITT, excluded from analyses based on PP, but will be reported under the treatment they actually received for all safety, PK, immune responses, and anti-tremelimumab or anti-PF-0680159 immunogenicity analyses.

5.8. Protocol Deviations

Major protocol deviations will be determined on an ongoing basis per medical data review. Any major protocol deviation will prevent the patient from being included in the per-protocol population.

A full list of major protocol deviations will be compiled prior to database closure. Once the final list of major protocol deviations is determined the per-protocol population flag will be updated.

6. ENDPOINTS AND COVARIATES

6.1. Part A Primary Endpoint

- *Incidence and grade of treatment-emergent adverse events including DLTs as graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 4.03).*

6.2. Part A Secondary Endpoint

- *Immune response including T cells specific to the three selected prostate cancer tumor-antigens.* **T cell immune response** will be determined as the frequency of IFN-γ spot forming cells (SFC)/million peripheral blood mononuclear cells (PBMC) in response to Prostate-Specific Membrane Antigen (PSMA), Prostate Stem Cell Antigen (PSCA) and Prostate-Specific Antigen (PSA) as measured in the IFN-γ ELISPOT assay. For analysis in the IFN-γ ELISPOT assay, the PSMA antigen will

be divided into three sub regions (peptide pools) for which the IFN-γ spot forming cells/million PBMCs will be reported separately (ie, 5 reported values) or by antigen (3 PSMA summed) or all pooled. The PSCA and PSA assays will each be analyzed as a single antigen (ie, 1 reported value for each antigen). ELISPOT assay reported variables, by the Lab, are specified and listed in the respective CSAP document.

- ***Antibody response specific to the PSMA antigen*** (Geometric Means and Seroconversion): will be determined as the titer (U/mL) of serum IgG antibodies elicited against the PSMA antigen as measured in the Luminex-based assay.

- ***Laboratory abnormalities*** *as characterized by type, frequency, severity (as graded by NCI CTCAE v 4.03) and timing.*

- ***Tremelimumab single-dose PK parameters****, including the maximum concentration (C_{max}), time to maximum concentration (T_{max}), and area under the concentration versus time curve (AUC) from time zero to the last quantifiable time point prior to the second tremelimumab dose (AUC_{last}) and if data permit, AUC from time zero extrapolated to infinity (AUC_{0-inf}); and trough concentrations after multiple dosing (C_{trough}).*

- ***PF-06801591 single-dose PK parameters****, including C_{max}, T_{max}, AUC_{last}, and if data permit, AUC_{inf}; and C_{trough} after multiple dosing.*

- *Incidence and titers of ADA and neutralizing antibodies against tremelimumab.*

- *Incidence and titers of ADA and neutralizing antibodies against PF-06801591.*

6.3. Part A Exploratory Endpoints

- *Objective tumor response, as assessed using the Response Evaluation Criteria in Solid Tumor (RECIST) version 1.1 by calculating the Objective Response Rate (ORR) and radiographic Progression-Free Survival (PFS).*

- *Antitumor response based on total measurable tumor burden as assessed by the Immune-Related Response Criteria (irRECIST)* and irPFS.

- *Bone outcome according to Prostate Cancer Working Group 3 (PCWG3) criteria.*

[redacted]

[redacted]

6.4. Part B Primary Endpoint

- *Incidence and grade of treatment-emergent adverse events including DLTs as graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 4.03).*

6.5. Part B Secondary Endpoint

- *For Cohort 3B: Objective response rate (ORR) and duration of response, as assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.*
- *For Cohort 3B: Antitumor response and tumor control duration based on total measurable tumor burden as assessed by the Immune-Related Response Criteria Derived from RECIST 1.1 (irRECIST).*
- *For Cohort 3B: Bone outcome according to Prostate Cancer Working Group 3 (PCWG3) criteria.*
- *For Cohort 3B: Radiographic Progression-Free Survival (rPFS) by RECIST 1.1, irRECIST and PCWG3 Criteria in participants with mCRPC.*
- *PSA-50 response rate and duration of response.*
- *Baseline and changes from baseline for PSA, PSA velocity, PSA slope and PSA doubling time (PSADT).*
- *Trough concentrations after multiple dosing (Ctrough).*
- *Ctrough after multiple dosing.*
- *Incidence and titers of ADA and neutralizing antibodies against tremelimumab.*
- *Incidence and titers of ADA and neutralizing antibodies against PF-06801591.*

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6.7. Covariates

Analyses of immune response and analyses of pharmacodynamic parameters may be adjusted (or stratified) by demographic and prognostics variables (eg, age, gleason score, baseline PSA, baseline PSADT etc).

7. HANDLING OF MISSING VALUES

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All the safety, immune responses, pharmacokinetic, pharmacodynamics, and immunogenicity analyses and summaries will be based on data as observed and no explicit imputation will be applied.

7.2. Immune Responses Lower Limit of Quantitation

Titers below the assay detection limit will be assigned a value of one-half that limit.

7.3. Pharmacokinetic Concentrations and Parameters

Drug concentrations below the limit of quantification

In all data presentations (except listings), drug concentrations below the limit of quantification (BLQ) will be set to zero. In the listings BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification.

Deviations, missing concentrations and anomalous values

Patients who experience events that may affect their PK (eg, incomplete dosing) may be excluded from the PK analysis.

In summary tables and plots of mean profiles of PK, statistics will be calculated having set concentrations to missing if 1 of the following cases is true:

1. A concentration has been collected as ND (ie, not done) or NS (ie, no sample);

2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the PK analyst.

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

An anomalous concentration value is one that, after verification of bioanalytical validity, is grossly inconsistent with other concentration data from the same individual or from other patients. For example, a BLQ concentration that is between quantifiable values from the same dose is considered as anomalous. Anomalous concentration values may be excluded from PK analysis at the discretion of the PK analyst.

Pharmacokinetic parameters

Actual PK sampling times will be used in the derivation of PK parameters.

If a PK parameter cannot be derived from a patient's concentration data, the parameter will be coded as NC (ie not calculated).

In summary tables, statistics will be calculated by setting NC values to missing; and statistics will not be presented for a particular treatment if more than 50% of the data are NC. For statistical analyses (ie analysis of variance), PK parameters coded as NC will also be set to missing.

7.4. Efficacy Data

For the time-to-event endpoints, the missing data handling method will be censoring. Censoring rules for time-to-event endpoints are detailed in Appendix 10.3.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

In general, all continuous endpoints will be summarized descriptively by cohort. If data are categorical then the standard contingency tables with counts and percent by group will be displayed.

8.1. Statistical Analyses

8.1.1. Baseline Evaluations

The baseline evaluation summaries and listings will display patient demographics, including age and performance status, PSA and Gleason score at the time of diagnosis. Details and dates of prior hormonal and nonhormonal therapies should be displayed, along with additional PSA measurements that can be used to estimate PSA doubling times (PSADT). Evaluable data on screened, but not enrolled patients, will be presented as listings.

8.1.2. Safety Analysis

The main analyses of DLTs will be based on the Per Protocol analysis set. Patients not meeting the criteria for inclusion in the Per Protocol Analysis set (ie, not evaluable for assessment of DLTs) will be replaced. Summaries and analyses of other safety parameters will include all patients in the Safety Analysis Set. Note only the safety data before the start of any new anti-cancer systemic therapy will be included in safety analysis.

8.1.2.1. Analysis of Primary Endpoint

DLT is a primary endpoint of the study. The properties of the statistical methods for the analyses of DLTs are described in Section 4. Adverse events constituting DLTs are detailed in Section 3.2 of the study protocol. Adverse events and *adverse events constituting DLTs will be listed per dose level* (ie, by cohort). A binary variable will be created at the patient level to indicate whether or not a patient has experienced any of the adverse events that are considered DLT. If required, a summary table will be created by cohort to present number and percentage of patients experiencing DLT and each specific adverse event constituting DLT.

Adverse Events (AEs) will be graded by the investigator according to the CTCAE version 4.03 and coded using the Medical Dictionary for Regulatory Activities (MedDRA). The focus of AE summaries will be on Treatment Emergent Adverse Events, those with initial onset or increasing in severity after the first dose of study treatment and before the start of any new anti-cancer systemic therapy. *The number and percentage of patients who experienced any AE, SAE, treatment related AE, and treatment related SAE will be summarized. The summaries will present AEs both on the entire study period by dose* (i.e., by cohort) *and by cycle.* The number and percentage of patients who discontinued from study medications due to AE or discontinued from the study due to AEs will also be presented.

AE, SAE, treatment related AE, treatment related SAE will be presented by system organ class and preferred term for each cohort. Severity summary tables will also be presented where AE, SAE, treatment related AE, and treatment related SAE will be presented for each cohort by system organ class and preferred term according to the worst toxicity grade.

Treatment related AE in this study refers to those adverse events that are determined by investigator as related to either adenovirus, or plasmid DNA, or tremelimumab, or PF-06801591. If deemed necessary, further summary of component-related AE may be presented.

8.1.2.2. Analysis of Secondary Safety Endpoints

These safety endpoints will be analyzed according to the Pfizer Data Standard. To better characterize the safety profile of the different regimen components analyses of safety will be conducted by cohort, by cycle (i.e., Cycle 1,2, maintenance cycle) and by period (Day 1 to Day 28, Day 29 to Day 85).

Laboratory Tests Abnormalities

The number and percentage of patients who experienced laboratory test abnormalities will be summarized according to worst toxicity grade observed for each lab assay. The analyses will summarize laboratory tests by cohort and visit. The most recent measurement prior to dosing is considered as baseline.

For laboratory tests without CTCAE grade definitions, results will be categorized as normal, abnormal or not done.

ECG

The analysis of ECG results will be based on patients in the safety analysis set with baseline and on-treatment ECG data. Baseline is defined as the pre-dose ECG collected before the first dose of any component of the study treatment.

ECG measurements (an average of the triplicate measurements) will be used for the statistical analysis and all data presentations. Any data obtained from ECGs repeated for safety reasons after the nominal time-points will not be averaged along with the preceding triplicates. Interval measurements from repeated ECGs will be included in the outlier analysis (categorical analysis) as individual values obtained at unscheduled time points.

QT intervals will be corrected for heart rate (QTc) using standard correction factors (ie, Frederica's). Data will be summarized and listed for QT, HR,PR, QRS, and QTcF by cohort. Individual QT intervals will be listed by time and cohort. Descriptive statistics (n, mean, median, standard deviation, minimum, and maximum) will be used to summarize the absolute corrected QT interval and changes from baseline in corrected QT after treatment by cohort and time point. For each patient and by treatment, the maximum change from baseline will be calculated as well as the maximum post-baseline interval across time-points. Categorical analysis will be conducted for the maximum change from baseline in corrected QT and the maximum post-baseline QT interval.

Shift tables will be provided for baseline vs worst on treatment corrected QT.

If applicable, the effect of relevant drug concentrations on corrected QT change from baseline will be explored graphically. Additional concentration-corrected QT analyses may be performed. Data may be pooled with other study results and/or explored further with PK/PD models.

8.1.2.3. Analysis of other Secondary Endpoints

1. **T cell immune response**

The number of antigen-specific T cells (expressed as SFC/million PBMC) for each prostate cancer antigen (or antigenic sub region) will be compared between pre-treatment and subsequent post treatment time points in two rounds of analysis. The first round (Cycle 1 analysis) will compare the pretreatment response (Cycle1 Day 1) to Cycle 1 Day 15, Cycle 1 Day 29, Cycle 1 Day 43 and Cycle 1 Day 71; the second round (Cycle 2 analyses) will compare Cycle 2 Day 1 with remaining timepoints as Cycle 2 Day 29 and Cycle 2 Day 99, or End of Treatment, and Cycle 1 Day 1). Data from the maintenance period may be analyzed in the same manner if deemed necessary. As appropriate, analysis of the immune response endpoints will include fold-change from baseline by patient and fold-change in geometric mean (GMFR) by cohort (by Cycle). The immune response against each peptide pool (5 values: 1 for PSA, 1 for PSCA and 3 for PSMA) may be analyzed separately or per antigen (eg 3 PSMA summed) or all pooled to provide the overall T cell response to the selected prostate cancer antigens.

In case there is failure for Cycle 1 Day 1, the Screening sample would be used as the pretreatment sample.

- Number of treatment-induced immune responses against the prostate cancer antigens will be defined as the number of patients with an X-fold (eg, where X could defined as 2-fold, 4-fold) increase in at least Y sampling time points (eg, Y=one time, Y=two timepoints) after treatment as compared to the Cycle 1 Day 1 (or Screening/Baseline sample if failure for Cycle 1 Day 1) for the same patient.

- Negative (media only) and positive (anti-CD3) control responses, as well as specific T cell responses observed to CMV, EBV, Flu and Tetanus Toxoid (CEFT) will be reported in a similar manner to the prostate cancer antigen data (ie, in two rounds of analysis).

2. **Antibody response specific to the PSMA antigen (Geometric Mean and Seroconversion)**

The LLOQ for the assay will be provided by the testing labs. Titers below the assay detection limit will be assigned a value of one-half that limit.

Geometric Mean Titer: For the analyses on antibody titer, least squares (ie, estimated) Geometric mean titer (GMT) will be calculated at baseline and post baseline. All statistical analyses will be performed on the logarithmically (natural base) transformed titer values. See 10.1.

Geometric Mean Fold Rise: The least squares (ie, estimated) GMFR of the post-vaccination titer value to the previous titer level (other than at baseline) will be calculated, as well as the associated confidence interval and the median, minimal, and maximal n-fold increase. See 10.1.

Seroconversion: Number of patients from negative to positive (eg, k fold above the assay detection) or with at least an X-fold increase from baseline (eg, k=2,3; X=2,4).

8.1.3. Exploratory (Part A) / Secondary (Part B) Efficacy Analyses

In this First-In-Patient study anti-tumor activity is an exploratory objective. The main analysis populations will be based on mITT and EP.

Tumor response, using RECIST, irRECIST*, will be presented in the form of patient data listings which include, but are not limited to tumor response assessed in comparison to baseline. Traditional response measures in solid tumors, such as ORR and PFS were developed to evaluate chemotherapies and can be unreliable for tracking response to* immunotherapies such as *cancer vaccines. For example, apparent tumor burden may increase during the first few months of cancer vaccine treatment because of inflammation/T cell infiltration into tumors even in patients who go on to have durable responses. Time to progression and number of patients with progression could be calculated from first vaccination (Cycle 1 Day 1), but may also be calculated excluding those progressions that happened before some specific timepoints (eg, Day 57, Day 113). Time to response will be calculated from first vaccination (Cycle 1 Day 1) to time of first response (ie, CR or PR).*

Additionally, total measurable tumor burden will be evaluated by the irRECIST criteria that include responses after disease progression that are not captured by RECIST evaluation criteria in solid tumors.

For tumor response data, the following analyses may be performed:

1. Tumor response (CR, PR, SD, PD, etc.) by cohort and visit, separately for RECIST 1.1 and irRECIST. Investigator provided tumor response will be presented by descriptive statistics (frequency and percentage).

2. Best overall response, by cohort, **across all available tumor assessments from both treatment period and maintenance period**, separately for RECIST 1.1 and irRECIST. Best overall response will be derived programmatically. In the tabular data presentation, a row of CR+PR will be added to show the results of ORR. With RECIST 1.1, tumor response (CR or PR) confirmation is optional as response rate is not the primary endpoint of the study, hence unconfirmed best overall response will be derived and presented. With irRECIST, tumor response (irCR or irPR) and disease progression (irPD) are required to be confirmed, however in this study tumor response confirmation is not required, therefore unconfirmed best overall response will be derived and presented. Descriptive statistics (frequency and percentage) will be provided.

Selected ad-hoc efficacy analyses (eg, ORR and radiographic PFS) combining participants from Cohorts 7A and 3B may be conducted, as participants in those two cohorts have identical or similar eligibility criteria for study entry and are scheduled to receive the same regimen.

Swimmer plots may be used to display duration of treatment and tumor response at each applicable time point. Waterfall plot for individual tumor size percent change from baseline, and spider plot for individual tumor size percent change from baseline over time may be presented. These plots, if generated, will be presented for RECIST 1.1 and irRECIST separately.

For the rPFS analysis in Part B, the Kaplan-Meier analysis may be performed if deemed necessary. If the Kaplan-Meier analysis is performed for radiographic progression-free survival, an event is defined as the first occurrence of "PD" status by investigator, or death, or a PCWG3 defined bone disease progression, whichever comes first, under RECIST. Time zero for all time-to-event analyses will be defined as the first vaccination on Cycle 1 Day 1. This analysis may be performed for the combined cohorts 7A and 3B. CCI [redacted]

For duration of response per RECIST and time to response (from the first vaccination date to the time of the first response, CR or PR) in Part B, descriptive statistics will be provided. Kaplan-Meier analysis may be performed if deemed necessary. These analyses may be performed for the combined cohorts 7A and 3B.

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PSA kinetics

PSA, PSA velocity, PSA slope and PSA doubling time (PSADT); PSA will be provided from laboratory data. PSA velocity is defined as the slope of the linear regression line of PSA against time in month. PSA slope is the slope of the linear regression line of natural log of PSA against time in month. PSA doubling time is defined as the natural log of 2 divided by the slope of the linear regression line of the natural log of PSA against time in month (see 10.2). These analyses will be conducted both for Central PSA and Local PSA in separate tables. PSA kinetics will be presented at individual level by timepoint (listings and plots). Mean % and absolute change from baseline in PSA kinetics may be presented by cohort. To report PSA-based outcomes, PCWG3 recommends that the percentage of change in PSA from baseline, as well as the maximum decline in PSA, PSA velocity, PSADT and PSA slope that occurs at any point after treatment be reported for each patient using a waterfall plot. Waterfall plots provide a broader and more sensitive display of data, and are more informative until a validated surrogate of clinical benefit is available.

A PSA-50 response rate may be calculated by cohort. PSA-50 response rate is defined as the proportion of patients whose on-study PSA declined from baseline by at least 50% at two consecutive measurements at least 3 weeks apart, prior to other systematic anti-cancer therapy. For the subset of PSA-50 responders, a duration of PSA-50 response will be summarized. Duration of PSA-50 response is defined as the period between the first

measurement when PSA-50 response was achieved to the measurement when PSA-50 response no longer holds.

Swimmer plots may be used to display duration of treatment and PSA values at each applicable time point.

CTC

The frequency of "traditional" and "all candidate" CTCs in whole blood each expressed as CTC/mL will be assessed in pre treatment (Screening, Cycle 1 Day 1) and post treatment (eg. Cycle 1 Day 71, Cycle 2 Day 1, Cycle 2 Day 29, Cycle 2 Day 99, and End of Treatment) samples. Analysis of the CTC endpoints may include absolute CTC and fold-change from baseline (pretreatment) by patient and % change and mean fold-change by cohort.

MDSC

The percentage of MDSCs will be assessed in pretreatment (Screening, Cycle 1 Day 1) and post treatment samples. MDSC levels will be presented per patient and per cohort by GMTs and GMFRs. Analyses of MDSC endpoints may include absolute and fold-change from baseline (pretreatment) by patient and % change and mean fold-change by cohort.

Bone scan assessment

Given the frequency of bone involvement in patients with progressive, castration-resistant disease, the decreased emphasis of early changes in PSA, and the increased availability of cytostatic agents, reliable methods to assess changes in bone are of increasing importance. PCWG3 recognizes that standards for using MRI and PET to assess bone metastases are under active investigation, so only radionuclide bone scans are considered here. PCWG3 also recognizes that there are no validated criteria for response on radionuclide bone scan.

For control/relieve/eliminate end points, the PCWG3 recommends that post-treatment changes be recorded simply as either "no new lesions" or "new lesions." However, progression at the first scheduled assessment should be confirmed on a second scan performed 6 or more weeks later, in the absence of clearly worsening disease or disease-related symptoms. In the event of visible lesions disappearing, this should also be confirmed at the next scheduled assessment.

For prevent/delay end points, progressing disease on bone scan is considered when a minimum of two new lesions is observed. PCWG3 does not recommend performing a follow-up bone scan before 12 weeks of treatment unless clinically indicated. At the first 12-week reassessment, defining disease progression requires a confirmatory scan (which shows additional new lesions compared with the first follow-up scan) performed 6 or more weeks later, because lesions visible at the first 12-week assessment may represent disease that was not detected on the pretreatment scan. When further progression is documented on the confirmatory scan, the date of progression recorded for the trial, is the date of the first scan that shows the change.

Correlative analyses

Plots may be generated to display PSA kinetics vs. PSA antibodies (serology: Luminex assay). Similar plots may be generated to evaluate individual patient trends on MDSC, CTC and T-cell response.

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8.1.5. Pharmacokinetics

Patients who receive the designated investigational product of interest and have at least one post-dose drug concentration measurement will be included in the PK data analysis.

The actual time of sample collection will be used in PK parameter calculation. In the event that the actual sampling time is not available, the nominal time may be used if there is no evidence that the actual sampling time deviates substantially from the nominal time.

8.1.5.1. Tremelimumab and PF-06801591 Pharmacokinetics

Presentation of Tremelimumab and PF-06801591 concentration-time data

The concentration-time data of tremelimumab and PF-06801591 will be presented as below:

- a listing of all concentrations by cohort, subject ID and nominal time for each compound. The concentration listings will also include the actual times. Deviations from the nominal time will be given in a separate listing for each compound.

- a summary of concentrations for each compound by cohort and nominal time, where the set of statistics will include n, mean, standard deviation, median, coefficient of

variation (cv), minimum, maximum and the number of concentrations above the lower limit of quantification.

- for the concentration-time data after the first dose median concentrations time plots (on both linear and semi-log scales) against nominal time postdose by cohort (all cohorts on the same plot per scale, based on the summary of concentrations by cohort and time postdose) for tremelimumab (for Cohorts 3A and 4A only) and PF-06801591 (for Cohorts 6A through 9A).

  for the concentration-time data after the first dose mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose by cohort (all cohorts on the same plot per scale, based on the summary of concentrations by cohort and time postdose) for tremelimumab (for Cohorts 3A and 4A only) and PF-06801591 (for Cohorts 6A through 9A).

For drug concentration summary statistics, median and mean plots by sampling time, the nominal PK sampling time will be used.

Calculation of tremelimumab PK parameters

For patients of Cohorts 3A and 4A, the concentration-time data of tremelimumab after the first dose will be analyzed individually by non-compartmental methods to determine the PK parameters. For patients of Cohorts 6A through 9A, the concentration time data of PF-06801591 after the first dose will be analyzed individually by non compartmental methods to determine the PK parameters. For each compound, *the PK parameters to be estimated will include the maximum drug concentration (C_{max}), time to maximum drug concentration (T_{max}), and area under the concentration versus time curve (AUC) from time zero to the last quantifiable time point prior to the second tremelimumab* or PF-06801591 *dose (AUC_{last}), and if data permit, AUC from time zero extrapolated to infinity (AUC_{inf}), terminal elimination half-life ($t_{1/2}$), and apparent clearance (CL/F). In addition, the accumulation ratio (Rac) as calculated by the ratio of the trough concentration prior to the fifth tremelimumab* or PF-06801591 *dose (on Cycle 2 Day 1) to the concentration prior to the second tremelimumab* or PF-06801591 *dose (on Cycle 1, Day 29) will be determined individually if data permit.*

PK parameters will be calculated using standard non-compartmental methods:

Parameter	Method of Determination
AUC_{last}	Linear/log trapezoidal method
AUC_{inf}[a]	$AUC_{last} + (C_{last}*/k_{el})$, where $C_{last}*$ is the predicted concentration at the last quantifiable time point (T) estimated from the log-linear regression analysis, and k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. The terminal log-linear phase will be determined from a minimum of 3 concentration-time data points, and will be verified with the r2 value.
C_{max}	Observed directly from data
CL/F[a]	$Dose/AUC_{inf}$
$t_{½}$[a]	$ln2/k_{el}$
T_{max}	Observed directly from data

[a] if data permit.

The PK parameters of each compound will be summarized as below:

Parameter	Summary statistics
AUClast, AUC_{inf}, C_{max}, and CL/F	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean.
$t_{1/2}$	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum.
T_{max}	N, median, minimum, maximum.

8.1.6. Tremelimumab and PF-06801591 Immunogenicity

For patients receiving tremelimumab, the percentage of subjects with positive ADA and neutralizing antibodies will be summarized by dosing cohort. For patients with positive ADA, the magnitude (titer), time of onset, and duration of ADA response will also be described, if data permit.

For patients receiving PF-06801591, the percentage of subjects with positive ADA and neutralizing antibodies will be summarized by dosing cohort. For patients with positive ADA, the magnitude (titer), time of onset, and duration of ADA response will also be described, if data permit.

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9. REFERENCES

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2. Nishino M, Gargano M, Suda M, Ramaiya NH, Hodi FS. Optimizing immune-related tumor response assessment: does reducing the number of lesions impact response assessment in melanoma patients treated with ipilimumab? J Immunother Cancer 2014;2:17.

10. APPENDICES

10.1. Appendix: Statistical Methods for Immunogenicity (serology)

Definitions:

Let x_i, i=1, 2, n, be the titer for subject i where n is the number of subjects. Then the geometric mean titer (GMT) is defined as follows

$$GMT=\exp[(1/n)\Sigma\log(x_i)],$$

where log is the natural logarithm.

Within each vaccine group and for each antibody, geometric mean titers (GMTs) will be calculated. Each titer will be logarithmically transformed for analysis. Two (2)-sided, 80% confidence intervals will be constructed by back transformation of the confidence intervals for the mean of the logarithmically transformed assay results.

Let x_i, i=1, 2, n, be the titer for test group and let y_i, i=1, 2, m, be the titer for reference group. Then the geometric mean ratio (GMR) is defined as follows:

$$GMR=\exp[(1/n)\Sigma\log(x_i) - (1/m)\Sigma\log(y_i)].$$

Also for the GMRs, the confidence intervals will be constructed by back transformation of the confidence intervals for the mean difference of the logarithmically transformed assay results (*test* relative to *reference*).

Let x_i, i=1, 2, n, be the titer prior vaccination and let y_i, i=1, 2, n, be the titer post vaccination. Then the geometric mean fold rise (GMFR) is defined as follows:

$$GMFR=\exp[(1/n)\Sigma\log(y_i/x_i)].$$

For the geometric mean fold rise, the confidence intervals will be constructed by back transformation of the confidence intervals for the mean difference of the logarithmically transformed assay results (*post* to *prior*).

GMTs, GMRs and GMFR will be estimated by an ANCOVA model with natural log transformed anti-body titer as outcome variable, and treatment group as factor and baseline (in log scale) as covariates at each of the post-dose measurement. These analyses based on log-transformed data, the LSMEAN estimates and confidence interval will be back-transformed (exponentiated) and presented. The estimated treatment difference and corresponding confidence intervals from the model will also be back-transformed.

-For GMTs the variable of interest, that will be back transformed, is $\log(x_i)$

x_i, i=1, 2, n, be the titer for subject i

-For GMFRs the variable of interest, that will be back transformed, is $\log(y_i) - \log(x_i)$

x_i, i=1, 2, n, be the titer prior vaccination and let y_i, i=1, 2, n, be the titer post vaccination

Back-transformation of estimated difference in GMTs (with contrasts) is interpreted as GMR between treatment and placebo group.

In addition to the adjusted analysis for GMTs, GMRs and GMFRs between groups through analysis of covariance, unadjusted (crude) GMTs, GMRs and GMFRs will be calculated as a sensitivity analysis.

For GMTs, two (2)-sided, 80% confidence intervals will be constructed by back transformation of the confidence intervals for the mean of the logarithmically transformed assay results computed using the Student t distribution.

For the GMRs, the confidence intervals will be constructed by back transformation of the confidence intervals for the mean difference of the logarithmically transformed assay results (*test* relative to *reference*) computed using the Student t distribution.

For reporting purposes, 1 decimal place will be used for geometric mean titers (GMTs), and 2 decimal will be used for geometric mean ratios (GMFRs, GMRs).

10.2. Appendix: PSA kinetics_AppendiCES

PSA velocity is defined as the slope of the linear regression line of PSA against time in month.

PSA slope is the slope of the linear regression line of natural log of PSA against time in month.

PSA doubling time is defined as the natural log of 2 divided by the slope of the linear regression line of the natural log of PSA against time in month

As a numerical example

Date	**PSA**	**Log_n**	**Log_2**
5/1/2016	0.5	-0.69	-1.00
6/1/2016	1	0.00	0.00
7/1/2016	0.6	-0.51	-0.74
8/1/2016	1	0.00	0.00
10/1/2106	1.6	0.47	0.68
PSA Slope	0.20	PSA Doubling Time	3.49 months
PSA Velocity	0.19 ng/ml/mo		

10.3. Appendix: Time to Event Data Analysis Censoring Rules

The specific programming specifications and censoring rules for PFS/rPFS will be defined in the Analysis Programming Specification.

Additionally, During the global pandemic of COVID-19, if a patient has missed 2 or more tumor assessments for causes related to COVID-19 (if the causes were identifiable and verified from any source), the patient may not be censored at the last objective tumor assessment, and the subsequent available tumor assessments can be used to determine an event of disease progression or as the censoring point.

10.4. Appendix: Immune-related Response Criteria per RECIST 1.1 (irRECIST)

Immune-related progressive disease (irPD) needs to be confirmed by a second, consecutive assessment at least 4 weeks apart. irPD is also considered to be confirmed if the patient.

- dies within 12 weeks after the initial observation of irPD, or
- discontinues treatment due to disease progression (clinical deterioration without radiological documentation) prior to or within 12 weeks after the assessment of irPD.

Immune-related Best Overall Response (irBOR) will be derived based on reported lesion responses at different evaluation time points from the start date until confirmed immune-related disease progression per irRECIST, according to the following rules.

- Confirmed irCR = at least two determinations of irCR at least 4 weeks apart before irPD.
- Unconfirmed irCR = one determination of irCR before irPD (and not qualifying for confirmed irCR or irPR).
- Confirmed irPR = at least two determinations of irPR or better at least 4 weeks apart before irPD (and not qualifying for confirmed irCR).
- Unconfirmed irPR = one determination of irPR before irPD (and not qualifying for confirmed irCR or irPR or unconfirmed irCR).
- irSD = at least one irSD or irPD assessment ≥6 weeks after start date and before confirmed irPD (and not qualifying for confirmed/unconfirmed irCR or irPR).
- irPD = ≤12 weeks after the first dose of study treatment and at least two consecutive determinations of irPD at least 4 weeks apart. irPD is also considered to be confirmed if the patient.
    - dies within 12 weeks after the initial observation of irPD, or
    - discontinues treatment due to disease progression (clinical deterioration without radiological documentation) prior to or within 12 weeks after the assessment of irPD.
- NE: All other cases.

Only tumor assessments performed before the start of any further anti-cancer treatment will be considered in the assessment of irBOR.

Immune-related Objective Response (irOR) is defined as irCR or irPR according to irRECIST from start date until confirmed irPD or death due to any cause. Both irCR and irPR must be confirmed by repeat assessments performed no less than 4 weeks after the criteria for immune-related response are first met. Also, irPD must be confirmed by a

second, consecutive assessment at least 4 weeks apart. Immune-related OR rate (irORR) is the proportion of patients with irOR in the analysis set.

CCI [redacted] Immune-relate DC rate (irDCR) is the proportion of patients with irDC.

Immune-related Duration of Response (irDR) is defined, for patients with an immune-related objective response, as the time from first documentation of immune-related objective response (irCR or irPR) to the date of first documentation of irPD (which is subsequently confirmed) or death due to any cause. If a patient has not had an event (irPD or death), irDR is censored at the date of last adequate tumor assessment. The censoring rules for irDR are as described below for irPFS.

irDR (months) = [date of event or censoring– first date of irOR +1]/30.4375

Immune-related Time to response (irTTR) is defined, for patients with an irOR, as the time from the start date to the first documentation of immune-related objective tumor response (irCR or irPR) which is subsequently confirmed.

irTTR (in months) = [first date of irOR – start date +1]/30.4375

Immune-related Progression-Free Survival (irPFS) is defined as the time from start date to the date of irPD or death due to any cause, whichever occurs first. irPFS data will be censored on the date of the last adequate tumor assessment for patients who do not have an event (confirmed irPD or death), for patients who start a new anti-cancer therapy prior to an event or for patients with an event after two or more missing tumor assessments. Patients who do not have a baseline tumor assessment or who do not have any post-baseline tumor assessments will be censored on the start date unless death occurred on or before the time of the second planned tumor assessment in which case the death will be considered an event.

The censoring and event date options to be considered for the irPFS and irDR analysis are presented in Table 2.

Table 2. Outcome and Event Dates for irPFS and irDR Analyses

Scenario	Date of event/censoring	Outcome
No baseline assessment	Start date	Censored [a]
irPD (subsequently confirmed) or death ≤16 weeks after last tumor assessment or ≤16 weeks after start date	Date of first irPD or death	Event[b]
irPD (subsequently confirmed) or death >16 weeks after the last tumor assessment	Date of last adequate assessment	Censored
Treatment discontinuation due to 'Disease progression' (clinical deterioration without radiological documentation) prior to or within 12 weeks after the assessment of first irPD	Date of first irPD	Event
New anti-cancer therapy prior to confirmed irPD	Date of last adequate assessment before anti-cancer therapy is given	Censored
irPD not confirmed or no irPD	Date of last adequate assessment	Censored

[a] However if the patient dies ≤8 weeks after start date the death is an event with date on death date

[b] irPD is also considered to be confirmed if the patient dies within 12 weeks after the initial documentation of irPD

The analyses for irBOR, irOR, irDR, irDC, irTTR will follow the methodology outlined for the RECIST 1.1 endpoints as follows

- irOR will be summarized but no formal test of hypotheses will be performed (only 95% CIs will be reported)

- irBOR, irOR, irDC, irDR, irTTR and irPFS will be summarized as described in Sections xx, for RECIST endpoints but irDR and irPFS will be analyzed using the censoring rules described in Table 2.