APPENDIX A

ADDITIONAL GUIDANCE FOR COMPLETING SECTION D, "BIOMARKER STATISTICAL PLAN"

Statistical analysis plans for biomarker studies should describe how the primary biomarker objectives will be addressed in a quantifiable and statistically evaluable way.

- The statistical plan should indicate the specific quantities or parameters of biomarkers that will be evaluated and the general statistical framework (e.g., estimation, association, comparison, prediction).
- **The statistical plan should align with the biomarker study objectives**.

The Biomarker Statistical Plan may require the following elements:

- Statistical methods for the primary analyses (e.g., Cox proportional hazards regression, conditional or unconditional logistic regression, etc.)
- Which biomarkers will be employed, and how they will be included in the analyses.
- If cutpoints will be used, specify the cutpoint(s). Provide the rationale for the cut-point(s) selected.
  - What proportion of subjects is expected to have values above and below the proposed assay value cut-points?
  - What magnitude of effect (e.g., treatment benefit) or outcome (e.g., prognosis) is expected for patients with assay results above and below the proposed cutpoint(s)?
- Criteria and metrics for defining significant changes (e.g., between timepoints, between responders and non-responders).
- Multiple-comparisons adjustment methods to prevent false positives, especially if many biomarkers are proposed.
- Scoring system(s).
- Variable selection procedures.
- Will the analysis involve validation? (for example, of a prognostic signature)
- List of standard clinical variables to be incorporated into models or other analyses.
- Transformations applied to variables.
- If only certain cohorts will be analyzed, please specify which cohorts and their sample size.
- If a subset of cases will be used, please describe the sampling plan and how bias will be avoided.

- Power calculation and sample size(s) and rationale:
  - Although power calculations may not be needed for all early-phase trials, especially small trials, it can still be useful to calculate the power to detect a given effect size with the samples that will be available.
  - The sample size rationale should include a clear explanation (or cited reference) for the method of sample size determination along with a statement of all assumptions required to perform that calculation so that an independent statistician would be able to reproduce the estimates from the information provided in the application.
  - Typically, a sample size estimate will require assumptions about the following:
    - Anticipated distribution of marker values in the targeted population(s)
    - (e.g., marker positivity rate if the marker is dichotomous)
    - Anticipated assay success rates
    - Event rates or number of events anticipated
    - Expected effect size(s)

EXAMPLE OBJECTIVE, HYPOTHESIS, AND CORRESPONDING STATISTICAL PLAN

Integrated biomarker: PD-L1

Objective: Demonstrate changes in PD-L1 that correlate with response to pembrolizumab.

Hypothesis: Response to pembrolizumab therapy will be associated with an increase in the percentage of cells showing PD-L1 positive staining.

Supporting assay(s): PD-L1 Singleplex IHC (FDA-approved PD-L1 kit 22C3 pharmDx by Dako)

Statistical plan:

- **Clinical endpoints to be used in the correlative analysis for this biomarker:** The primary endpoint will be proportion of patients experiencing an overall response (CR or PR) at 6 months. Response evaluation will be determined using the RECIST criteria.
- **Statistical analysis plan for biomarker:**
    - All patients with available tumor samples will be included in this analysis.
    - PD-L1 will be described as percentage of cells showing any positive staining. Additional analyses will look at PD-L1 as a binary endpoint with cut points of 1% and 50%.
    - Analyses will be descriptive in nature. Box plots describing PD-L1 by response group and average PD-L1 % staining will be compared for patients experiencing a response and not experiencing a response. A logistic regression will be employed to correlate binary ORR with continuous PD-L1%. Additional exploratory analyses will assess proportion of patients experiencing an ORR in PD-L1 low and PD-L1 high groups, as defined by both a 1% and 50% cut point. A two-sided Fisher's Exact test will be used to compare ORR rate in the low versus high groups.