Protocol A9001502

TREATMENT RESISTANCE FOLLOWING ANTI-CANCER THERAPIES

(TRANSLATE)

Statistical Analysis Plan
(SAP)

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1. VERSION HISTORY

Version	Date	Author(s)	Summary of Changes/Comments
3	June 02, 2020	PPD	• This SAP amendment is driven by protocol amendment 2, dated on April 3, 2020. • SAP template is updated and TOC is included for easy navigation. • Table 1 and Figure 1 are removed from Section 2.2. Appropriate table and figure from the protocol are referenced. This will prevent future amendment of SAP due to minor changes in the Table 1 and Figure 1 of the study protocol. • Language in Section 2.2 is updated to reflect the above change. • Minor language edits for other sections for better readability.

2	July 20, 2019	PPD	• Added a definition of the fully biomarker evaluable (FBE) population in Section 4.2. • Added Cohen's Kappa method for assessment of agreement between two samples on a categorical endpoint in 7.1.2. • Added the count of subjects carrying the alterations for both archival and denovo samples in BET(WETD) and BET(TTD) population in 7.2.1. • Added p values in report of the estimate of change of a continuous endpoint between archival and denovo samples in 7.2.3 and 7.2.4. • Section 7.2.4, "For overall agreement … will be generated for each subject in the BE (TBD) and BET (TBD) population" changed to "For overall agreement … will be generated for each subject in the BE population". • Added 7.2.6 Other analysis.
1	October 18, 2018	PPD	Version 1

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study A9001502. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

Texts copied from the protocol are italicized.

2.1. Clinical Study Background

The overall objective of this study is to better understand why tumors become resistant to treatment with standard of care (SOC) therapies that target critical aspects of tumor biology. Limited availability of post-progression tumor tissue has compromised efforts to rigorously characterize the tumor genomic and microenvironmental factors that drive resistance. Understanding mechanisms of resistance is critical to developing better anti-cancer treatments and treatment combinations. This study specifically addresses the key challenges associated with studying clinical mechanisms of resistance.

Intrinsic or baseline tumor genetic heterogeneity, reflecting varying degrees of both intra- and inter-patient biologic diversity, represents a defining characteristic of cancer biology. In addition, the host response to cancer further defines the tumor microenvironment within which each patient's cancer is subject to further selection, establishing distinct immune landscapes that reflect the complex tumor-immune cell interactions. The introduction of targeted therapeutic agents that potently modulate tumor and/or immune cell biology impose additional, very specific, selection pressures that further define the evolving biology of each patient's cancer, frequently resulting in resistance following an initially beneficial treatment. Given the underlying genetic heterogeneity and the diversity of potential immune landscapes in cancer, informative analysis of mechanisms of resistance requires the application of robust platform technologies supporting immuno-genomic profiling of post-progression tumor tissue applied uniformly across a large series of patients.

Understanding clinical mechanisms of resistance has proven challenging because obtaining the required clinical tumor samples once resistance is established is difficult and the rationale for patients to undergo a de novo tumor biopsy after progression has been limited. As a result, studies of clinical mechanisms of resistance often derive at best from very limited number of studies or case reports, each describing results from analysis of small numbers of patients. Furthermore, these independent studies do not employ common or broad molecular profiling technologies, further limiting the utility of the information. This information gap compromises efforts to develop therapeutic agents or combinations of therapeutic agents that might overcome these mechanisms of resistance to initially effective anti-cancer therapies. Despite these limitations initial progress has been made in identification of clinical mechanisms of resistance to different therapeutic agents, and this has been complemented by studies of resistance in preclinical models, as summarized below. The development of high quality clinical Next Generation Sequencing (NGS) tests, together with the availability of a broad spectrum of targeted therapeutic agents that are approved or in clinical development, provides additional rationale to pursue a de novo tumor biopsy for patients in whom a biopsy procedure can be safely performed. Broad and deep genomic characterization by NGS in larger cohorts increases the probability of identifying clinically relevant resistance mechanisms.

2.2. Biomarker Study Design

This is a multi-national, multi-center study to collect and analyze archival pre-treatment tumor and post-progression biospecimens (de novo tumor biopsy and blood) from patients with advanced or metastatic cancer whose disease has progressed on selected SOC therapy. Evaluable patients are defined as those patients for whom paired archived and post-progression tumor tissue is sufficient for the planned translational analyses.

An initial pre-screening informed consent will be obtained to allow assessment of archival tumor tissue availability and adequacy. Only those patients who meet archival tumor tissue pre-screening requirements will be eligible to participate in the main study consisting of study-related procedures, post-biopsy follow-up visit and assessments. Molecular profiling, including NGS analysis, will be performed on the archival and post-progression biospecimens.

As part of study participation, results from the NGS analysis of the de novo post-progression biopsy and/or blood biospecimens will be returned to the patient's physician/health care provider. Results will come from tests performed in a College of American Pathologists (CAP) CAP-accredited, Clinical Laboratory Improvements Amendments (CLIA)-certified, and good clinical practice (GCP)-compliant clinical reference third-party laboratory using a commercially available NGS test. A study-specific follow-up consultation between the treating physician and patient will be conducted to discuss the NGS results and potential treatment options (see Schedule of Activities in the protocol). All treatment decisions, including potential referral to a clinical trial, will be per the treating physician as part of the patients' clinical care and are not dictated or defined by the study. Further details of the study design are described in Figure 1 and section 3 (Study Design) of the study protocol.

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Target areas of biology, disease cohorts, prior SOC therapy, indications and cohort sizes are described in Table 1 and Section 3 (Study Design) of the study protocol. The total number of patients in this sudy depends on number of cohorts and cohot sizes. All cohorts will enroll concurrently. Individual cohorts and cohort sizes may be adjusted based upon emerging data. Cohort size refers to evaluable patients as defined above.

2.3. Biomarker Study Objectives

Table 1 Study Objectives

Primary Objective:
• *To obtain and analyze archival pre-treatment tumor samples and post-progression tumor biopsies to identify molecular markers of resistance to selected anti-cancer therapies.*
Secondary Objectives:
• *To evaluate the success rate in obtaining paired archival and post-progression tumor biopsies that are adequate to meet the objectives of the study;* • *To determine concordance in gene alterations identified by NGS analysis of post-progression tumor tissue and blood.*
Secondary Objectives by Target Biology:*
PD-1/-L1 inhibition: • *To evaluate alterations in genes encoding HLA, β2-Microglobulin, Signal transducer and activator of transcription 1(STAT1), JAK1, JAK2, IFN-γ, and interferon-gamma receptor (IFNGR) as mechanisms of resistance to PD-1/PD-L1 checkpoint blockade.*
CDK4/6 inhibition: • *To evaluate alterations in the Rb gene as a mechanism of resistance to palbociclib.*
AR inhibition: • *To evaluate AR gene alterations as mechanisms of resistance to enzalutamide or abiraterone;* • *To evaluate changes in expression of nuclear hormone receptor genes as a mechanism of resistance to enzalutamide or abiraterone.*
PARP inhibition: • *To evaluate somatic reversion of germline BRCA (gBRCA) gene alterations as a mechanism of resistance to monotherapy PARP inhibition.*
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*All abbreviations are explained in the Appendix 1 of study protocol.

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

A formal interim analysis will not be performed for this study. However, multiple snapshots of the data will be taken for data review based on accrual (eg, at 25%, 50% and 75% of cohort and/or study accrual).

4. HYPOTHESES AND DECISION RULES

4.1. Biological and Statistical Hypotheses

No formal hypothesis testing will be performed.

4.2. Quantitative Decision Rules

Not applicable.

4.3. Operational Hypothesis

Not applicable.

5. ANALYSIS POPULATIONS

5.1. Safety Analysis (SA) Population

The SA population is defined as all patients who were enrolled and for whom a de novo biopsy procedure or research blood draw was performed.

5.2. Biomarker Evaluable Patient Analysis

The Biomarker Evaluable (BE) population is defined as patients in the SA population who have at least one biomarker result from analysis of collected biospecimen. The fully biomarker evaluable (FBE) population is defined as the patients in the SA population who have every biomarker result from analysis of collected biospecimen.

The Biomarker Evaluable Target (BET) population is defined as patients in the BE population who have a targeted tumor DNA panel biomarker result from both the archival and de novo biopsy tumor tissue biospecimen.

Additional subset populations include:

- BET (TTD), defined as all patients in the BET population who have results of targeted tumor DNA (TTD) NGS gene panel sample analysis from both the archival and de novo biopsy tumor tissue biospecimen.
- BET (TTR), defined as all patients in the BET population who have results of targeted tumor RNA (TTR) sample analysis from both the archival and de novo biopsy tumor tissue biospecimen.
- BET (WETD), defined as all patients in the BET population who have results of whole exome tumor DNA (WETD) NGS sample analysis from both the archival and de novo biopsy tumor tissue biospecimen.

- BET (WTTR), defined as all patients in the BET population who have results of whole transcriptome tumor RNA (WTTR) NGS sample analysis from both the archival and de novo biopsy tumor tissue biospecimen.

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- BET (TTD-TTR), defined as all patients in the BET population who have both evaluable targeted tumor DNA (TTD) NGS gene panel data and targeted tumor RNA (TTR) sample analysis from both the archival and de novo biopsy tumor tissue biospecimen.

- BE (TBD), defined as all patients in the BE population who have results of targeted blood cfDNA (TBD) NGS gene panel sample analysis from the post- progression blood sample.

- BET (TBD), defined as all patients in the BET population who have results of targeted blood cfDNA (TBD) NGS gene panel sample analysis from the post- progression blood sample.

5.3. Derivations and Excluded Data

All data will be included for statistical analysis except data resulting from sample analyses that fail QC Biomarker assays or tests from different bioanalytical laboratories and employing different platform technologies will have different QC standards and metrics that are defined as part of the assay or test validation. Exclusion of biomarker data for statistical analysis will reflect pre-defined QC criteria, established prior to final analyses, as reflected in bioanalytical reports from the bioanalytical laboratory.

6. ENDPOINTS AND COVARIATES

6.1. Endpoints

Table 2 Primary, Secondary and Exploratory Endpoints

Primary Endpoint:
• *Change in the frequency of gene alterations between pre-treatment tumor samples and post-progression tumor biopsies.*
Secondary Endpoints:
• *Proportion of patients with fully evaluable archival and post-progression tumor biopsy (eg, sample sufficient for all intended analyses at all measured time points);* • *Overall agreement rates of gene alterations between post-progression biopsy tissue and blood NGS results.*
Secondary Endpoints by Target Biology:*
PD-1/-L1 inhibition: • *Change in the frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFN-γR between pre-treatment archival and post-progression samples;* • *The frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFNGR in cfDNA.*
CDK4/6 inhibition: • *Change in the frequency of RB1 gene alterations between pre-treatment archival and post-progression samples.* • *The frequency of RB1 gene alterations in cfDNA and tumor tissue.*
AR inhibition: • *Change in the frequency of AR gene alterations between pre-treatment archival and post-progression samples;* • *The frequency of AR gene alterations in cfDNA and tumor tissue;* • *Changes in the expression of nuclear hormone receptors or related RNA signatures reflecting nuclear receptor pathway activity between pre-treatment archival and post-progression samples.*
PARP inhibition: • *Change in the frequency of somatic reversion alterations in gBRCA mutant allele between pre-treatment archival and post-progression samples.*
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*All abbreviations are explained in the Appendix 1 of study protocol.

6.2. Clinical Data Included in the Analysis

No clinical data will be analysed as dependent variables in this study.

6.3. Covariates

Additional covariates may be considered if regression models are used, including for example age, gender, race, cancer related medical history, duration of most recent cancer treatment and stage of tumor.

7. DATA PREPARATION

7.1. Biomarker Sample Analysis

- Biomarkers measured in both archival tumor tissue and the de novo tumor biopsy may include, but may not be limited to, the following:
    - Targeted tumor DNA (TTD) NGS gene panel (Tempus xT; 595 gene panel);
    - Whole exome tumor DNA (WETD) NGS (Tempus Whole Exome Sequencing);
    - Targeted tumor RNA (TTR) panel (HTG 2560-transcript Oncology Biomarker Panel (OBP));
    - Whole transcriptome tumor RNA (WTTR) NGS (Tempus RNAseq);
    - CCI [redacted]
- Biomarkers measured in blood may include, but may not be limited to, the following:
    - Targeted blood cfDNA (TBD) NGS gene panel (Guardant 360 and/or Guardant OMNI).

7.2. Biomarker Data Management

Data generated by analytical laboratories will be delivered through secure FTP or through the best practices of the industry to ensure high level data protection. The data received will be stored in the sponsor's secure server.

Data QC document from each analytical laboratory will be delivered to the project team and stored in the sponsor's secure server. Additional QC will be performed by the computational biology team, and the QC methods will be defined in the supplemental statistical analysis plan.

7.3. Handling of Missing Values

Assayed values that are below the lower limit of quantification (LLOQ) will be set to LLOQ. Imputation will not be performed in the case of missing values.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methods

8.1.1. Statistical Methods for Continuous Variables

For the continuous variables, summary statistics including mean, min, max, quartiles, and 95% confidence intervals (CIs) of the mean will be reported per tumor type and cohort. For the continuous variables that are measured from two samples from the same subject, change between the two samples will be calculated. Summary statistics including mean, min, max, quartiles and 95% CIs of the change will be calculated for each cohort. As appropriate paired t test (or non-parametric equivalent) will be performed for the change and p values will be reported. For high dimensional data such as WES, RNASeq, Gene panel etc., a false discovery rate (FDR) based method (qvalue package in R or proc multtest in SAS) will be used for multiplicity adjustment (Storey, 2015).[3]

8.1.2. Statistical Methods for Categorical Variables

Regular Wald CIs suffer from low coverage probabilities that may be below nominal level. An improved CI estimation for the difference of proportions between matched pair will be employed (Agresti and Min, 2005; Tango 1998).[1,4]

For n matched pairs on a binary response, denote the probability of outcome i for the first observation and outcome j for the second observation by πij, where outcome 1 = 'positive' and 2 = 'negative'. Denote the four corresponding sample proportions by p_{11} = a/N, p_{12}= b/N, p_{21}= c/N, and p_{22} =d/N. Let $\pi_1 = \pi_{11} + \pi_{12}$, $\pi_2 = \pi_{21} + \pi_{22}$. Denote the sample proportions of positives by p_1 and p_2, $p_1 = p_{11} + p_{12}$, $p_2 = p_{21} + p_{22}$. Notations are illustrated in the table below:

	Positive	Negative	Total
Positive	a (p_{11})	b (p_{12})	a + b (p_1)
Negative	c (p_{21})	d (p_{22})	c + d
Total	a + c (p_2)	b + d	N

For the matched pairs, the score interval consists of the set of $\Delta = \pi_1 - \pi_2$ values for which

$$\frac{|(p_2 - p_1) - \Delta|}{\sqrt{[(\hat{\pi}_{12}(\Delta) + \hat{\pi}_{21}(\Delta)) - \Delta^2]/n}} < z_{\alpha/2}$$

where $\pi_{jk}(\Delta)$ denotes the ML estimate of π_{jk} under the constraint that $\pi_1 - \pi_2 = \Delta$. There is not a closed-form expression for the resulting interval, but it can be obtained using iterative methods. The iteration stops when the score is close to Zα/2 within defined margin 1e-06. The calculation of the score based interval can be performed with function scoreci.mp() in R package PropCIs or equivalent SAS macro program. McNemar test (mcnemar.test() in R or proc freq in SAS) will be used to compare the proportions between archival and de novo samples, and p values will be reported. For high dimensional data such as WES, RNASeq,

Gene panel etc., an FDR based method (qvalue package in R or proc multtest in SAS) may be used for multiplicity adjustment (Storey, 2015).[3]

For the CI estimation of proportion from one sample, Wilson score method will be used (Wilson, 1927). The calculation of the score based interval can be performed with function scoreci in R package PropCIs or proc freq in SAS.

$$\frac{\hat{p} + \frac{z^2}{2n}}{1 + \frac{z^2}{n}} \pm \frac{z}{1 + \frac{z^2}{n}} \sqrt{\frac{\hat{p}(1-\hat{p})}{n} + \frac{z^2}{4n^2}}$$

Assessment of agreement between two samples on a categorical endpoint can be calculated using Cohen's kappa (Cohen, 1960) in Proc Freq.[2]

8.2. Statistical Analysis

8.2.1. Analysis of Primary Endpoint

The primary endpoint is the change in the frequency of gene alterations associated with disease progression. The analysis of primary endpoint will be performed on data set in the BET (TTD) and BET (WETD) population defined in Section 5.2. As described in Section 8.1.2, score based method and McNemar test will be used for CI estimation and hypothesis testing. For each gene alteration in each cohort, the frequency of alteration is calculated as # of patients who carry the alteration divided by the total number of patients in the cohort. For both archival and denovo samples in BET(WETD) and BET(TTD) population, the count of subjects carrying the alterations, estimates and 95% CI of the frequency of alterations, the change in the frequency of alterations, p values and qvalues of the change in frequency will be reported.

8.2.2. Analysis of Secondary Endpoints

Summary statistics will be generated for the secondary endpoints. For the proportion of patients with fully evaluable archival and post progression tumor biopsies, proportions and 95% CI will be calculated using the method described in Section 8.1.2. For the overall agreement rates of gene mutations between post progression biopsy tissue and blood NGS results, basic summary statistics such as mean, median, and range of count of alterations per patient across all patients will be generated for each cohort. In addition, mean and median count of alterations in agreement between biopsy and blood samples will be calculated for each cohort. The frequency of alterations detected from post progression biopsy tissue, blood and the corresponding 95% CIs will be generated. In the BE (TBD) and BET (TBD) population, *the concordance between both matched biopsy tissue and blood samples and the 95% CI and Cohen's Kappa will also be calculated to evaluate the agreement.*

8.2.3. Analysis of Asset Specific Secondary Ednpoints

Methods described in Section 8.1.2 will be used for the analyses defined in the table below on the BET (TTD) and BET (WETD) population.

Table 3 Analysis Methods for Secondary Endpoints by Target Biology

Target Biology	Secondary Endpoints	Analysis Methods
PD-1/-L1 inhibition	• *Change in the frequency of alterations in genes encoding HLA, β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFNGR between pre-treatment archival and post-progression samples.* • *The frequency of alterations in genes encoding HLA.* • *β2-Microglobulin, STAT1, JAK1, JAK2, IFN-γ and IFNGR.* • *in cfDNA.*	• *For each alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated; A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples.* • *For each alteration in cfDNA, the estimate of the frequency and the 95% CI will be generated.*
• *CDK4/6 inhibition.*	• *Change in the frequency of RB1 gene alterations between pre-treatment archival and post-progression samples.* • *The frequency of RB1 gene alterations in cfDNA, and tumor tissue.*	• *For each alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples.* • *For each alteration in cfDNA, the estimate of the frequency and the 95% CI will be generated.*

• *AR inhibition.*	• *Change in the frequency of AR gene alterations between pre-treatment archival and post-progression samples.* • *The frequency of AR gene alterations in cfDNA, and tumor tissue.* • *Changes in the expression of nuclear hormone receptors or related RNA signatures reflecting nuclear receptor pathway activity between pre-treatment archival and post-progression samples.*	• *For each alteration in cfDNA, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples.* • *For each alteration, the estimate of the frequency and the 95% CI will be generated.* • *Estimates of mean expression and 95% CIs at pre-treatment archival and post progression will be generated; Estimates of the difference in expression and the 95% CI between pre-treatment archival and post-progression samples will be generated. P values will also be reported.*
• *PARP inhibition.*	• *Change in the frequency of somatic reversion alterations in gBRCA mutant allele between pre-treatment archival and post-progression samples.*	• *For each somatic reversion alteration, the estimate of the frequency and the 95% CI at pre-treatment archival and post-progression will be generated. A score based 95% CI will be generated for the difference in frequency between pre-treatment archival and post-progression samples.*

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8.3. Analysis of Safety Data

Formal statistical analysis, eg, statistical testing, will not be performed on the safety data. However, descriptive statistics will be generated for adverse events in the SA population.

8.4. Other Analysis

Demographics and baseline characteristics summary in the SA population will be provided. Summary of sample sizes from each population by cohort will be performed.

9. REFERENCES

1. Agresti A, Min Y. 2005. Simple improved confidence intervals for comparing matched proportions. Stat Med 2005; (24):729 40.

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5. Wilson, E.B. (1927) Probable inference, the law of succession, and statistical inference J. Amer. Stat. Assoc 22, 209-212.