STATISTICAL ANALYSIS PLAN

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A Phase 2, Open-Label, Single-Arm, Multicenter Study to Assess the Safety and Efficacy of ASP1650, a Monoclonal Antibody Targeting Claudin 6 (CLDN6), in Male Subjects with Incurable Platinum Refractory Germ Cell Tumors

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
3xW	Three times a week
ACT	Anti-cancer therapy
AE	Adverse event
AEoSI	Adverse event of special interest
AFP	Alpha-fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
ASCM	Analysis set classification meeting
AST	Aspartate aminotransferase
AT	Aminotransferases
AUC	Area under the concentration-time curve
AUC_{336}	AUC from time 0 to 336 hrs after dosing
βhCG	Beta human chorionic gonadotropin
BOIN	Bayesian Optimal Interval
BUN	Blood urea nitrogen
C1D1	Cycle 1/day 1
C2D14	Cycle 2/day 14
CBR	Clinical benefit rate
CDC	Complement-dependent cytotoxicity
CK	Creatine phosphokinase
CL	Total clearance after intravenous dosing
CLDN6	Claudin 6
C_{max}	Maximum concentration
CS	Classification Specifications
CSR	Clinical study report
CR	Complete response
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
C_{trough}	Trough concentration
CV	Coefficient of variation
DEAS	Dose limiting toxicity evaluation analysis set
DEC	Dose Evaluation Committee
DLT	Dose limiting toxicity
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
FAS	Full analysis set
FFPE	Formalin fixed paraffin embedded
GCP	Good Clinical Practice

Abbreviations	Description of abbreviations
Hct	Hematocrit
Hgb	Hemoglobin
ICF	Informed consent form
ICH	International Council for Harmonisation of technical requirements for registration of pharmaceuticals for human use
IND	Investigational New Drug
INR	International normalized ratio
IRR	Infusion-related reaction
IRT	Interactive response technology
ISN	International study number
kg	Kilograms
LDH	Lactate dehydrogenase
mAb	Monoclonal antibody
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
µg/mL	Micrograms/milliliter
mg	Milligrams
mmHg	Millimeter of mercury
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCA	Noncompartmental analysis
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria for Adverse Events
NE	Not evaluable
ORR	Objective response rate
PD	Progressive disease
PGx	Pharmacogenomics
PFS	Progression-free survival
PKAS	Pharmacokinetic analysis set
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
Q2W	Every 2 weeks
Q3W	Every 3 weeks
RBC	Red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
RDI	Relative dose intensity
RP2D	Recommended phase 2 dose
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical Analysis Plan

Abbreviations	Description of abbreviations
SD	Stable disease
T4	Thyroxine
TBL	Total bilirubin
TNM	TNM Classification of Malignant Tumors
TP	Total protein
TSH	Thyroid stimulating hormone
TEAE	Treatment-emergent adverse event
$t_{1/2}$	Terminal elimination half-life
t_{max}	Time of maximum concentration
ULN	Upper limit of normal
Vz	Volume of distribution after intravenous dosing during the terminal elimination phase
WBC	White blood cell

List of Key Terms

Terms	Definition of terms
Baseline	Assessments of subjects as they enter a trial before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a trial.
Enroll	To register or enter a subject into a clinical trial. NOTE: Once a subject has received the study drug or placebo, the clinical trial protocol applies to the subject.
Intervention	The drug, device, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study. (e.g., health-related quality of life, efficacy, safety and pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the test drug or comparative drug.
Post investigational period	Period of time after the last assessment of the protocol. Follow-up observations for sustained adverse events and/or survival are done in this period.
Screening	A process of active consideration of potential subjects for enrollment in a trial.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a trial.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

This Statistical Analysis Plan (SAP) contains technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes procedures for executing the statistical analysis to fulfill the objectives of the study.

The final SAP will be approved prior to database hardlock.

Changes from the planned analyses in the final SAP that impact the statistical analyses will be documented in the Clinical Study Report (CSR).

2 STUDY OBJECTIVE(S) AND DESIGN

2.1 Study Objective(s)

2.1.1 Primary Objective

- To establish the recommended phase 2 dose (RP2D) of ASP1650 in subjects with incurable platinum refractory germ cell tumors (Safety Lead-in Phase)
- To evaluate the efficacy of ASP1650 as measured by confirmed Overall Response Rate (ORR), as assessed by modified Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (RECIST 1.1 and serum tumor biomarker [beta human chorionic gonadotropin [βhCG]] and alpha-fetoprotein [AFP] response criteria), in subjects with incurable platinum refractory germ cell tumors (phase 2)

2.1.2 Secondary Objectives

- To evaluate the following efficacy measures:
    - Confirmed ORR by RECIST 1.1
    - Clinical benefit rate (CBR), as assessed by modified RECIST 1.1 and RECIST 1.1
    - Duration of response (DOR), as assessed by the modified RECIST 1.1 and RECIST 1.1
    - Progression-Free Survival (PFS), as assessed by modified RECIST 1.1 and RECIST 1.1
- To evaluate safety and tolerability of ASP1650
- To evaluate the effect of ASP1650 on changes in serum βhCG and AFP
- To evaluate the pharmacokinetics of ASP1650

2.1.3 Exploratory Objectives

- To evaluate the immunogenicity profile of ASP1650
- To evaluate potential genomic and/or other biomarkers that may correlate with treatment outcome to ASP1650

2.2 Study Design

This is a phase 2, open-label, single-arm, multicenter study to assess the safety and efficacy of ASP1650, an mAb targeting CLDN6, in male subjects with incurable platinum refractory germ cell tumors. Up to 46 subjects at approximately 3 centers located in the United States will participate in the study.

The study consists of 2 phases: Safety Lead-in phase and phase 2. For all subjects, the study will consist of the following periods: Screening, Enrollment, Treatment and Follow-up. Each subject may complete a maximum of 12 treatment cycles. One treatment cycle is 14 days. After discontinuation of study drug treatment, all subjects will complete a study treatment discontinuation visit and a safety Follow-up visit.

1. Screening and Enrollment Period

Screening will take place up to 45 days prior to enrollment. Re-screening may be allowed 1 time per subject upon discussion with the medical monitor.

2. Treatment Period

Safety Lead-in Phase

The Safety Lead-in phase of this study is to establish the tolerability of RP2D (ASP1650 1500 mg/m^2 once every 2 weeks [Q2W]). An initial dose level of ASP1650 1000 mg/m^2 Q2W will be evaluated in a 3-subject cohort (cohort 1), and if well tolerated, a new subject cohort (minimum 3 and up to 4 subjects) will be opened to evaluate a dose level of ASP1650 1500 mg/m^2 Q2W (cohort 2) according to the Bayesian Optimal Interval (BOIN) Design. [Liu et al, 2015]. Based on tolerability observed in cohort 2, an additional subject cohort (cohort 3) will be opened to evaluate 1500 mg/m^2 Q2W (minimum 3 and up to 4 subjects) or de-escalation to 1250 mg/m^2 Q2W if 1500 mg/m^2 Q2W is not tolerable (minimum 6 and up to 8 subjects). Nine to 18 subjects will be enrolled in the Safety Lead-in phase. The RP2D determination will be based on at least 6 evaluable subjects at the RP2D as determined by the Dose Evaluation Committee (DEC).

There will be at least 3 calendar days between the treatment initiation of the first subject and treatment initiation of all subsequent subjects at the same dose level.

The dose limiting toxicity (DLT) observation period will be from cycle 1/day 1 (C1D1) through C2D14. Evaluable subjects are defined as subjects who experience a DLT or in the absence of DLT, complete the DLT observation period. Subjects who are later discovered not to meet eligibility criteria or are not evaluable for DLT may be replaced. If no DLTs are observed in the first 6 evaluable subjects (cohorts 1 and 2), the DLT observation period for cohort 3 may be reduced to ClD1 through C1D14 (one cycle).

Study Design Overview continued:

Dose evaluation rules based on the BOIN design with target DLT rate of 0.30 and optimal interval of (0.236, 0.359) are as follows:

	Number of Subjects Treated at Current Dose Level						
Action	3	4	5	6	7	8	9
Escalate dose if number of subjects with DLT ≤	0	0	1	1	1	1	2
Stay at current dose level if number of subjects with DLT =	1	1	-	2	2	2	3
De-escalate if number of subjects with DLT =	2*	2	2 or 3	3	3 or 4	3 or 4	4
Stop if number of subjects with DLT ≥	3	3	4	4	5	5	5

DLT: dose limiting toxicity.

*The study may be terminated instead of de-escalated upon discussion with the DEC.

The DEC will be responsible for the review of individual subject safety data in order to provide an assessment of whether reduction or escalation should occur within the next cohort and/or to determine when maximum tolerated dose has been reached in a given dose level. Additional details regarding responsibilities and membership requirements will be included in the DEC Charter.

Proposed Dose Levels of ASP1650

Dose Level	ASP1650	Planned Number of Subjects
1	1000 mg/m^2	3
2	1500 mg/m^2	6-8
De-escalated	1250 mg/m^2	Only if necessary based on DLTs of Dose Level 2

DLT: dose limiting toxicity

At minimum, safety data from the DLT observation period are needed for the DEC meeting; however, all available safety findings, including those occurring after the designated DLT observation period that meet DLT criteria ("delayed DLT"), will be considered.

Subjects who are tolerating study drug at a dose level concurrently under review due to DLTs in another subject are allowed to continue dosing through week 24, as tolerated unless otherwise directed by the DEC.

Enrollment of 3 subjects at the target RP2D (1500 mg/m^2 Q2W) will begin once the 1000 mg/m^2 Q2W dose level has been deemed tolerable. A de-escalation dose cohort will be opened at 1250 mg/m^2 Q2W if 1500 mg/m^2 Q2W has been deemed not tolerable. Subjects enrolled in the 1000 mg/m^2 Q2W dose level will continue treatment at that dose level (unless they meet study treatment discontinuation criteria) and will be dose escalated to the RP2D after the RP2D is deemed tolerable.

2.2.1.1 Dose Limiting Toxicity Criteria

A DLT is defined as any of the following adverse events (AEs; graded using National Cancer Institute Common Toxicity Criteria for Adverse Events [NCI-CTCAE] version 5.0) or laboratory findings that the investigator (or sponsor) cannot clearly attribute to a cause other than study drug:

- Grade 4 neutropenia or grade ≥ 3 febrile neutropenia

- Grade 4 thrombocytopenia; or grade 3 thrombocytopenia accompanied by bleeding that requires any transfusion
- Grade 4 anemia or grade 3 anemia requiring transfusion
- Grade ≥ 3 non-hematological AE
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 5 x upper limit of normal (ULN; grade ≥ 3) without liver metastases
- AST or ALT > 8 x ULN in subjects with liver metastases
- AST or ALT > 3 x ULN and total bilirubin > 2 x ULN (in subject with Gilbert syndrome: AST or ALT > 3 x ULN and **direct** bilirubin > 1.5 x ULN)
- Total bilirubin > 3 x ULN (grade ≥ 3)
- Amylase or lipase > 2 x ULN (grade ≥ 3)
- IRR that requires the infusion to be permanently discontinued

Phase 2

Once RP2D has been established as tolerable, up to 34 subjects will be enrolled in phase 2 to receive ASP1650 Q2W starting on C1D1 for up to a maximum of 12 cycles or until a study discontinuation criteria has been met, whichever occurs earlier.

A Simon's 2-stage design is implemented to allow for early termination. In stage I, a total of 13 subjects will be enrolled including the subjects from the RP2D cohort of the Safety Lead-in phase will be evaluated for response. If there is 1 or fewer responses among these 13 subjects, the study will be stopped early. Otherwise, an additional 21 subjects will be enrolled in stage II.

Safety Lead-in and Phase 2 Assessments

Radiologic disease assessment will be evaluated every 6 weeks + 7 days counting from C1D1 for the first 24 weeks and then every 12 weeks ± 7 days thereafter until the subject develops radiological disease progression per RECIST v1.1 by local investigator evaluation or starts other systemic anticancer treatment, whichever comes earlier. All measurable disease must be documented at Screening and re-assessed at each subsequent radiologic evaluation. Imaging will include computed tomography (CT) scans with contrast of the thorax, abdomen and pelvis. If the CT scan with contrast is medically not feasible, a CT scan without contrast or magnetic resonance imaging may be used for imaging. Bone scans (or focal X-ray) or brain imaging may be performed if metastatic disease is suspected. The same mode of imaging should be utilized throughout the study unless medical necessity requires a change.

Blood will be drawn for the measurement of serum AFP and serum βhCG at day 1 of every cycle and during the post treatment period follow up period to align with imaging assessment (every 6 weeks + 7 days counting from C1D1 for the first 24 weeks and then every 12 weeks ± 7 days thereafter) until the subject develops radiological disease progression per RECIST v1.1 by local investigator evaluation or starts other systemic anticancer treatment.

Response assessments for the primary objective of this trial (ORR) will be made using modified RECIST v1.1. The same measurable and non-measurable lesions determined at baseline will be followed at subsequent timepoints according to RECIST v1.1. Response

assessments by RECIST v1.1 and modified RECIST v1.1 will be based upon local investigator evaluation.

Safety Assessments

Safety assessments will include AEs, vital signs, electrocardiograms (ECGs), physical exams, Eastern Cooperative Oncology Group (ECOG) performance status and laboratory assessments. Severity of AEs and laboratory abnormalities will be assessed based on NCI-CTCAE.

Biomarkers and Other Sampling

An archival tumor specimen will be collected prior to first dose of study treatment. If archival tissue is unavailable or insufficient, a tumor biopsy may be performed during the screening period if the subject is an appropriate candidate for tumor biopsy.

Samples for pharmacokinetics, immunogenicity and biomarkers will be collected. An optional on treatment tumor tissue sample may be collected. Pharmacogenomics and post-progression tumor samples may be collected for those subjects who sign separate informed consent forms.

Follow-up Period

Following discontinuation from study treatment, subjects will have a Study Treatment Discontinuation Visit ≤ 7 days after their last dose of study drug or decision by the investigator to discontinue treatment, in addition to a Safety Follow-up visit at 30 days (+7 days) after their last dose of study drug.

If a subject discontinues study drug prior to radiologic progression, the subject should enter the Post-Treatment Follow-up Period. The subject should continue to undergo imaging assessments and serum AFP and serum βhCG every 6 weeks + 7 days counting from C1D1 for the first 24 weeks and then every 12 weeks ± 7 days thereafter until 1 of the following events occurs:

- Radiological disease progression
- Subject starts another anticancer treatment

All post-progression anti-cancer therapies including date and site of progression will be recorded on the electronic case report form (eCRF).

2.2.2 Dose Rationale

The recommended target dose and schedule is 1500 mg/m^2, administered Q2W. This is based on consideration of both clinical and non-clinical efficacy and safety data.

With respect to efficacy, ASP1650 significantly inhibited tumor growth in a mouse testicular cancer xenograft model and prolonged survival over a dose of 4.375 mg/kg to 35 mg/kg three times a week (3xW) corresponding to estimated ASP1650 trough concentrations of 12.5 μg/mL to 100 μg/mL. Animals treated with 17.5 mg/kg (90.77%) or 35 mg/kg 3xW showed > 90% tumor growth inhibition (90.88%) with prolonged survival. The respective ASP1650 trough concentrations were estimated to be 50 and 100 μg/ml.

With respect to safety, no ASP1650-related toxicities were noted in either single dose study in cynomolgus monkeys or 28-day weekly intravenous dose study in mice (100 mg/kg). The MTD was not achieved in the 1650-CL-0101 study, as none of the AEs were considered dose limiting. Elevated amylase was reported in 3 subjects in the ovarian study, with 1 each in 100, 300 and 1000 mg/m^2 dose group. However, exposure safety analysis did not reveal any relationship between drug exposure and amylase values.

Using human pharmacokinetic data from the 1650-CL-0101 study, modeling and simulation results support a dosing schedule of 1500 mg/m^2 Q2W instead of the every 3 weeks (Q3W) schedule employed in the 1650-CL-0101 study. A dosing schedule of 1500 mg/m^2 Q2W is anticipated to lead to a mean trough concentration of 144 µg/mL with approximately 80 and 60% of subjects having trough values above 50 and 100 µg/mL, respectively.

A starting dose of 1000 mg/m^2 Q2W is recommended in this study, which represents a 30% higher dose intensity compared to 1000 mg/m^2 Q3W in the OVAR study. If no dose limiting toxicities are observed in 1000 mg/m^2 Q2W dose, the dose will be increased to 1500 mg/m^2 Q2W. This represents a 31% increase in C_{max}.

2.3 Randomization

N/A

3 SAMPLE SIZE

The sample size for the Safety Lead-in phase is not based on a statistical power calculation. The planned number of up to 18 subjects would provide adequate information for the objectives of the safety cohort.

Simon's optimal 2-stage design [Simon, 1989] will be used for conducting phase 2 of the study. The null hypothesis is that the true ORR is 10%, and the alternative hypothesis is that the true ORR is 25%. The study will be carried out in 2 stages. In stage I, a total number of 13 subjects treated at the RP2D will be evaluated.

If there is 1 or fewer responses among these 13 subjects, the study will be stopped early for futility. Otherwise, an additional 21 subjects will be enrolled in stage II, resulting in a total number sample size of 34. If there are 6 or more responses among these 34 subjects, we reject the null hypothesis and claim that the treatment is promising. The design controls the type I error rate at 10% and yields the power of 80%.

4 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

Detailed criteria for analysis sets will be laid out in classification specifications and the allocation of subjects to analysis sets will be determined prior to database hard-lock.

4.1 Full Analysis Set

The full analysis set (FAS) will consist of all enrolled subjects. This will be the primary analysis set for efficacy analyses.

4.2 Safety Analysis Set

The safety analysis set (SAF) consists of all subjects who took at least 1 dose of study drug, and will be used for safety analyses.

For the statistical summary of the safety data, the SAF will be used.

4.3 Pharmacokinetics Analysis Set

The pharmacokinetic analysis set (PKAS) will consist of the subset of the SAF for which at least 1 ASP1650 concentration measurement is available. Additional subjects may be excluded from the PKAS at the discretion of the pharmacokineticist. The PKAS will be used for description of pharmacokinetic data.

4.4 Dose Limiting Toxicity Evaluation Analysis Set (DEAS)

The dose limiting toxicity evaluation analysis set (DEAS) is defined as all subjects in SAF by excluding the subjects who meet the following criterion:

- A subject without a DLT who receives less than the planned ASP1650 dose during the DLT observation period.

DEAS will be used for the analysis of DLT data.

5 EFFICACY AND SAFETY ENDPOINTS

5.1 Primary Efficacy Endpoints

5.1.1 Objective Response Rate

ORR is defined as the proportion of subjects who have a best overall response of confirmed CR or confirmed PR, as assessed by modified RECIST 1.1. A response of CR or PR must be maintained for at least 4 weeks to be confirmed.

For subjects with measurable disease at baseline, the following criteria in Table 1 will be used to assess response using modified RECIST 1.1:

Table 1 Response Criteria Based on Modified RECIST v1.1

Serum Markers	Target Lesions	Non-Target Lesions		New Lesions	Overall Timepoint Response Assessment
	(target lesion response)	(non-target lesion response)		(unequivocal new lesions)	
Values that fall below the ULN for the assay employed	CR	CR		No or Equivocal	CR
	CR	Not Applicable		No or Equivocal	CR
	Not applicable	CR		No or Equivocal	CR
For both AFP and beta-hCG, no increase ≥ 50% in 2 samples at least 1 week apart compared to nadir values, at least one of the values are above ULN	CR	CR		No or Equivocal	PR
	CR	Not Applicable		No or Equivocal	PR
	Not applicable	CR		No or Equivocal	PR
For both AFP and beta-hCG, no increase ≥ 50% in 2 samples at least 1 week apart compared to nadir values	CR	Non-CR/Non-PD		No or Equivocal	PR
	CR	NE/ND		No or Equivocal	PR
	PR	CR	= not PD	No or Equivocal	PR
	PR	Non-CR/Non-PD	= not PD	No or Equivocal	PR
	PR	NE/ND	= not PD	No or Equivocal	PR
	PR	Not applicable	= not PD	No or Equivocal	PR
For both AFP and beta-hCG, no increase ≥ 50% in 2 samples at least 1 week apart compared to nadir values	SD	CR	= not PD	No or Equivocal	SD
	SD	Non-CR/Non-PD	= not PD	No or Equivocal	SD
	SD	NE/ND	= not PD	No or Equivocal	SD
	SD	Not applicable	= not PD	No or Equivocal	SD
Any	PD	Any		Any	PD
Any	Any	PD		Any	PD
Any	Any	Any		Yes or Unequivocal	PD
Increase ≥ 50% in serum tumor markers AFP or beta-hCG in 2 samples at least 1 week apart compared to nadir values	Any	Any		Any	PD
NE/ND	NE/ND	Not PD		No or Equivocal	NE

CR: complete response; ND: not done; NE: non-evaluable; PD: progressive disease; PR: partial response; SD: stable disease.

Confirmatory assessment for CR or PR should be done at least 4 weeks after the date of the CR or PR was first observed.

In subjects whose only evidence of disease is elevated serum tumor markers (> 2 x ULN AFP or > 2 x ULN βhCG) at baseline, the modified RECIST v1.1 will reduce to serum tumor marker response criteria. Serum marker criteria can be found in section 5.3.1.3 of the protocol. In subjects whose only evidence of disease is elevated serum tumor markers (> 2 x ULN AFP or > 2 x ULN βhCG) at baseline, confirmatory assessment for CR or PR is not required.

Best overall response is determined once all tumor response data for the subject is available. Subjects will be classified by best response on study as outlined in modified RECIST v1.1 criteria. For best overall response of stable disease (SD), SD must be documented as present at least once after study entry and maintained for at least 12 weeks.

ORR as assessed by RECIST 1.1 alone and by serum marker alone will be performed as a sensitivity analysis.

5.2 Secondary Efficacy Endpoints

5.2.1 Clinical benefit rate (CBR)

CBR is defined as the proportion of subjects for each dose level whose best overall response is rated as confirmed CR, PR or durable SD, as assessed by modified RECIST 1.1. Durable SD is a SD maintained for at least 12 weeks.

CBR will be assessed for subjects, using RECIST 1.1 alone, and using serum markers alone.

5.2.2 Duration of Response

Duration of Response (DOR) will be calculated only for the subgroup with confirmed response CR/PR.

DOR is defined as the time from the date of the first confirmed response CR/PR (whichever is first recorded) as assessed by modified RECIST 1.1 to the date of disease progression (assessed by modified RECIST 1.1) or date of censoring, whichever is earlier. If a subject has not progressed, the subject will be censored at the date of last disease assessment or at the date of first confirmed CR/PR if no post-baseline disease assessment is available. DOR will be derived for subjects with best overall response as confirmed CR or confirmed PR.

DOR (in days) is calculated as:

(Date of documented PD, death, or censoring) – (Date of the first CR/PR which is subsequently confirmed) +1.

To apply the cut-off date to DOR is to exclude disease assessments after cut-off date in the analysis.

DOR will be calculated for each subject using RECIST 1.1 alone, and serum markers alone.

Table 2 DOR Definition

Situation	Date of Event or Censor	Outcome
Subject with confirmed CR/PR:		
Disease progression documented per modified RECIST v1.1	Date of PD	Event
No disease progression	Date of last efficacy assessment or at the date of first CR or PR if no postbaseline efficacy assessment is available	Censor

DOR = Date of Event or Censor – Date of first CR or PR +1

5.2.3 Progression-Free Survival (PFS)

PFS is defined as the time from the date of first dose until the date of disease progression as assessed by modified RECIST 1.1, or until death due to any cause. If a subject has neither progressed nor died, the subject will be censored at the date of last disease assessment or at the date of registration if no post-baseline radiological assessment is available. Subjects who receive any further anticancer therapy for the disease before disease progression will be censored at the date of the last disease assessment before the anticancer therapy started. If progression or death occurs after missing 2 scheduled disease assessments, the subject will be censored at the date of last disease assessment or at the date of registration if no post-baseline disease assessment is available. A subject has missed 2 scheduled disease assessments if the gap between two disease assessments is more than 2.5 times the scheduled disease assessment interval. During treatment, this will be 105 days (2.5 x 42 days during treatment for the first 24 weeks); during the follow-up period this will be 210 days (2.5 x 84 days during follow-up).

PFS (in days) is calculated as:

(Date of death, or Progression assessed by modified RECIST 1.1, or censoring) – (Date of first dose) +1.

To apply the cut-off date to PFS is to exclude disease assessments, death and anti-cancer therapy date after cut-off date in the analysis.

Note: Patient cannot be censored at “Not Evaluable (NE)”. If NE is the only previous assessment, then PFS will be censored at Day 1.

PFS will be calculated for each subject using RECIST 1.1 alone, and serum markers alone.

Table 3 PFS Definition

Situation	Date of Event or Censor	Outcome
No evaluable post-baseline efficacy assessments, no death	Date of first dose	Censor
Subject did not receive new anti-cancer therapy:		
Disease progression documented per modified RECIST v1.1	Date of PD	Event
No disease progression, but death recorded on eCRF	Date of death	Event
No PD nor death	Date of last efficacy assessment or at the date of first dose if no postbaseline efficacy assessment is available	Censor
Subject received new anti-cancer (ACT) therapy:		
Disease progression documented per modified RECIST v1.1 after new ACT	Date of last efficacy assessment before start of new anti-cancer therapy or at the date of first dose if no post-baseline efficacy assessment is available	Censor
Disease progression documented per modified RECIST v1.1 before new ACT	Date of PD	Event
No disease progression before new ACT but death recorded	Date of last efficacy assessment before start of new anti-cancer therapy or at the date of first dose if no post-baseline radiological assessment is available	Censor
No disease progression nor death	Date of last efficacy assessment before start of new anti-cancer therapy or at the date of first dose if no post-baseline efficacy assessment is available	Censor
Missed 2 scheduled efficacy assessments:		
If disease progression or death occurs after missing 2 scheduled efficacy assessments	Date of last efficacy assessment or at the date of first dose if no postbaseline efficacy assessment is available	Censor

PFS = Date of Event or Censor – Date of First Dose +1

PFS by RECIST1.1 will be also be derived as described in Table 3.

5.3 Safety Endpoints

Safety endpoints such as DLTs, AEs, laboratory tests, vital signs, ECGs and ECOG performance status are secondary endpoints of the study.

Safety will be assessed by evaluation of the following variables:

5.3.1 Adverse Events

5.3.1.1 Dose-Limiting Toxicities

DLT criteria are defined in section 2.2.1.1.

5.3.1.2 Treatment-Emergent Adverse Events

Treatment-emergent adverse events (TEAEs; frequency, severity, seriousness, and relationship to study drug).

TEAE is defined as an adverse event observed after starting administration of the study drug, and up to 30 days after the last dose of study drug. If the adverse event occurs on Day 1 and the onset check box is marked “Onset after first dose of study drug” or the onset check box is left blank, then the adverse event will be considered treatment-emergent. If the adverse event occurs on Day 1 and the onset check box is marked “Onset before first dose of study drug”, then the adverse event will not be considered treatment-emergent. If a subject experiences an event both during the preinvestigational period and during the investigational period, the event will be considered as TEAE only if it has worsened in severity (i.e. it is reported with a new start date). All adverse events collected that begin within 30 days after taking the last dose of study drug will also be counted as TEAE. Any AEs with onset dates completely missing will be considered TEAEs in summaries. AEs with partially missing onset dates will be assumed TEAEs unless the available portion of the date indicates that the onset was strictly before start of study medication.

A drug-related TEAE is defined as any TEAE with at least possible relationship (possibly or probably related) to study treatment as assessed by the investigator or with missing assessment of the causal relationship

5.3.1.3 Serious Adverse Events

Serious Adverse Events (SAEs) include adverse events that are flagged as serious by the investigator on eCRF, or upgraded by the Sponsor based on review of the Sponsor’s list of Always Serious term.

5.3.1.4 Clinical Laboratory Variables

Below is a table of the laboratory tests that will be performed during the conduct of the study.

Additional laboratory tests should be performed according to institutional standard of care. Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or delegated sub-investigator who is a qualified physician.

Table 4 Laboratory Assessments

Panel/Assessment	Parameters to be Analyzed	NCI-CTCAE Grading (Hyper/Hypo)
Hematology	Hematocrit (Hct)	N/A
	Hemoglobin (Hgb)	Both
	Red Blood Cell Count (RBC)	N/A
	White Blood Cell Count (WBC)	Both
	WBC differential	Both
	Absolute Neutrophil Count (ANC)	Hypo
	Platelets	Hypo
	Mean Corpuscular Volume (MCV)	N/A
	Mean Corpuscular Hemoglobin (MCH)	N/A
	Mean Corpuscular Hemoglobin Concentration (MCHC)	N/A
Biochemistry	Albumin	Hypo
	Blood Urea Nitrogen (BUN)	N/A
	Calcium	Both
	Bicarbonate	Hypo
	Chloride	N/A
	Creatinine	Hyper
	Estimated glomerular filtration rate	N/A
	Glucose	Hypo
	Magnesium	Both
	Phosphate	N/A
	Potassium	Both
	Sodium	Both
	Total Bilirubin	Hyper
	Total Protein	NA
	Alanine Aminotransferase (ALT)	Hyper
	Alkaline Phosphatase (ALP)	Hyper
	Aspartate Aminotransferase (AST)	Hyper
	Amylase	Hyper
	Lipase	Hyper
Table continued on next page		

Panel/Assessment	Parameters to be Analyzed	NCI-CTCAE Grading (Hyper/Hypo)
Urinalysis	Color	N/A
	Clarity/turbidity	N/A
	pH	N/A
	Specific gravity	N/A
	Glucose	N/A
	Ketones	N/A
	Nitrites	N/A
	Leukocyte esterase	N/A
	Bilirubin	N/A
	Urobilinogen	N/A
	Blood	N/A
	Protein	N/A
	Microscopic urinalysis to be performed if abnormal dipstick	N/A
Grade 3 or 4 IRR	Cytokine/Chemokine Panel*	N/A
Any reaction with features of anaphylaxis	Serum Total Tryptase*	N/A
Biomarkers	Alpha-fetoprotein (AFP)	N/A
	Beta human chorionic gonadotropin (βhCG)	N/A
Coagulation	Prothrombin time (PT) (sec)	Hyper
	Partial Thromboplastin Time (PTT)	Hyper
	International normalized ratio (INR)	Hyper
Thyroid Function Test	Thyroid stimulating hormone (TSH)	N/A
	Free T4 (thyroxine)	N/A

5.3.1.5 Vital signs

Vital signs include systolic and diastolic blood pressures (mmHg), radial pulse rate (beats/minute) and body temperature (degrees Celsius).

5.3.1.6 Other

- 12-lead electrocardiogram (ECG)
- ECOG performance scores
- Body Mass Index (BMI)

  $BMI = weight\ (kg) / [height\ (m)]^2$

- Duration of exposure

  Duration of exposure to a study drug will be calculated in days, using the following formula:

(Date of last dose of study drug – Date of first dose) + 1

When the start or stop date is missing, for example if the subject is ongoing, then the exposure will be treated as missing.

- Cumulative Actual Dose

  Total amount of study drug actually taken by the patient from first dose date to last dose date

- Planned Dose Intensity

  Planned dose intensity for ASP1650 is 1000 mg/m^2 Q2W or 1500 mg/m^2 Q2W.

- Actual Dose Intensity

  Defined as the cumulative actual dose in mg divided by duration of exposure in days.

- Relative Dose Intensity (RDI)

  Actual Dose Intensity

  ---------- x 100

  Planned Dose Intensity

- Previous and concomitant medication
  - Previous medication is defined as medication with at least one dose taken before the date of the first dose of study drug.
  - Concomitant medication is defined as medication with at least one dose taken between the date of first dose (inclusive) and the date of last dose (inclusive) of study drug.

5.4 Major Protocol Deviations and Other Analyses

Protocol deviations as defined in the study protocol (Section 8.3 Protocol Deviations) will be assessed for all subjects registered. The number and percentage of subjects meeting any criteria will be summarized for each criterion and overall, by cohort and dose level and overall as well as by study site. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion. Any subjects who have more than one protocol deviation will be counted once in the overall summary. A data listing will be provided by site and subject.

The protocol deviation criteria will be uniquely identified in the summary table and listing. The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of subjects (n), mean, standard deviation, median, minimum and maximum. Categorical data will be summarized by frequencies and percentages. Percentages by categories will be based on the number of subjects with no missing data, i.e. the percentages for the non-missing categories will add up to 100%.

All data summarization and analyses will be performed using SAS® Version 9.4 or higher on Linux. Specifications for table, figures, and data listing formats can be found in the TLF specifications.

6.2 Study Population

6.2.1 Disposition of Subjects

The number and percentage of subjects who completed and discontinued treatment and reasons for treatment discontinuation will be presented for all FAS subjects and for subjects in the SAF by dose level and overall. For the discontinuation, the primary reason reported by the investigator will be summarized. Similar tables for screening disposition, as well as both 30-day and long term follow-up disposition will also be presented. All disposition details and dates of first and last evaluations for each subject will be listed.

6.2.2 Protocol Deviations

The number and percentage of subjects with the following protocol deviation criteria will be summarized for each criterion and overall, by treatment group and total as well as by investigative site. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion.

The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

6.2.3 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics including age, sex, race, ethnicity, height, weight, baseline BMI and BSA, time in days from diagnosis to registration, tumor location, tumor type, number of prior lines of medication therapy, presence of visceral metastases, late relapse, and prior high dose chemotherapy will be summarized by dose level and overall for FAS and SAF.

TNM staging will also be listed for the SAF.

6.2.3.1 Medical History

Medical history will be coded by MedDRA and listed and summarized by SOC and PT, by cohort and dose level and overall for the SAF.

6.2.3.2 Substance Use

History of substance use, including use of alcohol, tobacco, or drugs will be listed for the SAF.

6.2.3.3 Prior Procedures

Prior Procedures, Prior Therapy including best response, and Prior Radiation for cancer will be listed and summarized by dose level and overall for the SAF.

6.2.3.4 Liver Abnormality

Liver abnormalities will be listed for the SAF.

6.2.4 Previous and Concomitant Medications

Previous and concomitant medications will be summarized by preferred WHO name by dose level group for the SAF. Subjects taking the same medication multiple times will be counted once per medication and investigational period. Previous medications are defined as medications that patients started and ended prior to first administration of study medication. Concomitant medications are defined as any medications that patients took after the first dose of study medication and through 90 days from last dose of study drug. Medications that started prior to first administration of study drug and continued while study drug was given will be counted in both previous and concomitant medications.

Previous and concomitant medications will be listed.

6.2.4.1 Prior Radiation Therapy

Frequency tabulations of subjects with prior radiation therapy will be presented for SAF.

6.2.4.2 Prior Procedures for Tumors

Frequency tabulations of subjects with prior procedures for tumors will be presented for SAF.

6.2.4.3 Prior Cancer Therapy

Summary of prior cancer therapy will be presented for SAF.

6.2.4.4 Disease History

Summary of disease history will be presented for SAF.

6.2.4.5 New Anti-Cancer Therapy

Summary of new anti-cancer therapy will be presented for SAF.

6.3 Study Drug

Summaries of drug exposure and relative dose intensity (RDI) by dose level and overall will be presented using each subject's assigned dose at treatment start.

6.3.1 Exposure

Summary statistics (n, mean, standard deviation, median, minimum and maximum) will be presented for the following continuous variables:

- Duration of exposure
- Total (mg) ASP1650 used per subject
- Average dose (mg/m^2 Q2W) per subject

As a categorical variable: number and percentage of subjects in each of the following duration categories:

- Number of dose delay, dose reduction, dose discontinuation, dose interruption
- Number of cycles initiated/completed

Reasons for dose delay, reduction, discontinuation or interruption will be presented in a listing.

6.3.2 Relative Dose Intensity

RDI will be summarized by dose level and overall for subjects in the SAF whose total study drug infused and first and last days of treatment are known. RDI will be presented in two ways:

- As a continuous variable, summary statistics will be presented.
- As a categorical variable, RDI will be summarized using the following categories:
  <50
  50 – < 75
  75 – < 90
  90 – <= 100
  > 100
  Unknown

6.4 Analysis of Efficacy

6.4.1 Analysis of Primary Efficacy Endpoint

Efficacy analyses will be conducted on the FAS.

6.4.1.1 Best Overall Response

Best Overall Response both with and without subsequent confirmation will be presented for the Full Analysis Set.

Best percentage change from baseline in the size of the target lesions will be presented in a waterfall plot. A swimmer plot will display study events by participant.

6.4.1.2 Objective Response Rate

ORR is defined as the proportion of subjects for each dose level whose best overall response is rated as confirmed CR or PR by modified RECIST 1.1. ORR by dose level will be calculated and its 90% confidence interval will be constructed by Clopper-Pearson method.

6.4.1.3 Sensitivity Analysis for Primary Efficacy Endpoint

ORR as assessed by RECIST 1.1 alone and by serum markers alone will be calculated by dose level and its 90% confidence interval will be constructed by Clopper-Pearson method.

6.4.2 Analysis of Secondary Efficacy Endpoints

6.4.2.1 Clinical Benefit Rate

CBR is defined as the proportion of subjects for each dose level whose best overall response is rated as confirmed CR, PR or durable SD. Durable SD is a SD maintained for at least 12 weeks. CBR by dose level will be calculated and its 90% confidence interval will be constructed

6.4.2.2 Duration of Response

DOR will be calculated only for the subgroup with confirmed response CR/PR. The distribution of DOR will be estimated for phase 2 subjects only using Kaplan-Meier methodology.

6.4.2.3 Progression-Free Survival

PFS is defined as the time from the date of first dose until the date of disease progression, or until death due to any cause. If a subject has neither progressed nor died, the subject will be censored at the date of last disease assessment or at the date of registration if no post-baseline radiological assessment is available. Subjects who receive any further anticancer therapy for the disease before radiological progression will be censored at the date of the last radiological assessment before the anticancer therapy started. If progression or death occurs after missing 2 scheduled radiological assessments, the subject will be censored at the date of last radiological assessment or at the date of registration if no post-baseline radiological assessment is available. A subject has missed 2 scheduled disease assessments if the gap between two disease assessments is more than 2.5 times the scheduled disease assessment interval. During treatment, this will be 105 days (2.5 x 42 days during treatment for the first 24 weeks); during the follow-up period this will be 210 days (2.5 x 84 days during follow-up).

PFS will be derived for phase 2 subjects only. The distribution of PFS will be estimated for each dose level using Kaplan-Meier methodology.

PFS (in days) is calculated as:

(Date of death or documented Progression or censoring) – (Date of first dose date) +1.

To apply the cut-off date to PFS is to exclude tumor assessments, death and anti-cancer therapy date after cut-off date in the analysis.

Note: Patient cannot be censored at “Not Evaluable (NE)”. If NE is the only previous assessment, then PFS will be censored at Day 1.

6.4.2.4 βhCG and AFP

Levels of βhCG and AFP, as well as change from baseline, during the study will be tabulated and presented in line charts for FAS and SAF.

6.5 Analysis of Safety

6.5.1 Dose-Limiting Toxicities

All dose-limiting toxicity (DLT) events, as defined in the DLT criteria in section 2.2.1.1, will be summarized by dose level using DEAS. Details of DLTs will be presented in listings and subject narratives.

6.5.2 Adverse Events

The coding dictionary for this study will be MedDRA v21.1. It will be used to summarize AEs by SOC and PT. AEs will be graded using National Cancer Institute's Common Terminology Criteria for AEs (NCI-CTCAE).

An overview table will include the following details by treatment group and overall:

- Number and percentage of subjects with TEAEs,
- Number and percentage of subjects with causally drug related TEAEs,
- Number and percentage of subjects with serious TEAEs and Astellas upgraded serious TEAE,
- Number and percentage of subjects with serious drug related TEAEs and Astellas upgraded serious drug related TEAE,
- Number and percentage of subjects with TEAEs leading to death
- Number and percentage of subjects with drug-related TEAEs leading to death
- Number and percentage of subjects with TEAEs leading to permanent discontinuation of study drug,
- Number and percentage of subjects with drug related TEAEs leading to permanent discontinuation of study drug, and
- Number and percentage of subjects with grade 3 or higher TEAE
- Number of deaths.

The number and percentage of subjects with TEAEs, as classified by SOC and PT will be summarized by treatment group and overall. Summaries will be provided for:

- TEAEs
- drug related TEAEs,
- serious TEAEs and Astellas upgraded serious TEAE,
- drug related serious TEAEs and drug related Astellas upgraded serious TEAE,
- TEAEs leading to permanent discontinuation of study drug,
- drug related TEAEs leading to permanent discontinuation of study drug,
- TEAEs excluding serious adverse events that equal to or exceed a threshold of 5% in any treatment group, and
- Common TEAEs that equal to or exceed a threshold of 10% in the total group.
- TEAE with CTCAE Grade 3 or higher

The number and percentage of subjects with TEAEs and TEAEs leading to death, as classified by PT only, will be summarized by treatment group and overall.

AE summary tables will include subject counts as opposed to AE counts. If a subject experiences more than one episode of a particular AE, that subject will be counted only once for that event. If a subject has more than one AE that code to the same preferred term, the subject will be counted only once for that preferred term. Similarly, if a subject has more than one AE within a body system, the subject will be counted only once in that body system.

TEAEs and the number and percentage of subjects with TEAEs, as classified by SOC and PT will also be summarized by NCI-CTCAE severity grade and by relationship to study drug. If an adverse event changes in severity grade or relationship, then the subject will be counted only once with the worst severity grade and highest degree of relationship. The adverse event however will be presented in each category they were classified to. If a subject has an event more than once with missing severity grade/relationship and with non-missing severity grade, then the subject will be counted as the highest non-missing grade. If a subject has an event more than once with missing relationship and with non-missing relationship, then the subject will be counted as related. Drug related TEAEs will be presented in a similar way by severity only.

The number and percentage of subjects with treatment-emergent adverse events of special interest (AEoSI), as classified by SOC and PT will also be summarized by treatment group and overall. The list of AEoSI to be summarized may change during the course of the study due to ongoing pharmacovigilance. It will be finalized before the database hard lock.

All AEs, deaths, SAEs and withdrawals due to adverse events will be displayed in listings.

6.5.3 Clinical Laboratory Evaluation

The baseline visit is the last measurement taken prior to initial study drug administration.

Selected quantitative clinical laboratory variables, i.e. hematology, biochemistry, coagulation and urinalysis will be summarized using mean, standard deviation, minimum, maximum and median by cohort and dose level at each visit. Additionally, a within-subject change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way. Changes in laboratory values will be summarized for baseline values versus minimum, maximum and last post-baseline values. Plots of median lab values at each scheduled assessment time by cohort and dose level may be provided for each laboratory parameter.

Frequency tabulations of selected qualitative clinical laboratory variables (i.e. urinalysis) will be presented by cohort and dose level at each visit.

Laboratory results will also be graded using NCI-CTCAE, where possible. Parameters that have criteria available for both low and high values, i.e., hypo- and hyper-, will be summarized for both criteria. The same subject can be counted for both values if the subject has different laboratory values meeting each criterion. NCI-CTCAE grade of laboratory evaluations will be summarized by number and percentage of subjects for each visit. Shift

tables of NCI-CTCAE grade change from baseline to worst post-baseline grade for selected laboratory parameters will also be presented.

The list of laboratory parameters to be summarized may change during the course of the study due to ongoing pharmacovigilance. It will be finalized before the database hard lock.

Laboratory data will be displayed in listings.

6.5.3.1 Liver Safety Assessment

The following potentially clinically significant criteria in liver function tests for Alkaline

Phosphatase (ALP), Alanine Transaminase (ALT), total bilirubin, Aspartate Transaminase (AST) and their combinations are defined. The subject's highest value during the investigational period will be used.

Table 5 Potentially Clinically Significant Criteria

Parameter	Criteria
ALT	> 3xULN > 5xULN > 10xULN >20xULN
AST	> 3xULN > 5xULN > 10xULN >20xULN
ALT or AST	> 3xULN > 5xULN > 10xULN >20xULN
Total Bilirubin	> 2xULN
ALP	> 1.5xULN
ALT and/or AST AND Total Bilirubin(*)	(ALT and/or AST > 3xULN) and Total Bilirubin > 2xULN
ALT and/or AST AND Alkaline Phosphatase AND Total Bilirubin(*)	ALT and/or AST > 3xULN AND Alkaline Phosphatase < 2xULN AND Total Bilirubin > 2xULN

* Combination of values measured on the same day or within one calendar day.

The number and percentage of subjects with potentially clinically significant values in liver enzymes and total bilirubin during the investigational period will be presented by cohort and dose level.

6.5.4 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from pre-dose on the day of infusion for subjects in the SAF by dose level and time point. Weight and BSA

results and change from baseline will also be summarized with descriptive statistics and listed.

Potentially clinically significant vital signs will be tabulated with summary statistics. The following potentially significant clinically significant criteria are defined for each parameter:

Vital Sign Variable	**Criteria**
SBP	>= 180 mmHg AND >= 20 mmHg change from baseline
SBP	<= 80 mmHg
DBP	>= 105 mmHg AND >= 15 mmHg change from baseline
Pulse Rate	>= 120 bpm AND >= 15 bpm change from baseline

Vital signs data will be displayed in listings.

6.5.5 Electrocardiograms

The 12-lead ECG results will be summarized by dose level and time point. A shift analysis table showing shifts from baseline in overall ECG (normal and abnormal) will be provided.

6.5.6 Eastern Cooperative Oncology Group Performance Status

Summary statistics (number and percent of subjects) for each category of the ECOG performance status at each assessment will be provided. The change from baseline to final visit or early termination will also be summarized. Negative change scores indicate an improvement. Positive scores indicate a decline in performance.

6.5.7 Immunogenicity

Immunogenicity of ASP1650 will be summarized for each dose level and scheduled time point using the frequency of antidrug antibody (ADA) positive subjects. The potential relationship between ASP1650 immunogenicity and ASP1650 pharmacokinetics, efficacy and safety profiles in subjects may be assessed.

6.6 Analysis of Pharmacokinetics

Descriptive statistics will include the number of subjects (n), mean, standard deviation, coefficient of variation (CV), geometric mean, geometric CV, median, minimum, maximum.

6.6.1 Serum Concentrations

Serum concentrations of ASP1650 will be listed and summarized using descriptive statistics by dose and scheduled time point. Standard graphics including mean serum concentration-time profiles and overlay (spaghetti) plots will be produced. Steady state of ASP1650 will be

evaluated using a visual inspection of individual participant trough concentrations versus visit overlaid with a mean profile.

6.6.2 Estimation of Pharmacokinetic Parameters

Noncompartmental (NCA) analysis will be performed to calculate ASP1650 pharmacokinetic parameters using Phoenix® WinNonlin® version 6.4 or higher (Certara USA, Inc., Princeton, NJ). Descriptive statistics will be provided for the NCA-based parameters whenever applicable.

PK parameters will be listed and summarized by dose level and visit. The following parameters will be summarized:

- AUC_{336}, C_{max}, t_{max}, C_{trough}
- Additional PK parameters as applicable: AUC_{inf}, AUC_{inf} [%extrap], AUC_{last}, $t_{1/2}$, t_{last}, CL, V_z

6.7 Analysis of Biomarkers

Additional post hoc statistical analyses may be outlined in a biomarker SAP.

Results of microscopic examination of CLDN6 will be summarized and listed for subjects with available results.

6.8 Other Analysis

Due to COVID-19, the assessments of some subjects may be affected. All visit-based and non-visit-based assessments affected by the COVID-19 pandemic will be listed.

6.9 Subgroup Analysis for Primary Efficacy Endpoint

The following subgroups may be explored:

- Tumor type: Seminoma vs non-seminoma or relapsed primary mediatinal non-seminomatous germ cell tumor vs mixed
- Prior lines of medication therapy: 2 vs ≥3
- Primary testicular site vs other (mediastinal or retroperitoneum)
- Presence of visceral metastases: yes vs no
- Late relapse: yes vs no
- Prior high-dose chemotherapy: yes vs no

6.10 Interim Analysis (and Early Discontinuation of the Clinical Study)

No formal interim analysis is planned during the Safety Lead-in phase. Safety, pharmacokinetic and other clinical data will be reviewed on an ongoing basis.

A futility analysis will be conducted 24 weeks after the first dose of the 13th subjects are enrolled in stage I of phase 2 of the study. The study may be stopped if we observed less than 2 confirmed response (confirmed CR or confirmed PR by modified RECIST 1.1) are observed 24 weeks after the first dose of the 13th subject.

6.11 Additional Conventions

6.11.1 Missing Data

Every effort will be made to resolve missing or incomplete dates for adverse events, concomitant medications, anti-cancer therapy, and death. If a partial date cannot be resolved, the most conservative imputation methods will be used to complete the missing information.

Missing or partial start and stop dates of adverse events and concomitant medication will be imputed using the following algorithm:

- Imputation rules for partial or missing stop dates:
  - If the month and year are present, then impute as the last day of that month.
  - If only the year is present, impute as December 31 of that year.
  - If the stop date is entirely missing, assume the event or medication is ongoing.

Imputation rules for partial or missing start dates:

Start Date		Stop Date: Complete: *yyyymmdd*		Stop Date: Partial: *yyyymm*		Stop Date: Partial: *yyyy*		Stop Date: Missing
		< 1st dose	≥ 1st dose	< 1st dose *yyyymm*	≥ 1st dose *yyyymm*	< 1st dose *yyyy*	≥ 1st dose *yyyy*	
Partial: *yyyymm*	= 1st dose *yyyymm*	2	1	2	1	n/a	1	1
	≠ 1st dose *yyyymm*		2		2	2	2	2
Partial: *yyyy*	= 1st dose *yyyy*	3	1	3	1	n/a	1	1
	≠ 1st dose *yyyy*		3		3	3	3	3
Missing		4	1	4	1	4	1	1

1 = Impute as the date of first dose; 2 = Impute as the first of the month; 3 = Impute as January 1 of the year; 4 = Impute as January 1 of the stop year

The imputed dates will be used to determine whether an AE is/is not treatment emergent. Listings of AEs and concomitant medications will present the actual partial dates; imputed dates will not be shown.

For secondary endpoint PFS or DOR, missing or incomplete death date will be imputed as the earliest feasible date on or after the date last known to be alive as the examples shown in the table below.

Incomplete Date of Death (YYYY MMM DD)	Date Last Know to be Alive (YYYY MMM DD)	Imputed Date of Death (YYYY MMM DD)
2005 APR ??	2005 MAR 31	2005 APR 01
2005 ??? 13	2005 MAR 31	2005 APR 13
2005 ??? ??	2005 MAR 31	2005 MAR 31
???? APR ??	2005 MAR 31	2005 APR 01
???? APR 13	2005 MAR 31	2005 APR 13
???? ??? ??	2005 MAR 31	2005 MAR 31

6.11.2 Outliers

All values will be included in the analyses.

7 REVISION AND RATIONALE

Version	Date	Changes	Comment/rationale for change
1.0	02Nov2018	started	
2.0	10Feb2021	updated	clarified blood draw schedule for post treatment period; allowed cohort 2 and beyond to have up to 4 subjects derivation of PFS inaccurately had noted registration date as first date; clarified to be first dose date Updated to align with protocol version 3.1 incorporating Nonsubstantial Amendment 1 Updated to describe biomarker analysis

8 REFERENCES

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports,

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Liu S, Yuan Y. Bayesian optimal interval designs for phase I clinical trials. Appl Statist. 2015; 64(Part 3):507–23.

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9 APPENDICES

9.1 Appendix 1: Key Contributors

PPD	Phastar, Inc. (Biometrics)
PPD	Data Science
PPD	Medical Science
PPD	CPED
PPD	CPED
PPD	Clinical Science
PPD	Medical Safety

9.2 Appendix 2: Author and Approver Signatures

(E-signatures are attached at end of document)

PPD was author of this Statistical Analysis Plan.

PPD, Astellas Pharma Global Development was the biostatistics peer reviewer of this Statistical Analysis Plan

This Statistical Analysis Plan was approved by:

PPD, Astellas Pharma Global Development

(E-signatures are attached at the end of document.)