STATISTICAL ANALYSIS PLAN

Version 3.0

30-Aug-2022

A Phase 2 Open-label, Dose-finding Study to Determine the Optimal dose for Lymph Node Visualization using ASP5354 in Participants with Breast Cancer or Melanoma Undergoing Sentinel Lymph Node Biopsy

ISN/Protocol 5354-CL-1201

IND 154703

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ASP5354	Astellas Development Code for Pudexacianinium Chloride
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical classification system
CI	Confidence Intervals
CRF	Case Report Form
CSR	Clinical Study Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ISN	International Study Number
MedDRA	Medical Dictionary for Regulatory Activities
LN	Lymph node
mg	Milligrams
mL	Milliliter
PD	Protocol Deviation
PK	Pharmacokinetic
PKAS	Pharmacokinetic Analysis Set
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SBR	Signal Background Ratio
SLN	Sentinel Lymph Node
TEAE	Treatment Emergent Adverse Event
TLF	Tables, Listings and Figures
WHO	World Health Organization

Version History

SAP Version History Summary

The changes from the prior approved SAP that impact analyses are listed with the rationale in the table below.

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
1.0	03-Dec-21		Not Applicable	Original Version
1.1	07-Feb-22	4.2.6 4.2.8	Sections 4.2.6 and 4.2.8 added.	Prior history imaging medication treatment and SoC Visualization Agent used during biopsy details added in the document.
		4.4.1.2	Histopathologic confirmation information added at patient level	Histopathologic confirmation aspects added at patient level as secondary objective.
		4.5.2	Additional details for metastatic spread defined in 4.5.2	Details for comparing LNs detected with metastatic spread at lymph node level added as exploratory objective.
2.0	07-Mar-22	4.2.7	Additional details for study drug dosing defined in section 4.2.7.	Details for drug dosing information for volume prepared and administered from syringe added.
		4.7.1	TEAE of Interest: Hypersensitivity SMQ by NCI-CTC Grade added.	Hypersensitivity SMQ by NCI-CTC Grade details will be summarized as TEAE of Interest.
2.1	09-Aug-22	1.2	Dose Arm/IP Name	Dose Arm/IP Name updated according to Protocol Amendment 2.
		4.5.1	SBR added	SBR definition and imputation in case of 'Not Visualized' added.
		4.6.1	Green Coloration of Urine or Skin	Green Discoloration was replaced by Green Coloration of urine or skin according to Protocol Amendment 2.

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 fulfil the objectives of the study.

The final SAP will be approved before first subject screened.

If there are any changes from the planned analyses in the final version of the SAP that impact the statistical analyses, then it will be documented in the Clinical Study Report (CSR).

1.1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To determine the optimal dose of locally administered ASP5354 for LN visualization in participants undergoing SLN biopsy.	• Optimal dose determination by the VRC based on: o LN tissue visualized (Yes/No) o Visualized tissue is lymphatic in origin based on pathologic confirmation o The Likert Scale determination of the intensity of fluorescence (0 to 3) o Proportion of identified LN with histopathologic confirmation of LN tissue by ASP5354 compared with SoC treatment with either Tc-99mSC (approved drug) or Lymphoseek (generic name tilmanocept)
Secondary	
• To evaluate LN visualization of locally administered ASP5354 in participants undergoing SLN biopsy	• Proportion of participants with at least 1 LN detected by visualization (Yes/No) with histopathologic confirmation of LN tissue using ASP5354
• To compare LN detection for ASP5354 with the technetium-based treatment in breast cancer and melanoma	• Proportion of identified LN with histopathologic confirmation of LN tissue by ASP5354 compared with SoC treatment with either Tc-99mSC or Lymphoseek

Objectives	Endpoints
• To investigate the safety and tolerability of locally administered ASP5354 in participants undergoing SLN biopsy	• Vital signs (blood pressure, pulse and respiratory rate) • Routine 12-lead ECGs • Clinical laboratory tests (hematology [complete blood count], serum chemistry and urinalysis) • Nature, frequency and severity of TEAEs and SAEs
• To investigate the pharmacokinetics of locally administered ASP5354 in participants undergoing SLN biopsy	• Plasma concentrations of ASP5354
Exploratory	
• To explore the visualization characteristics of ASP5354	• The Likert Scale for qualitative response about the intensity of fluorescence • Signal background ratio • Transdermal visualization of lymphatic spread of ASP5354 after injection (Yes/No) • Time from injection to surgical identification of LN(s) • Number of LNs visualized by ASP5354
• To compare LNs detected with metastatic spread for ASP5354 with the technetium-based treatment	• Proportion of LN with metastatic spread, based on histopathologic review, detected by ASP5354 compared with SoC treatment with either Tc-99mSC or Lymphoseek

Abbreviations from table above: ECG: electrocardiogram; LN: lymph node; SAE: serious adverse event; SLN: sentinel lymph node; SoC: standard of care; Tc-99mSC: Technetium-99m sulfur colloid; TEAE: treatment-emergent adverse event; VRC: Visualization Review Committee.

1.2 Study Design

The study is an open-label, dose-finding study to determine the optimal dose for LN visualization, as part of lymphatic mapping, in adult participants with breast cancer and melanoma undergoing SLN biopsy as part of cancer treatment.

Enrollment of breast cancer and melanoma participants will be performed independently and simultaneously. Dose level 2 (0.2 mg) will enroll the first 3 participants and then the VRC will review the data for the breast cancer group and the melanoma group separately. If LN visualization, with histopathologic confirmation, occurs in 3 participants, then 3 participants each will be enrolled first in 0.05 mg, and then in 0.6 mg dose levels. An interim analysis will then be performed across initial dose levels (0.05, 0.2 and 0.6 mg) in each cohort for breast cancer and melanoma by the VRC prior to further enrollment of participants. If < 3 participants at 0.2 mg have LN visualization with histopathologic confirmation on VRC review, then 3 participants will be enrolled sequentially in 0.6 mg and 1 mg. An interim analysis will then be performed across initial dose levels (0.2, 0.6 and 1 mg) in each cohort for breast cancer and melanoma by the VRC prior to further enrollment of participants.

Breast Cancer

Dose Arm/IP Name	ASP5354 Dose Level	ASP5354 Dose Conc and Volume	Number of Participants
Breast Cancer 0.05 mg/ ASP5354	**0.05 mg**	**1.0 mg/mL, 0.05 mL in total**	**Up to 12**
Breast Cancer 0.2 mg/ ASP5354	**0.2 mg**	**1.0 mg/mL, 0.2 mL in total**	**Up to 12**
Breast Cancer 0.6 mg/ ASP5354	**0.6 mg**	**1.0 mg/mL, 0.6 mL in total**	**Up to 12**
Breast Cancer 1 mg/ ASP5354	1 mg	1.0 mg/mL, 1 mL in total	Up to 12
Breast Cancer 2 mg/ ASP5354	2 mg	1.0 mg/mL, 2 mL in total	Up to 12
Breast Cancer 4 mg/ ASP5354	4 mg*	1.0 mg/mL, 4 mL in total	Up to 12

IP: investigational product

* The 4 mg dose level will be added only for breast cancer participants.

Melanoma

Dose Arm/IP Name	ASP5354 Dose Level	ASP5354 Dose Conc and Volume	Number of Participants
Melanoma 0.05 mg/ ASP5354	**0.05 mg**	**1.0 mg/mL, 0.05 mL in total**	**Up to 12**
Melanoma 0.2 mg/ ASP5354	**0.2 mg**	**1.0 mg/mL, 0.2 mL in total**	**Up to 12**
Melanoma 0.6 mg/ ASP5354	**0.6 mg**	**1.0 mg/mL, 0.6 mL in total**	**Up to 12**
Melanoma 1 mg/ ASP5354	1 mg	1.0 mg/mL, 1 mL in total	Up to 12
Melanoma 2 mg/ ASP5354	2 mg	1.0 mg/mL, 2 mL in total	Up to 12

IP: investigational product

Conc: concentration

* The 4 mg dose level will be added only for breast cancer participants

Depending on the outcome of the interim VRC data reviews, additional participants may be added to the same dose level, a dose may be closed, or a new dose (1 mg, 2 mg or 4 mg) will begin enrollment. Up to 12 participants will be assigned at each dose level for breast cancer and melanoma.

Details of the schedule of clinical assessments are available in the protocol (section 1.3).

1.3 Randomization

This is an open-label study. Participant enrollment and dispensation of IP will be performed via the interactive response technology (IRT) system. Prior to initiation of treatment, study site personnel will obtain the participant number and treatment assignment from the IRT system. Specific IRT procedures will be described in the respective study manual.

2 STATISTICAL HYPOTHESES

No statistical hypothesis is planned.

3 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.

The determination of whether participants are included or excluded from the safety analysis sets will be made prior to database lock, except the pharmacokinetic analysis set.

Population	**Description**
Enrolled	All participants who sign the ICF
Full Analysis Set (FAS)	All participants who are enrolled and receive study IP. Participants will be analyzed according to the IP they actually received.
Safety Analysis Set (SAF)	All participants who are enrolled and receive study IP. Participants will be analyzed according to the IP they actually received. SAF is the same as FAS for this study.
Pharmacokinetic analysis set (PKAS)	All participants who receive at least 1 dose of IP for which at least 1 plasma concentration data are available with the time of dosing and sampling. Inclusion of participants in the PKAS with missing data or important protocol deviations will be considered by the pharmacokineticist on a case-by-case basis.

4 STATISTICAL ANALYSES

A Statistical Analysis Plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. Changes from the planned analyses in the final SAP that impact the statistical analyses will be justified in the Clinical Study Report (CSR).

4.1 General Considerations

For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation, median, minimum and maximum. When needed, the use of other percentiles (e.g.10%, 25%, 75% and 90%) will be mentioned in the relevant section.

Summaries based on FAS (e.g. efficacy data) will be presented by planned dose level, unless specifically stated otherwise. Safety analysis and other summaries based on SAF will be presented by actual dose level received.

The 95% confidence interval (95% CI) in proportion is based on exact method unless specifically stated otherwise.

Pharmacokinetic summaries based on PKAS will be presented by actual dose level received.

All data processing, summarization, and analyses will be performed using SAS® Version 9.3 or higher on Unix. Specifications for table, figure, and data listing formats can be found in the TLF specifications for this study.

Unless otherwise specified, all summaries will be presented by tumor type and dose level.

4.2 Study Participants

Participant disposition, demographics and baseline characteristics will be summarized by tumor type, dose level and overall for FAS population.

Baseline will be defined as the last no missing observation prior to administration of IP, unless otherwise specified.

4.2.1 Participant Disposition

The number and percentage of participants who completed and discontinued screening period and reasons for discontinuation will be presented for all participants who signed the ICF.

A similar table for treatment and follow-up disposition will also be presented for the FAS by dose level and overall.

All disposition details and dates of first and last evaluations for each participant will be listed.

Number and percentage of participants for each analysis set will be summarized by dose level and overall.

4.2.2 Protocol Deviations

The number and percentage of participants with the following important protocol deviation criteria will be summarized for each criterion and overall, by dose level and overall, and

tumor type. Participants deviating from a criterion more than once will be counted once for the corresponding criterion.

The unique identifiers will be as follows:

PD 1: Inclusion/Exclusion

PD 2: Withdrawal Criteria

PD 3: Study Intervention

PD 4: Excluded Concomitant Medications

PD 5: Informed Consent

PD 6: Safety Reporting

PD 7: Procedures/Tests

4.2.3 Demographic and Other Baseline Characteristics

Demographic, and other baseline characteristics will be summarized descriptively by tumor type, dose level, and overall for the FAS.

Descriptive statistics for age, age categories (<65, >=65 - <75, >=75), body mass index (BMI), weight and height will be presented. Frequency tabulations for sex, ethnicity and race will be presented.

Medical history is coded in MedDRA, and will be summarized by System Organ Class (SOC) and Preferred Term (PT), by tumor type, dose level, and overall for the FAS.

Primary diagnosis of the target disease will be listed including T categorization in TNM classification by tumor type and dose level. For breast cancer laterality (left/right) will be presented by dose level. The location of tumor (extremities: upper/lower, left/right), trunk (front, back or side [L or R]), head, neck, other, tumor thickness, and ulceration status will be presented by melanoma and dose level.

4.2.4 Previous and Concomitant Medications

Previous and concomitant medications will be summarized by therapeutic subgroup (ATC 2nd level), chemical subgroup (ATC 4th level) and preferred WHO name (active ingredients for combination drugs) by tumor type and dose level for the SAF.

Participants taking the same medication multiple times will be counted once per medication and investigational period. A medication which can be classified into several chemical and/or therapeutic subgroups is presented in all chemical and therapeutic subgroups.

Previous medications are defined as medications that participants started prior to IP administration period. Concomitant medications are defined as any medications that participants took after IP administration until the end of follow up period. Medications that started prior to and continued after IP administration will be counted in both previous and concomitant medications.

4.2.5 Non-medication therapies

Frequency tabulations of subjects with non-medication therapy and reason for use will be presented by tumor type and dose level and overall for SAF.

4.2.6 Prior history imaging medication treatment

Frequency tabulations of subjects with prior history imaging medication treatment will be presented by tumor type and dose level and overall for SAF.

4.2.7 Extent of Exposure

Since this is a single dose study, the analysis of duration of exposure is not applicable. Study drug dosing information which include dosing date and time, volume prepared and administered from syringe, total drug used (derived following the pharmacy manual), compliance, number of injection sites, route of administration, directionality of the anatomical location, and location of administration will be listed.

4.2.8 SoC Visualization Agent used during biopsy

SoC visualization agent used during biopsy at Day 1 intraoperative will be summarized.

4.3 Primary Endpoint Analysis

The primary analysis will be conducted on the FAS. No statistical testing will be performed.

4.3.1 Definition of Endpoints

Optimal dose determination will be assessed based on the totality of collected data by the VRC based on:

- LN tissue visualized (Yes/No)
- Visualized tissue is lymphatic in origin based on pathologic confirmation
- The Likert Scale determination of the intensity of fluorescence (0 to 3)
- Proportion of identified LN with histopathologic confirmation of LN tissue by ASP5354 compared with SoC treatment with either Tc-99mSC or Lymphoseek

The optimal dose is defined as the dose that provides a better visualization compared with lower doses and a comparable visualization to the next higher dose. In the case where 2 doses performed equally, the lower dose would be selected. Cumulative visualization data from all treated participants will be used for optimal dose determination.

4.3.2 Main Analytical Approach

The optimal dose will be determined by VRC based on the totality of the data and including the following assessments: LN tissue visualized (Y/N), visualized tissue is lymphatic in origin based on pathologic confirmation, the Likert scale determination of the intensity of fluorescence, and proportion of identified LN with histopathologic confirmation of LN tissue by ASP5354 compared with SoC treatment with either Tc-99mSC or Lymphoseek. The individual listings for the visualization parameters not limited to the parameters above are created.

4.4 Secondary Endpoints Analysis

The secondary analysis will be conducted on the FAS. No statistical testing will be performed.

4.4.1 Definition of Endpoints

4.4.1.1 Lymph Node Level

- The number of LN identified intraoperatively.
- The number and percentage [a)] of LN identified intraoperatively by histopathologic confirmation.
- The number of sentinel LN identified intraoperatively.
- The number and percentage [b)] of sentinel LN identified intraoperatively by histopathologic confirmation.

a) The denominator is the number of LN identified intraoperatively.

b) The denominator is the number of sentinel LN identified intraoperatively.

Sentinel LN detailed:

- The number of sentinel LN identified by ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek.
- The number of sentinel LN identified by ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek.
- The number and percentage [c)] of sentinel LN identified by ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation.
- The number of sentinel LN identified by ASP5354.
- The number and percentage [c)] of sentinel LN identified by ASP5354 with histopathologic confirmation and 95% CI.
- The number of sentinel LN identified by SoC treatment with either Tc-99mSC or Lymphoseek.
- The number and percentage [c)] of sentinel LN identified by SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation and 95% CI.
- The difference in proportion of identified sentinel LN with histopathologic confirmation of LN tissue between ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI.

c) The denominator is the number of sentinel LN identified intraoperatively by histopathologic confirmation.

LN detailed:

- The number of LN identified by ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek.
- The number of LN identified by ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek.
- The number and percentage [d)] of LN identified by ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation.
- The number of LN identified by ASP5354.
- The number and percentage [d)] of LN identified by ASP5354 with histopathologic confirmation* and 95% CI.
- The number of LN identified by SoC treatment with either Tc-99mSC or Lymphoseek.
- The number and percentage [d)] of LN identified by SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation* and 95% CI.
- The difference in proportion of identified LN with histopathologic confirmation of LN tissue between ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI.

d) The denominator is the number of LN identified intraoperatively by histopathologic confirmation

4.4.1.2 Patient Level

- The number of LN identified intraoperatively per patient.
- The number of LN identified by histopathologic confirmation per patient.
- The number of LN identified by ASP5354 with histopathologic confirmation per patient.
- The number of LN identified by SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation per patient.
- The number of LN identified by ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation per patient.
- The number of LN identified by ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek with histopathologic confirmation per patient.
- Proportion of participants with at least 1 LN detected with histopathologic confirmation of LN tissue using ASP5354 and 95% CI.
- Proportion of participants with at least 1 LN detected with histopathologic confirmation of LN tissue using SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI.

- The difference in proportion of identified LN with histopathologic confirmation of LN tissue between ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI.
- Proportion of participants with at least 1 LN with histopathologic confirmation detected by the method other than ASP5354 or SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI.

4.4.2 Main Analytical Approach

4.4.2.1 Lymph Node Level

The number of and percentage for lymph nodes or sentinel lymph nodes will be summarized per the specified denominator.

4.4.2.2 Patient Level

The number of and percentage for the detection of lymph nodes or sentinel lymph nodes per patient will be summarized with specified statistics.

4.5 Exploratory Endpoints Analysis

4.5.1 Patient Level

- The Maximum of Likert Scale of the intensity of the fluorescence in ASP5354 will be summarized by frequency and percentage.
- The average score of Likert Scale (0- None, 1- Mild, 2- Moderate, 3- Strong) in sentinel lymph nodes per patients for the intensity of the fluorescence in ASP5354 will be summarized by descriptive statistics.
- The Signal Background Ratio (SBR) in ASP5354 will be summarized using descriptive statistics.

  The SBR will be defined as follows:

$$SBR = \frac{Mean\ region\ of\ interest}{Mean\ signal\ background}$$

based on Hoogstins et al, 2019. For SBR records where images are reviewed and no visualization occurs, the SBR will be imputed as 1 for the summary tables.

- The transdermal visualization of lymphatic spread of ASP5354 after injection will be summarized by frequency and percentage.
- The time from injection to surgical identification of LN(s) will be summarized by descriptive statistics.

4.5.2 Lymph Node Level

- The number and percentage [e)] of LN with metastatic spread, based on histopathologic review, detected by ASP5354

- The number and percentage [e)] of LN with metastatic spread, based on histopathologic review, detected by SoC treatment with either Tc-99mSC or Lymphoseek
- The difference in proportion of LN with metastatic spread, based on histopathologic confirmation of LN tissue between ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek and 95% CI

e) The denominator is the number of LN identified intraoperatively by histopathologic confirmation

4.6 Other Endpoints

4.6.1 Green Coloration of Urine or Skin

The frequency and percentage of patients who experience green coloration of urine will be summarized by dose level and tumor type only for subjects who used urethral catheter. The duration of green coloration of urine or skin (in hours) will be summarized using descriptive statistics by dose level and tumor type. The duration is calculated from first date/time point of green coloration of urine or skin to last date/time point of green skin coloration. If the green coloration of urine or skin is still present at discharge, the duration is from first date/time point of green coloration to the date/time of discharge.

Additional to descriptive statistics, if applicable, the duration will be analyzed using Kaplan Meier estimation. For this purpose, subjects with green coloration of urine or skin at discharge are censored at the time point of discharge.

4.7 Safety Analyses

The safety and tolerability will be summarized by descriptive statistics for the SAF population.

4.7.1 Adverse Events

AEs will be coded using MedDRA and graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE version 5.0).

A TEAE is defined as an AE observed after administration of the IP and up to the follow-up period. An IP-related TEAE is defined as any TEAE with a causal relationship assessed as “yes” by the investigator.

The number and percentage of participants with TEAEs, IP-related TEAEs, serious TEAEs and IP-related serious TEAEs will be summarized by system organ class, preferred term and dose level. The worst grade will be summarized if the same AE is recorded more than once for a participant.

AE data will be listed.

An overview table will include the following by tumor type and dose level:

- TEAEs
- Drug related TEAEs
- Serious TEAEs

- Drug related serious TEAEs
- TEAEs leading to death
- TEAEs of Interest: Hypersensitivity SMQ by NCI-CTC Grade
- Drug-related TEAEs leading to death
- Deaths
- Drug-related Grade 3 or higher TEAE
- Drug-related Grade 3 or higher: Local Site Reaction
- Drug-related Grade 3 or higher: Infusion Related Reactions
- Drug-related Grade 3 or higher: Hypersensitivity SMQ

4.7.2 Clinical Laboratory Evaluation

For quantitative clinical laboratory measurements, descriptive statistics will be used to summarize results and change from baseline by dose level and time point.

Shifts from baseline to the post baseline worst grade based on NCI-CTCAE until the follow up period in clinical laboratory tests will be tabulated. Quantitative laboratory test results by NCI-CTCAE Grade (version 5.0) will be summarized with frequencies and percentages by dose level and time point.

Laboratory data will be listed.

4.7.3 Liver Safety Assessment

The liver safety assessments will be summarized by the categories below based on the measurements from Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Total Bilirubin, Aspartate Transaminase (AST) and their combination. These parameters will be based on measurements from a local laboratory.

The participant's highest value during the course of the study will be used; upper limit of normal (ULN).

- ALT: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- AST: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALT or AST: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALP: > 1.5xULN
- Total Bilirubin: > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN and ALP < 2xULN

The last 2 criteria where 2 or more parameters are evaluated will use the measurements on the same day or up to 1 day apart. The number and percentage of subjects meeting the criteria post-baseline will be summarized.

Information on alcohol, other substance use will be listed by dose level and tumor type.

4.7.4 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline by dose level and time point. Vital signs data will be listed.

A table for potentially clinically significant vital signs (blood pressure >150/90 mmHg or < 70/60 mmHg; pulse > 100 per min or <60 per min; respiratory rate <12 and >16) will be generated by tumor type and dose level.

4.7.5 Electrocardiograms

The routine 12-lead ECG results and intraoperative ECG monitoring will be summarized by dose level and time point.

12-lead ECG and intraoperative ECG data and interpretations will be listed.

4.7.6 COVID-19 Impact Assessment

Assessments affected by the COVID-19 pandemic will be listed for visit-based assessments and for non-visit-based assessments.

For visit-based assessments affected by COVID-19, the listing shows if an assessment was not performed due to COVID-19, if it was out of window, if the assessment was performed at an alternative location or if it was a virtual assessment. Other information and comments reported on assessments affected by COVID-19 are also included.

For non-visit-based assessments affected by COVID-19, subjects who experience any of the following items: COVID-19 adverse event, hospitalization due to COVID-19, or COVID-19 death, will be flagged in a listing.

Any events like adverse events, hospitalization, or death, which are related to COVID-19, will be flagged in the corresponding listing.

4.8 Analysis of PK

PK analysis will be conducted on the PKAS.

4.8.1 Estimation of PK parameters

The plasma PK parameter calculation will be performed using noncompartmental method in Phoenix® (Certara St. Louise, MO, USA, version 6.3 or higher) software. For AUC determination, linear up and log down analysis will be used.

4.8.2 Plasma Concentration and PK parameters

Plasma concentrations and PK parameters of ASP5354 will be summarized by tumor type and dose level and, where appropriate, by nominal time points and days using descriptive statistics, including n, mean, SD, coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum. For the pharmacokinetic parameters t_{max} only n, median, minimum and maximum will be calculated. For induction phase nominal days for Ctrough concentration based on start of ASP5354 administration will be used.

4.8.3 Analysis of Pharmacokinetics

A listing and summary statistics of the individual plasma concentrations of ASP5354 and sampling times by treatment and assessment time interval will be prepared.

4.9 Interim Analysis

VRC review will be held after the initial 3 participants in 0.2 mg in each tumor type have data for review. Three participants each will then be enrolled in the 2 additional dose levels in each tumor type. The determination of dose(s) to stop, continue or define as optimal dose will start when 3 participants assigned to each dose level have their data for visualization reviewed by the VRC. This process will continue for every additional 3 participants assigned to each dose level until reaching the maximum of 12 participants. A new dose level might be added.

4.10 Sample Size Determination

The sample size is not based on statistical power calculation. The sample size is expected to provide adequate information to determine the optimal dose ASP5354 for LN visualization in breast cancer and melanoma. Approximately up to 12 participants will be assigned to each 3 dose levels of ASP5354 in each tumor type, and then an estimated total of up to 36 participants will be required in each tumor type. In case some dose levels are stopped based on VRC, additional dose levels might be added.

4.11 Additional Conventions

4.11.1 Missing Data

As a general principle, no imputation of missing data will be done. Exceptions are the start and stop dates of AEs and concomitant medications if they are missing

In case of missing or partial start and stop dates for concomitant medications, the following rules will be used:

If the start date is missing or partial:

- if the month is missing, use January
- if the day is missing, use the first day of the month under consideration
- if the year is missing, use year of the informed consent date
- if the entire date is missing, use informed consent date

If the stop date is missing or partial:

- if the month is missing, use December
- if the day is missing, use the last day of the month under consideration
- if the year or the entire date is missing, set the stop date to December 31st, 2099

If the imputed start date is after the stop date, then the imputed start date will be 1 day prior to the stop date.

For AEs, a missing or incomplete onset date will be imputed according to the following conventions.

If only the year is known for the AE onset date, the imputed onset date will be the latest of the following non-missing dates:

- Date of first dose of study drug
- January 1 of the year of AE onset date

If only the month and year is known for the onset date, set the surrogate onset date to the first day of that month and then apply the following rules.

- If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the surrogate onset date will be the imputed onset date.
- If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the latest of the following non-missing dates:
    - Date of first dose of study drug
    - Surrogate onset date

If the imputed onset date is after the adverse event end date, the imputed onset date will be the same as the adverse event end date.

4.11.2 Analysis Windows

Nominal visits will be used by visit summary.

5 SUPPORTING DOCUMENTATION

Please refer to protocol 5354-CL-1201 for all supporting documents and references.

6 SIGNATURE

Prepared by: *E-signatures are attached at end of document* Date:

PPD

Date (DD Mmm YYYY)

Approved by: *E-signatures are attached at end of document* Date:

PPD

Date (DD Mmm YYYY)

Approved by: *E-signatures are attached at end of document* Date:

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Date (DD Mmm YYYY)