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

Amendment 3 [Nonsubstantial]

11 MAY 2023

IND 154703

Sponsor:

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Protocol history:
Original protocol [26 APR 2021]
Amendment 1 [Nonsubstantial] [30 AUG 2021]
Amendment 2 [Nonsubstantial] [01 AUG 2022]

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CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL

24-hour Contact for Serious Adverse Events See [Section 10.3.7 Reporting Procedures for Serious Adverse Events]	**Please fax or email the serious adverse events/special situations worksheet to:** Astellas Pharma Global Development Inc. US Pharmacovigilance **North America fax number: +1-888-396-3750** **North America alternate fax number: +1-847-317-1241** **Email: safety-us@astellas.com**
Medical Monitor/Study Physician	PPD ICON Clinical Research, US PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES

DOCUMENT HISTORY	
Document	**Date**
Amendment 2 [Nonsubstantial]	01 AUG 2022
Amendment 1 [Nonsubstantial]	30 AUG 2021
Original Protocol	26 APR 2021

Amendment 3 [Nonsubstantial] 11 MAY 2023

This amendment is considered to be nonsubstantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and EU Clinical Trial Regulation because it neither significantly impacts the safety or physical/mental integrity of participants nor the scientific value of the study.

Overall Rationale for the Amendment:

The primary rationale for this amendment is to add the clarification to the study procedure throughout the protocol, and to provide the latest study status.

Summary of Changes

Nonsubstantial Changes

Section Number	Description of Change	Brief Rationale
1.1	The planned study period end date is updated from 4Q2023 to 1Q2025	To align with current planned study period
1.1	The planned total number of study sites is updated from 8 to 4	To align with the current planned total number of study sites

Section Number	Description of Change	Brief Rationale
1.1, 1.3 (Table 1, footnote no. 11), 3 (Table 3), 9.3.5.4, 10.7 (Table 10, footnote no. 6)	*Throughout* Routine 12-lead ECGs is updated from "routine" to "**standard**" *1.3 (Table 1, footnote no. 11) and10.7 (Table 10, footnote no. 6)* The following text is added: "..**as per the site's standard of care routine for cardiac monitoring**." *1.3 (Table 1, footnote no. 11) and 7.2.4* Text is updated to the following: ~~Routine~~ **Standard** 12-lead ECGs will be taken per site's standard procedure at screening, preoperative and safety follow-up period. Intraoperative ECG monitoring will occur at 30 minutes postdose (15 min ≤ and < 45 min). ~~During the surgery,~~**A** 12-lead ECG is not mandatory **for intraoperative and postoperative ECG** and assessment can be based on the rhythm strip or other site standard ECG **as per the site's standard of care routine for cardiac monitoring.**	For further clarification

1.1, 4.1	Language for the visualization data to be collected is updated: ● The visualization of the LN(s) will be assessed by the investigator intraoperatively using a binary "Yes or No" question. o **If** "Yes," the time of surgical identification of LN(s) will be collected by the investigator. o **If** "Yes," the number of LNs visualized and the number of LNs visualized with histopathology confirmed LN will be also assessed by the investigator. **Note: Histopathology review will be conducted per standard practice at each site**. ● Transdermal visualization of lymphatic spread of ASP5354 after injection of the drug will also be assessed using a binary "Yes or No" question. ● The fluorescence intensity of the LN will be qualitatively assessed using a Likert Scale (0 = None; 1 = Mild; 2 = Moderate; and 3 = Strong). ● **The radioactivity count will be collected for all the LNs identified by ASP5354 and/or SoC**.~~Recorded images will be used to determine the Signal Background Ratio (SBR).~~ ● The number of LN(s) with metastatic spread will be assessed based on histopathologic review. **Note: Histopathology review will be conducted per standard practice at each site**. ~~● Following proportions will be compared between ASP5354 and SoC treatment with either Tc-99mSC or Lymphoseek.~~ ~~o Proportion of participants with at least 1 LN detected by visualization with histopathologic confirmation of LN tissue~~ ~~o Proportion of identified LNs with histopathologic confirmation of LN tissue~~ ~~o Proportion of LNs with metastatic spread, based on histopathologic review~~ **During the surgery, video and/or image will be recorded to capture the lymphatic flow of ASP5354 and the signal intensity of each LN**	To clarify assessments that are done by the investigator

Section Number	Description of Change	Brief Rationale
	visualized. These images are used for Signal Background Ratio Analysis done by external reviewer (see [Section 7.1.2]). The visualization data **of the LNs** will be assessed by a VRC **and will be compared with the performance of SoC**.	
1.1, 4.1, 10.1.5.1	The following text is updated: The data for the participants, at each **subsequent dose level**, who completed the SLN biopsy will be assessed by the VRC to decide whether to: • Expand a dose **level if** additional participants are needed to increase confidence for the optimal dose selection; • Stop a dose **level if** 100% of participants lack visualization or all participants have a fluorescence intensity of **0 or** 1 **on the qualitative** Likert Scale, in which case additional participants would not be added to a dose level; or • Define an optimal dose (in the case of 2 doses performing equally, the lower dose will be selected). *Sections 1.1 and 4.1* The VRC assessment for optimal dose determination will be based on the totality of the collected data, including but not limited to: • **Cummulative** LN visualization (Yes/No)	For further clarification
1.1	The following text is added: **The optimal dose for breast cancer and melanoma will be selected separately.**	To align with text in Section 4.1

Section Number	Description of Change	Brief Rationale
1.1, 1.3 (Table 1, footnote no. 15), 4.1, 6.1.1	The following text is removed throughout: "..NIR-F imaging system ~~(with FDA 510[k] cleared optical device system)~~ followed by confirmation with the gamma probe." The following text in bold is added: **Once lymphatic mapping is completed, gamma probe will be used per SoC. For all the LNs observed by ASP5354, gamma probe will be attempted to measure radioactivity**. If the LN is not observed with NIR-F after approximately 15 minutes **following** injection **for breast cancer participants and approximately 15 to 30 minutes for melanoma participants depending on the location of the tumor and LNs**, the investigator can proceed to use the gamma probe for LN detection. When the LN is detected with the gamma probe **first, radioactivity will be measured and then** NIR-F imaging should be attempted again for LN visualization.	To add clarity to the study procedure relating to the biopsy.
1.2 (Figure 2)	• Bullet no. 2 is updated to provide clearer language. • Bullet no. 3 is updated from: "The second interim analysis will be performed by the VRC across initial dose levels…." To: "The second **VRC review** will be performed **for the** initial dose levels…."	For further clarification
1.3 (Table 1, new note)	The following text in bold is added as a new note: **†† The assessments scheduled for the screening period can be done on day 1 if all eligibility criteria can be confirmed prior to registration in the IRT and prior to treatment. If screening procedures are performed on day 1, physical examination, vital signs, clinical laboratory tests, ECG, previous/concomitant medications and AE assessment will be done once.**	To align with surgery procedures at clinical sites
1.3 (Table 1, footnote no. 4)	Body mass index is removed	Body mass index is not a procedure/assessment, but rather a calculated value derived from height and weight

Section Number	Description of Change	Brief Rationale
1.3 (Table 1, footnote no. 6), 7.2.3, 10.7 (Table 10, footnote no. 2)	The following text in bold is added for clarification: At visit 2 and follow-up (visit 3), **a full physical examination is not mandatory, but** a symptom-directed physical examination will be performed.	To provide explicit instruction about the required process of physical examination
1.3 (Table 1, footnote no. 7)	The following text is added to footnote no. 7: Vital signs include blood pressure, pulse and respiratory rate. All vital signs should be measured within 1 week prior to IP administration **at the screening visit. All vital signs** will be measured with the participant in the sitting or supine position. Height and weight will be measured using standard institution practice and equipment.	To provide clear instruction on how to perform and record vital signs
1.3 (Table 1, footnote no. 8)	Text is updated to the following: Postoperative vital signs and clinical laboratory tests will be collected within 2 hours ~~after~~**following** the **end of** surgery	For further clarification

1.3 (Table 1, footnote nos. 9 and 14), 7.2.6.1, 7.3.5, 9.3.5.1, 9.3.7.1, 10.7 (Table 10, footnote no. 7)	The following text is removed: *Section 1.3 (Table 1, footnote no. 9)* Clinical laboratory tests include blood collection for hematology (complete blood count) and serum chemistry and urine samples for urinalysis ~~and urine color evaluation~~. The following text in bold is added: *Section 1.3 (Table 1, footnote no. 14), 7.2.6.1 and 10.7 (Table 10, footnote no. 7)* The occurrence of green coloration of the urine **or skin** will be **monitored throughout the study. If observed, any visual green coloration abnormalities will be** recorded **as an AE**… The occurrence of green coloration of the skin will be recorded **as an AE** any time after dose administration The following text is removed: *Section 7.3.5* ~~Green coloration of the urine or skin will not be considered an AE as this is an expected, known effect of short duration without any expected untoward clinical symptoms; however, this still needs to be assessed and recorded. The assessment details are described in [Section 7.2.6.1 Green Coloration of Urine or Skin].~~ The following text in bold is added: *Section 9.3.5.1* The number and percentage of participants with TEAEs, IP-related TEAEs, serious TEAEs**,** ~~and~~ IP-related serious TEAEs **and green coloration of urine or skin** will be summarized by system organ class, preferred term and dose group. The following text is removed: *Section 9.3.7* ~~9.3.7.1 Green Coloration of Urine or Skin~~ ~~The frequency and percentage of participants who experience green coloration of urine or skin will be summarized by dose group. The duration of green coloration will be summarized using descriptive statistics by dose group.~~	To align with other ASP5354 studies to handle "Green Coloration" as an AE

Section Number	Description of Change	Brief Rationale
2.2	The reference details for NCCN guidelines are added	Reference is missing
5.1 (Inclusion criterion no. 2)	Text is updated from: "Participant has been diagnosed with localized breast cancer (female only, stage 1 to 2, N0 and M0) or….." To: "Participant has been diagnosed with localized breast cancer (female only, stage **0** to 2, N0 and M0) or….."	To allow participants with stage 0 breast cancer to participate in the study based on study site feedback that lymphatic mapping is a part of Standard Care in this population.
5.2 (Exclusion criterion no. 15)	A new exclusion criterion is added: **15. Participant has been diagnosed with bilateral breast cancer**	To exclude participants with bilateral breast cancer, because surgical removal of both breasts is expected and lymphatic mapping is also conducted to both sides. The dose setting for this study is planned for lymphatic mapping for one side of the body, and additional dosing is not allowed. Thus study team determined that participants with bilateral breast cancer is deemed ineligible.
6.1.1	The following text in bold is added for breast cancer and melanoma: **Please refrain from massaging the area of administration after injection of IP.**	To clarify the intraoperative procedure for the site to refrain from massaging the area of administration after the injection of study drug.
6.1.1	The following text in bold is added for melanoma: **Please note that the time from injection of IP to the first visualization may vary due to tumor and LN location**. In the event that NIR-F imaging does not detect LN after approximately 15 **to 30** minutes, the gamma probe should be used for LN identification**.**. The following text is removed: ~~Once identified with the gamma probe, NIR-F should again assess the LNs for fluorescence.~~	To clarify the intraoperative procedure for the site to understand the expected time for the visualization in the each indication
7.1	Text is updated to clarify the data collected in the eCRF for Sentinel LN or Non-sentinel LN	To correct the clerical error for the data points to be collected in the eCRF

Section Number	Description of Change	Brief Rationale
7.1.2	The following text in bold is added: **7.1.2 Signal Background Ratio** **Video recording by NIR-F device is also required to evaluate the visualization of LN by ASP5354. Start recording a video at the time of injection of ASP5354 and continue until the first lymph node is visualized. For the remaining lymph nodes visualized, it is recommended to use video recording, but capturing images with the device is also acceptable. For further details, please refer to the Imaging Charter**. The following text in bold is added and strikethrough text is removed: Images will be captured on a NIR-F and overlay view (if available) **for all the LNs identified by ASP5354**. SBR will be calculated ~~using fluorescence images at the time point when ASP5354 detect the first LN~~ as follows [Hoogstins et al, 2019]:	For futher clarification
7.2	The following strikethrough text is removed: ~~Procedures conducted as part of a participant's routine clinical management (i.e., SoC) obtained before signing the ICF may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame, as defined in the Schedule of Assessments [Table 1]~~.	To remove duplication
7.2.4	The following strikethrough text is removed: The ~~anonymized~~ ECG recordings will be printed, signed and saved in the source. ~~Alternatively, per time point, the ECGs can be stored electronically and reviewed in a timely manner by the investigator.~~	For futher clarification
7.3, 7.3.1.1, 7.3.4, 10.3, 10.3.3, 10.3.3.1, 10.3.3.2, 10.3.3.3, 10.3.3.4, 10.3.4, 10.3.6, 10.3.7	Language for the definition of an adverse device effect, along with collection and reporting details are added throughout	To add the details and requirements of adverse event relating to device

Section Number	Description of Change	Brief Rationale
9.2, 9.3.3, 9.3.4, 9.3.6	The pharmacokinetic analysis set population is removed as an analysis set and the analysis set population is clarified throughout. In the following sections, the text in bold is added: *Section 9.3.3* **The secondary endpoint analysis will be performed using the FAS.** *Section 9.3.4* **The exploratory analysis will be performed using the FAS.** *Section 9.3.6* **The pharmacokinetics analysis will be performed using the SAF.**	Pharmacokinetic analysis will be conducted on the SAF and PKAS will be removed from the analysis sets. Considering that PK blood sampling is designed in this study to confirm the exposure to systemic circulation and overall safety of ASP5354 in case of local administration, analysis based on SAF would be more appropriate for this study.
9.3.7	The following text in bold is added: **Other analyses such as the relationship between LNs visualization and pharmacokinetics or other exploratory endpoints may be explored when deemed appropriate. All details will be described in a separate analysis plan and results will be described in a separate report and will not be incorporated in the integrated CSR.**	To add the details of other analysis considered in the future
9.4	The following text in bold is added: **During the VRC review, enrollment at that indication will be closed until the determination of the next dose level to be made.** 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	For further clarification
8.2	The following strikethrough text is removed: ~~A discontinuation from the study is defined as a participant who enrolled in the study and for whom the study is permanently discontinued for any reason.~~	To remove duplication of text in Section 10.1.9
10.1.9	The following strikethrough text is removed: ~~The study start date is the date the first participant signs the ICF for the study (excluding screen failures).~~	To remove duplication of text in Section 4.4
Throughout	Minor administrative-type changes were made, e.g., consistency issues, typos, formatting, section and footnote numbering, and continuity throughout the protocol.	To provide clarifications to the protocol and ensure complete understanding of study procedures.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title of Study:

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

Planned Study Period:

From approximately 3Q2021 to 1Q2025.

Planned Total Number of Study Sites and Location(s):

Approximately 4 study sites in the US

Study Objectives, Endpoints and Estimands:

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

Table continued on next page

Objectives	Endpoints
Secondary *(continued)*	
● To investigate the safety and tolerability of locally administered ASP5354 in participants undergoing SLN biopsy	● Vital signs (blood pressure, pulse and respiratory rate) ● Standard 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.

Estimands

Estimand is not defined for any endpoint.

Study Population:

Male or female participants ≥ 18 years of age with either breast cancer (female only) or melanoma scheduled to undergo sentinel lymph node (SLN) biopsy.

Number of Participants:

Enrollment for breast cancer and melanoma population will be performed independently and simultaneously.

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

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

Study Design Overview:

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 Standard of Care (SoC).

Participants in each tumor type may be assigned to receive a single dose of ASP5354 (0.05, 0.2 or 0.6 mg or 0.2, 0.6 or 1 mg) with the first 3 participants starting at 0.2 mg, which will be locally administered as subcutaneous (breast cancer) or intradermal (melanoma) injection to evaluate the visualization of at least 1 LN, with histopathologic confirmation of LN tissue for each tumor type. Three participants will be assigned to each dose in sequential order after the first 3 participants are evaluated by the VRC. An interim analysis will then be performed across the initial three dose levels (0.05, 0.2 and 0.6 mg or 0.2, 0.6 and 1 mg) for breast cancer and melanoma by the VRC prior to further enrollment of participants.

Participants will receive technetium-99m sulfur colloid (Tc-99mSC) or Lymphoseek as part of SoC for SLN biopsy. In case the participant needs other surgery (e.g., lumpectomy, mastectomy etc.) on the same day of study drug administration, the other surgery is allowed once the SLN biopsy has completed. In the operating room, participants will receive ASP5354. After administration of ASP5354, visualization of the LN(s) will be assessed using a near-infrared fluorescence (NIR-F) imaging system followed by confirmation with the gamma probe. Once lymphatic mapping is completed, gamma probe will be used per SoC. For all the LNs observed by ASP5354, gamma probe will be attempted to measure radioactivity. If the LN is not observed with NIR-F after approximately 15 minutes following injection for breast cancer participants and approximately 15 to 30 minutes for melanoma participants depending on the location of the tumor and LNs, the investigator can proceed to use the gamma probe for LN detection. When the LN is detected with the gamma probe first, radioactivity will be measured and then NIR-F imaging should be attempted again for LN visualization. For participants with melanoma, usage of lymphoscintigraphy or single photon emission computed tomography (SPECT) as part of SoC is also allowed.

The visualization data to be collected will be consist of following:

- The visualization of the LN(s) will be assessed by the investigator intraoperatively using a binary “Yes or No” question.
  - If “Yes,” the time of surgical identification of LN(s) will be collected by the investigator.
  - If “Yes,” the number of LNs visualized and the number of LNs visualized with histopathology confirmed LN will be also assessed by the investigator.

    Note: Histopathology review will be conducted per standard practice at each site.
- Transdermal visualization of lymphatic spread of ASP5354 after injection of the drug will also be assessed using a binary “Yes or No” question.
- The fluorescence intensity of the LN will be qualitatively assessed using a Likert Scale (0 = None; 1 = Mild; 2 = Moderate; and 3 = Strong).
- The radioactivity count will be collected for all the LNs identified by ASP5354 and/or SoC.
- The number of LN(s) with metastatic spread will be assessed by the investigator based on histopathologic review.

  Note: Histopathology review will be conducted per standard practice at each site.

During the surgery, video and/or image will be recorded to capture the lymphatic flow of ASP5354 and the signal intensity of each LN visualized. These images are used for Signal Background Ratio Analysis done by external reviewer.

The visualization data of the LNs will be assessed by a VRC and will be compared with the performance of SoC. The VRC will be held at the following time points for each dose:

- Initial 3 participants at 0.2 mg in each tumor type have completed the SLN biopsies;
- Initial 3 participants at each subsequent dose levels (0.05 and 0.6 mg or 0.6 and 1 mg) in each tumor type have completed the SLN biopsies (total of 6 participants); and
- Additional 3 participants have completed SLN biopsies at the expanded or additional dose levels. Repeat this process until the optimal dose is defined but maximum participants for each cohort (i.e., 12) has not been reached.

The data for the participants, at each subsequent dose level, who completed the SLN biopsy will be assessed by the VRC to decide whether to:

- Expand a dose level if additional participants are needed to increase confidence for the optimal dose selection;
- Stop a dose level if 100% of participants lack visualization or all participants have a fluorescence intensity of 0 or 1 on the qualitative Likert Scale, in which case additional participants would not be added to a dose level; or
- Define an optimal dose (in the case of 2 doses performing equally, the lower dose will be selected).

The VRC assessment for optimal dose determination will be based on the totality of the collected data, including but not limited to:

- Cummulative LN visualization (Yes/No)
- Histopathologic confirmation of LN tissue visualized with ASP5354
- Fluorescence intensity based on the Likert Scale (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
- Safety data

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 of the LNs from all treated participants will be used for optimal dose determination.The optimal dose for breast cancer and melanoma will be selected separately.

Based on VRC review of the initial 3 dose levels, if none of the first 9 participants have LN visualization, then all 3 initial dose levels are stopped. The 1 and 2 mg dose levels or the 2 and 4 mg (breast cancer only) dose levels will be added dependant on the highest dose used in the 3 prior dose levels. If 2 of the initial 3 doses are stopped due to lack of visualization in the 6 participants in the dose levels, then the dose level will be expanded to the 1 and/or 2 mg dose level. The 4 mg dose level for breast cancer will only be added if the 2 mg dose level remained open. For further details of the VRC, refer to the VRC Charter.

If the investigator determines visualization of ASP5354 is not sufficient for LN imaging after local administration, the investigator can proceed with the use of other NIR-F imaging modalities or Blue dye for LN detection if indicated.

Safety and tolerability will be assessed by recording treatment-emergent adverse events and adverse reactions associated with the use of an investigational product, clinical laboratory evaluations (hematology, serum chemistry and urinalysis), 12-lead electrocardiograms, vital signs measurements and physical examination. Blood samples for pharmacokinetic assessment will be collected pre- and post-administration of ASP5354 at defined time points up to the end of surgery.

Treatment Groups and Duration:

ASP5354 lyophilization powder will be supplied by the sponsor or designee.

Breast Cancer

Arm/ IP Name	Breast Cancer 0.05 mg/ ASP5354	Breast Cancer 0.2 mg/ ASP5354	Breast Cancer 0.6 mg/ ASP5354
Use	test product	test product	test product
Dose	0.05 mg	0.2 mg	0.6 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	0.05 mL in total	0.2 mL in total	0.6 mL in total
Frequency	single dose	single dose	single dose
Route	subcutaneous injection	subcutaneous injection	subcutaneous injection
Location	subareolar	subareolar	subareolar
Injection site	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections
Duration	single dose	single dose	single dose

IP: investigational product

Arm/ IP Name	Breast Cancer 1 mg/ ASP5354	Breast Cancer 2 mg/ ASP5354	Breast Cancer 4 mg/ ASP5354
Use	test product	test product	test product
Dose	1 mg	2 mg	4 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	1 mL in total	2 mL in total	4 mL in total
Frequency	single dose	single dose	single dose
Route	subcutaneous injection	subcutaneous injection	subcutaneous injection
Location	subareolar	subareolar	subareolar
Injection site	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections
Duration	single dose	single dose	single dose

IP: investigational product

Melanoma

Arm/ IP Name	Melanoma 0.05 mg/ ASP5354	Melanoma 0.2 mg/ ASP5354	Melanoma 0.6 mg/ ASP5354
Use	test product	test product	test product
Dose	0.05 mg	0.2 mg	0.6 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	0.05 mL in total	0.2 mL in total	0.6 mL in total
Frequency	single dose	single dose	single dose
Route	intradermal injection	intradermal injection	intradermal injection
Location	peritumoral	peritumoral	peritumoral
Injection site	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections
Duration	single dose	single dose	single dose

Arm/ IP Name	Melanoma 1 mg/ ASP5354	Melanoma 2 mg/ ASP5354
Use	test product	test product
Dose	1 mg	2 mg
Frequency	single dose	single dose
Concentration	1.0 mg/mL	1.0 mg/mL
Volume	1.0 mL in total	2.0 mL in total
Frequency	single dose	single dose
Route	intradermal injection	intradermal injection
Location	peritumoral	peritumoral
Injection site	minimum of 2, up to 4, equally divided injections	minimum of 2, up to 4, equally divided injections
Duration	single dose	single dose

IP: investigational product

The anticipated duration of the study for each participant, including screening and follow-up, is approximately 10 days.

1.2 Study Schema

Figure 1 Study Schema

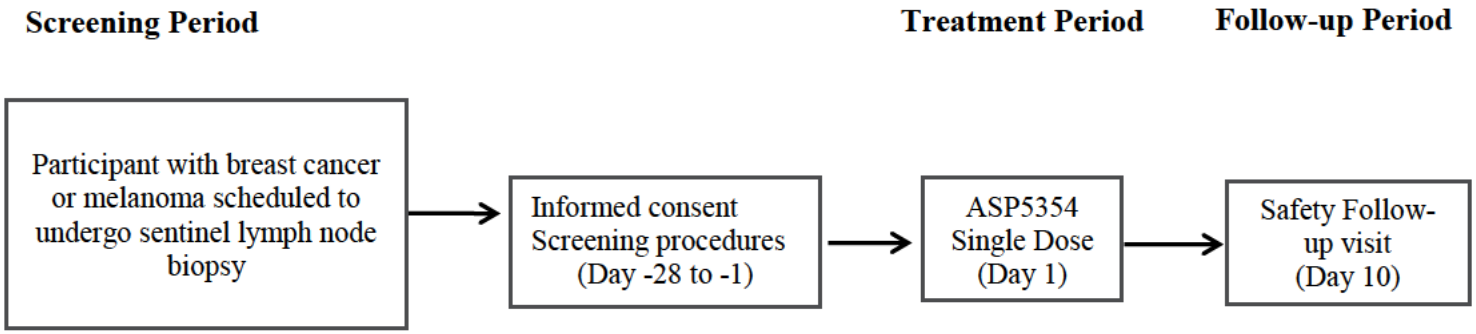
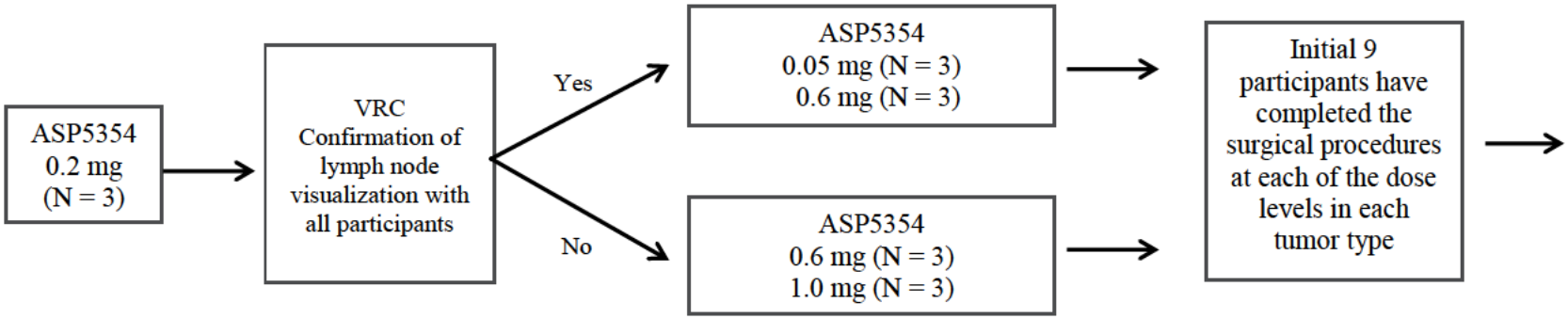
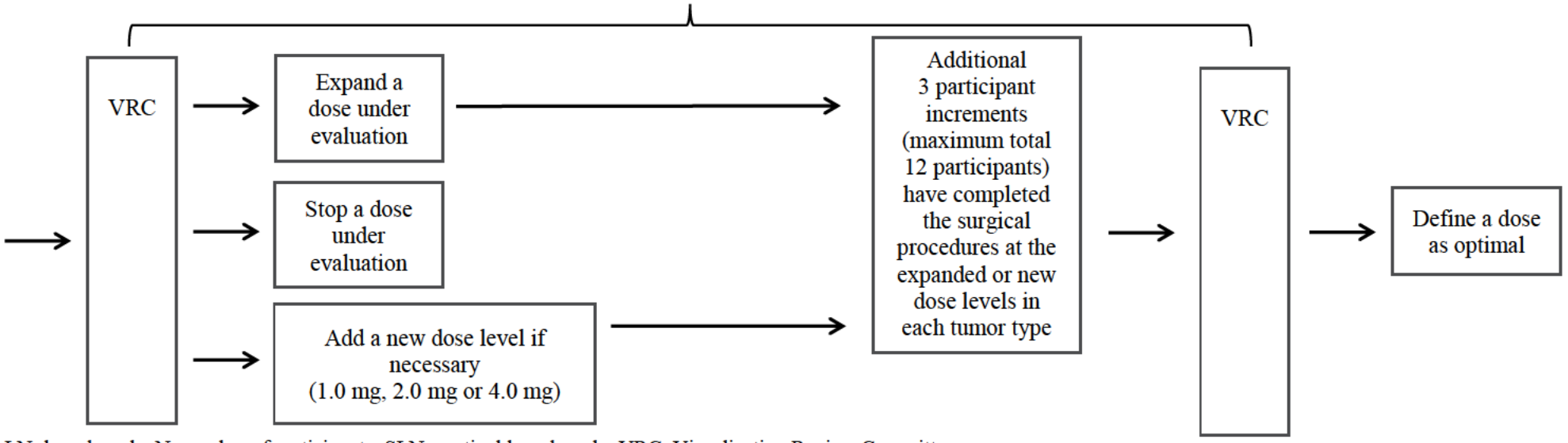


Figure 2 Dose Cohort Schema (Enrollment for breast cancer and melanoma population will be performed independently and simultaneously)



Repeat this process until a dose is defined as optimal (up to a total 12 participants at a dose)



LN: lymph node; N: number of participants; SLN: sentinel lymph node; VRC: Visualization Review Committee

Footnotes continued on next page

- Enrollment of breast cancer and melanoma participants will be performed independently and simultaneously. Starting dose will be 0.2 mg for the initial 3 participants in each tumor type.
- VRC review will be held when initial 3 participants have completed the surgical procedures at the 0.2 mg dose level to decide whether to enroll participants at the 0.05 mg and 0.6 mg dose levels or the 0.6 mg and 1 mg dose levels. If LN visualization with histopathologic confirmation occurs in the first 3 participants in each indication, then dose level expansion will occur and an additional 3 participants in each indication will be enrolled first with the 0.05 mg dose, followed by another 3 participants with the 0.6 mg dose. If < 3 participants at 0.2 mg dose level have LN visualization with histopathologic confirmation on VRC review, 3 participants will be enrolled sequentially in the 0.6 mg and 1 mg dose levels.
- The second VRC review will be performed for the initial dose levels (0.05, 0.2 and 0.6 mg or 0.2, 0.6 and 1 mg) for breast cancer and melanoma prior to further enrollment of participants. Data for the 9 participants in the first 3 dose levels who completed the SLN biopsy will be assessed to decide whether to: 1) expand a dose under evaluation in 3 participants increments up to 12 participants; 2) stop a dose in which case additional participants would not be added to a dose level; 3) add a new dose level(s); or 4) define a dose as optimal.
- Based on VRC review of the initial 3 doses levels of 3 participants:
    - If 0 doses remain, then a 1 and 2 mg or 2 and 4 mg (breast cancer only) dose will be added
    - If 1 dose remains, then the 1 and/or 2 mg dose will be added.
    - The 4 mg dose level will be added for breast cancer participants if only the 2 mg dose level has visualization.
- Up to 12 participants will be assigned to receive single doses of ASP5354 at each initial dose levels (0.05, 0.2 and 0.6 mg or 0.2, 0.6 and 1 mg) or added dose levels (1, 2 or 4 mg [breast cancer only]) for breast cancer or melanoma.

1.3 Schedule of Assessments

Table 1 Schedule of Assessments

Assessments	**Screening Period††**	**Treatment Period**			**Safety Follow-up Period**
Day	**-28 to -1**	**1 (Operation)**			**10 (Outpatient) [1]**
		Preoperative	**Intraoperative**	**Postoperative**	
Window	**-**	**-**	**-**	**-**	**±4**
Visit Number	**1**	**2**			**3**
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Primary Diagnosis of Target Disease [2]	X				
Medical History[3]	X				
Demographics [4]	X				
Drug and Alcohol Screen [5]	X†				
Physical Examination [6]	X	X		X	X
Vital Signs [7]	X	X		X [8]	X
Clinical Laboratory Tests [9]	X†	X		X [8]	X
Height [7]	X				
Body Weight [7]	X				X
Serum or Urine Pregnancy Test [10]	X†				X
ECG [11]	X	X	X	X	X
Dosing ASP5354 [12]			X		
Blood Sampling for ASP5354 Pharmacokinetics [13]		X	X		
Confirmation of Urine or Skin Coloration [14]			→	→	→
Investigator Evaluation for LN Visualization [15]			X		
Record Surgery [16]			X		
Previous/Concomitant Medications	X	X	X	X	X [17]
AE Assessment	X	X	X	X	X [17]
Study Participant Feedback Questionnaire	X				X

Footnotes and abbreviations on next page

AE: adverse event, ECG: electrocardiogram; IP: investigational product; IRT: interactive response technology; LN: lymph node; NIR-F: near-infrared fluorescence; TNM: tumor, nodes and metastases

† These assessments will be performed by a local laboratory and the results of these assessments must be obtained prior to participant enrollment.

†† The assessments scheduled for the screening period can be done on day 1 if all eligibility criteria can be confirmed prior to registration in the IRT and prior to treatment. If screening procedures are performed on day 1, physical examination, vital signs, clinical laboratory tests, ECG, previous/concomitant medications and AE assessment will be done once.

1. If a participant discontinues early from the study, day 10 outpatient visit procedures will be performed upon study discontinuation.
2. Primary diagnosis of the target disease will include:
   - Breast cancer: T categorization in TNM classification, location of tumor (left breast, right breast or other)
   - Melanoma: T categorization in TNM classification and location of tumor (Extremities [upper/lower, left/right], Trunk, Head and Neck, Other), Tumor Thickness and Ulceration status.
3. Medical History includes all significant medical conditions that have occurred or are currently ongoing. The medical condition, onset date, and recovery date will be collected.
4. Demographic information will be collected for all participants at screening and will include age, sex, race, ethnicity, weight and height.
5. Drug screen and alcohol screen will be performed by the study site's preferred method.
6. A full physical examination will be performed at screening (visit 1). Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular. At visit 2 and follow-up (visit 3), a full physical examination is not mandatory, but a symptom-directed physical examination will be performed.
7. Vital signs include blood pressure, pulse and respiratory rate. All vital signs should be measured within 1 week prior to IP administration at the screening visit. All vital signs will be measured with the participant in the sitting or supine position. Height and weight will be measured using standard institution practice and equipment.
8. Postoperative vital signs and clinical laboratory tests will be collected within 2 hours following the end of surgery.
9. Clinical laboratory tests include blood collection for hematology (complete blood count) and serum chemistry and urine samples for urinalysis. These assessments will be performed by a local laboratory.
10. This assessment will be required only for participants who are women of childbearing potential (see [Section 10.2, Appendix 2 Contraception Requirements]).
11. Standard 12-lead ECGs will be taken per site's standard procedure at screening, preoperative and safety follow-up period. Intraoperative ECG monitoring will occur at 30 minutes postdose (15 min ≤ and < 45 min).A 12-lead ECG is not mandatory for intraoperative and postoperative ECG and assessment can be based on the rhythm strip or other site standard ECG as per the site's standard of care routine for cardiac monitoring.
12. ASP5354 will be injected at a minimum of 2 and up to 4 sites of equally divided injections. The exact date and time and location (i.e., 3 and 9 o'clock position of tumor) of IP administration will be documented.
13. Blood samples for pharmacokinetics of IP and possible metabolite(s) (if applicable) will be collected from every participant. A single pharmacokinetic sample will be collected at each of the following time points: prior to dose, approximately 10 minutes, 30 minutes, 60 minutes and 90 minutes postdose, and at the end of surgery.
14. The occurrence of green coloration of the urine or skin will be monitored throughout the study. If observed, any visual green coloration abnormalities will be recorded as an AE. If it occurs for participants who have an urethral catheter in place, the color of urine will be assessed during surgery and every 120 minutes (± 30 minutes window) after the end of surgery until it resolves or the participant is discharged, whichever is earlier. If no urethral catheter is in place, voided urine will be assessed for green coloration and the time collected until it resolves or the participant is discharged, whichever is earlier. The start and stop date and time will also be recorded. The occurrence of green coloration of the skin will be recorded as an AE any time after dose administration. The start and stop date and time will be recorded. Skin coloration will be followed up until it resolves or the participant is discharged, whichever is earlier.

Footnotes continued on next page

15. Surgeon evaluation for LN(s) visualization includes incidence of visualization of the LN(s) and distal or second tier LN(s) and the Likert Scale for qualitative response of the intensity of fluorescence. After administration of ASP5354, visualization of the LN(s) will be assessed first using a NIR-F imaging system followed by confirmation with the gamma probe. Once lymphatic mapping is completed, gamma probe will be used per SoC. For all the LNs observed by ASP5354, gamma probe will be attempted to measure radioactivity.If the LN is not observed with NIR-F after approximately 15 minutes following injection for breast cancer participants and approximately 15 to 30 minutes for melanoma participants depending on the location of the tumor and LNs, the investigator can proceed to use the gamma probe for LN detection. When the LN is detected with the gamma probe first, radioactivity will be measured and then NIR-F imaging should be attempted again for LN visualization. During surgery, parameters will be recorded such as time of incision and number of LN(s).
16. Fluorescence images will be captured and recorded during the entire surgery and archived.
17. If a participant experiences an AE or change in concomitant medications during the follow up period, the participant should call the study site to inform any of these changes. Should the participant develop a hypersensitivity reaction, an additional blood sample for determination of histamine and tryptase concentrations should be taken as soon as possible after the onset of the hypersensitivity reaction.

1.3.1 Sample Collection and Timed Procedure Schedule

Table 2 Sample Collection and Timed Procedure Schedule

Day	Operation	Time Point	Sample Collection	Collection Window
			Blood Sampling for Pharmacokinetics	
Day 1	Preoperative	Predose	X	Within 1 h before dosing
	Intraoperative	10 min [a]	X	5 min ≤ and < 15 min
		30 min [a]	X	15 min ≤ and < 45 min
		60 min [a]	X	45 min ≤ and < 75 min
		90 min [a]	X	75 min ≤ and < 105 min
	End of surgery [b]	-	X	-

h: hour

a. If the end of surgery is earlier than this time point, this time point(s) will not need to be collected.

b. In case the participant needs other surgery following sentinel lymph node biopsy, this time point will be at the time of all surgeries end.

2 INTRODUCTION

2.1 Study Rationale

The currently approved agents for lymphatic mapping, while effective, have negative consequences such as allergic reaction, tissue staining, radiation exposure and/or require special handling and disposal. An agent that is safe, easy to use and may shorten operative times without negative effects would be beneficial for both patients and surgeons.

ASP5354 is a small-molecule, near-infrared fluorescence (NIR-F) imaging agent that is an indocyanine green (ICG)-derivative conjugated with cyclodextrin. The physical and chemical properties differ from ICG and allow shorter transit times from injection to lymph node (LN) uptake than ICG in non-clinical studies.

ASP5354 will be evaluated in patients undergoing Standard of Care (SoC) lymphatic mapping to determine safety, optimal dose and preliminary efficacy in lymphatic mapping.

2.2 Background

Lymphatic mapping is important in many cancer surgeries where detection of sentinel lymph nodes (SLNs) for removal is SoC and also for understanding lymphatic drainage in extremities post lymphadenectomy [Jeremiasse et al, 2020; Suami et al, 2018].

SLN resection has become an integral part of surgery for many localized cancers to diagnose the presence of lymphatic spread and secondarily to avoid the complications related to extensive removal of the lymphatics. It has been shown that the degree of lymphatic complications is related to the number of LNs removed [Herd-Smith et al, 2001]. For patients with localized breast cancer, melanoma and cervical cancer, it has become SoC [National Comprehensive Cancer Network (NCCN) Guidelines® for cutaneous melanoma, 2019; NCCN) Guidelines® for breast cancer, 2021; Dessources et al, 2020; Giuliano et al, 2011]. SLN removal has also been used in Head and Neck and gastrointestinal (GI) malignancies, although not uniformly [de Bree et al, 2020; Mueller et al, 2019].

Intraoperatively, identification of the lymphatics can be performed visually; however, improvements to optimize the detection of the lymphatics, especially for SLNs, can be done using NIR-F imaging with ICG, blue dyes or radioisotopes.

For breast cancer, surgeons primarily use radioisotope, blue dye or both for LN detection with rates of detection > 90%; both of which are FDA approved [Lymphazurin™ prescribing information, January 2012 and Lymphoseek prescribing information, June 2017]. For Melanoma, the FDA approved radioisotope that is typically used. This preference may be partly due to variability of lymphatic drainage for truncal lesions. For cervical or endometrial cancer, ICG is the preferred FDA approved modality for detection [Spy Agent™ Green prescribing information, November 2018]. In Head and Neck cancer, radioisotope has also been approved for use [Lymphoseek prescribing information, June 2017]. ICG use has also been reported for GI malignancies [Chen et al, 2020].

While each modality has high rates of SLN detection, they have some limitations. Radio-isotope cannot be used intraoperatively, is associated with radiation and requires special equipment to prepare and handle and a gamma counter to detect the radiation emitted from the isotope [Lymphoseek prescribing information, June 2017; Wallace et al, 2013; Sondak et al, 2013]. Blue dye can lead to interactions and adverse reactions, mainly allergic, and also can stain the tissue making determination of the LN harder and may lead to cosmetic issues [Isosulfan Blue prescribing information, September 2020] [Thevarajah et al, 2005; Saha et al, 2015]. ICG is considered safe but does have a 1 in 42,000 risk of allergic reaction, for which fatal outcomes have been reported [Spy Agent™ Green (indocyanine green) prescribing information, November 2018].

ASP5354 is a small-molecule NIR-F imaging agent that is an ICG-derivative conjugated with cyclodextrin. ASP5354 is currently being developed by Astellas Pharma Global Development Inc. as an imaging agent to be administered intravenously for intraoperative NIR-F guided identification of the ureters. The physical and chemical properties of ASP5354 are sufficiently different from ICG that non-clinical studies were performed to assess its utility in lymphatic mapping. Non-clinical studies have determined the transit time from injection to LN uptake is shorter than comparable doses of ICG; in addition, no local toxicity was seen. This data suggests that it might be advantageous for lymphatic mapping during breast cancer or melanoma surgery. The purpose of this phase 2 dose-range study is to characterize the safety, optimal dose and preliminary efficacy of ASP5354 in patients undergoing SLN biopsy as part of cancer treatment.

2.2.1 Nonclinical and Clinical Data

Nonclinical and clinical data for ASP5354 available as of the writing of this protocol are summarized below. Please refer to the current version of the ASP5354 Investigator's Brochure (IB) for the most recent data.

2.2.1.1 Summary of Nonclinical Data

ASP5354, ICG-derivative conjugated with cyclodextrins, is a NIR-F imaging agent with hydrophilic properties.

ASP5354 emitted NIR-F at wavelength 820 nm when irradiated with near-infrared excitation light at wavelength 780 nm in vitro. Moreover, results of an in vivo imaging study using Göttingen minipigs suggested that subcutaneous administration of ASP5354 at 0.01 mg/body or greater allows for sufficient intraoperative imaging of the lymphatic system.

ASP5354 at a concentration of 10 μmol/L did not have > 50% inhibition on radioligand binding to any of the 66 receptors, ion channels, transporters tested, nor did it affect the activity of enzyme reactions of 7 enzymes, suggesting that the potential off target activities of ASP5354 is low.

The plasma protein binding of ASP5354 at concentrations of 1 to 1000 μg/mL was 76.11% to 78.90% in rats, 68.50% to 73.84% in rabbits, 73.88% to 75.42% in Göttingen minipigs, 72.98% to 74.90% in cynomolgus monkeys and 74.17% to 77.25% in humans.

No ASP5354 metabolites were detected after incubation with cryopreserved hepatocytes or liver microsomes from rats, dogs, cynomolgus monkeys and humans. No ASP5354 metabolites were also detected in rat, cynomolgus monkey and human plasma and in rat, Göttingen minipig, cynomolgus monkey and human urine obtained after intravenous administration of ASP5354. These results suggest that ASP5354 is metabolically stable in these species.

After intravenous administration ASP5354 was mainly excreted into urine in rats and Göttingen minipigs.

ASP5354 up to 100 μmol/L have no inhibitory effects on cytochrome P450 (CYP) 1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptide (OATP) 1B1, OATP1B3, organic anion transporter (OAT) 1, OAT3, organic cation transporter 2 (OCT2), multidrug and toxin extrusion (MATE) 1 and MATE2-K. ASP5354 is not a substrate of P-gp, BCRP, OAT1, OAT3, OCT2, MATE1 and MATE2-K.

ASP5354 had no genotoxic, teratogenic, local irritative or phototoxic effects, nor did it effect the central nervous**,** cardiovascular or respiratory systems.

Subcutaneous or intramuscular administration of ASP5354 to rabbits resulted in no test article-related discernible gross or histopathological findings. Systemic exposure following subcutaneous to Göttingen minipigs at minimum effective dose (0.01 mg/body) was below the lower limit of quantification, and below the levels seen following intravenous dosing to rats and cynomolgus monkeys. Intravenous administration to rats or cynomolgus monkeys, showed no systemic toxicities at levels 15411-fold and 76712-fold the C_{max} observed following subcutaneous administration to Göttingen minipigs at pharmacologically sufficient dose (0.1 mg/body), respectively.

The intravenous pharmacokinetic and toxicity studies show that systemic exposure to ASP5354 is short and that high systemic exposure of the imaging agent had little or no discernible toxicities. These data, coupled with the absence of local irritation following subcutaneous or intramuscular administration, support expanding the clinical use of this product for lymphatic mapping in breast cancer or melanoma surgeries.

Administration of ASP5354 to pregnant rats or rabbits had no adverse effect on the pregnant animals nor did it result in embryofetal loss or fetal variations or malformations. Despite these findings, appropriate restrictions on administration of ASP5354 in clinical studies are recommended.

Please refer to the current ASP5354 IB for the most recent nonclinical data.

2.2.1.2 Summary of Clinical Data

2.2.1.2.1 Phase 1 Results (Study 5354-CL-0001)

The study, 5354-CL-0001, was a phase 1, double-blind, randomized, placebo controlled, single ascending dose study assessing the safety and pharmacokinetics of ASP5354 in

healthy adult volunteers. Investigational product (IP) was administered as a single intravenous bolus injection under fasting conditions.

2.2.1.2.1.1 Pharmacokinetics and Product Metabolism in Humans

Following a single intravenous administration, ASP5354 was rapidly eliminated. The observed pharmacokinetic parameters suggested a dose proportional increase in the investigated dose range from 0.1 to 24 mg. Almost all fraction of ASP5354 administered was excreted unchanged in urine. Renal clearance (CL_R) was nearly equivalent to the total body clearance.

2.2.1.2.1.2 Safety

Final safety data from Study 5354-CL-0001 show that of the 30 healthy participants enrolled, 2 (20%) participants who received placebo and 3 (15%) participants who received ASP5354 experienced at least 1 treatment-emergent adverse event (TEAE) (dysuria, headache, incontinence, nausea, oral herpes, presyncope, pyelonephritis, urinary tract infection and vomiting [each in 1 participant]). The TEAEs reported in participants who received placebo were nausea, vomiting and incontinence (each in 1 participant). The TEAEs reported in participants who received ASP5354 were oral herpes, pyelonephritis, urinary tract infection, headache, presyncope and dysuria (each in 1 participant). None of the participants experienced TEAEs considered to be causally related to ASP5354. One participant who received 24 mg ASP5354 experienced a ≥ grade 3 TEAE that began on day 2 following dosing; this grade 3 event of pyelonephritis was also a serious adverse event (SAE) that was considered by the investigator to be not related to ASP5354; rather, it was assessed as related to the use of a urinary catheter. At doses of 2, 8 and 24 mg, participants experienced a transient green coloration of urine, due to the color of the dye. This observation was not considered as an adverse event (AE).

2.2.1.2.2 Phase 1 Results (Study 5354-CL-0002)

The Japanese Phase 1 study (Study 5354-CL-0002) was a randomized participant- and investigator-blinded, placebo-controlled, single intravenous dose study in healthy adult Japanese participants.

2.2.1.2.2.1 Pharmacokinetics and Product Metabolism in Humans

Plasma exposure (AUC_{inf}, AUC_{last} and C_{max}) of ASP5354 following single intravenous doses increased proportional to increases in dose across the 0.5 to 8 mg dose range. Pharmacokinetics of ASP5354 appeared linear across the evaluated dose range. Urinary excretion of ASP5354 was rapid and complete urinary excretion was observed across the 0.5 to 8 mg dose range. CL_R estimates were nearly equivalent to observed total body clearance estimates and almost constant across the 0.5 to 8 mg dose range.

2.2.1.2.2.2 Safety

Single intravenous doses of ASP5354 0.5 mg, 2 mg or 8 mg in healthy adult Japanese male participants appeared to be safe and well tolerated. No TEAEs were reported in any of the 12 participants. There were no clinically significant changes in vital signs, 12-lead ECG,

clinical laboratory evaluation or physical examination. Green coloration of urine was observed in all participants in the ASP5354 2 mg and 8 mg groups, but was not observed in the ASP5354 0.5 mg group or the placebo group.

2.2.1.2.3 Phase 2 Results (5354-CL-0201)

The study, 5354-CL-0201, was a phase 2, randomized, open-label, dose-ranging study for ureter visualization, using ASP5354 in participants undergoing laparoscopic/minimally invasive colorectal surgery. Participants were randomly assigned to receive single doses of ASP5354 (0.3, 1, or 3 mg), which were administered as an intravenous bolus.

Safety data from Study 5354-CL-0201 revealed 1 nonserious TEAE (proteinuria grade 1 with a duration of 11 days in the 1 mg dose expansion group) reported by the investigator as having a reasonable possibility of being related to ASP5354. There were 2 grade 3 SAEs of rectal perforation and colon cancer, which were both in the same participant (0.3 mg group) and not considered related to study treatment. Another participant (0.3 mg group) experienced a grade 3 SAE of blood loss anemia that was not considered related to study treatment.

2.2.2 Summary of Key Safety Information for Investigational Product(s)

The safety profile of ASP5354 is based on the results of the nonclinical studies, clinical phase 1 studies (Study 5354-CL-0001 and Study 5354-CL-0002) and a clinical phase 2 study (5354-CL-0201). Please refer to the current version of the ASP5354 IB for the most recent information.

In nonclinical studies, ASP5354 caused very slight macrophage accumulation in the alveolus in the lungs of rats and increased plasma histamine levels in dogs. Abnormal urine or skin color (pale green, greenish yellow or green) and green coloration of the kidneys, epididymides and/or LNs in gross pathology were also observed in rats and monkeys. These findings disappeared with discontinuation of drug administration. These findings were considered to be caused due to the green color of ASP5354 and were not considered toxicologically significant.

ASP5354 has shown no local irritation, discernible genotoxicity, phototoxicity or embryofetal toxicity potential in the nonclinical species tested.

Since all the phase 1 and 2 studies were single-dose studies, TEAEs leading to withdrawal of study drug were not applicable. No deaths were reported in any of the studies. ASP5354 was well tolerated and had no clinically significant effect on vital signs, ECG, blood chemistry, hematology or urinalysis.

In conclusion, the nonclinical and phase 1 and 2 clinical safety data support the initiation of a phase 2 dose-finding study in patients with either breast cancer or melanoma scheduled to undergo SLN biopsy.

For full details please refer to the current version of the ASP5354 IB.

2.3 Risk/Benefit Assessment

2.3.1 Risk Assessment

Hypersensitivity is monitored as an important potential risk based on the safety profile of ICG, of which ASP5354 is a derivative. Hypersensitivity reactions, including anaphylactic reactions with fatal outcome, are reported to have occurred after treatment with ICG. The reported incidence for ICG-related anaphylactic reactions is 1:42000 administrations or lower. The risk with ASP5354 is estimated to be lower, as ASP5354 is not iodinated, whereas ICG is.

Hypersensitivity reactions are well known to occur, in varying frequencies, with all kinds of contrast agents (iodinated contrast agents for computed tomography, gadolinium-based contrast agents for magnetic resonance imaging and contrast agents for image enhancement during ultrasound). No such reactions have been observed for ASP5354 during the completed phase 1 studies (Study 5354-CL-0001 and Study 5354-CL-0002) or during the phase 2 study (Study 5354-CL-0201).

2.3.2 Benefit Assessment

FDA approved imaging agents for lymphatic mapping are available; however, they have been associated with undesirable effect such as allergic reactions, skin staining or radiation exposure. ASP5354 provides a way to visualize LNs intraoperatively. ASP5354 has been safely administered up to 24 mg intravenously. ASP5354 was well tolerated, with no IP-related TEAEs reported. As such, there are no safety concerns for its use in participants undergoing SLN biopsy. The benefits of using ASP5354 intraoperatively may decrease operating times, avoid radiation exposure.

2.3.3 Overall Risk-Benefit Conclusion

The benefit of a single administration of ASP5354 outweighs the anticipated risk. No toxicities have been seen in intravenous doses up to 24 mg and no local toxicity was observed in non-clinical evaluations. Safety will be monitored during the study for all enrolled participants.

3 OBJECTIVES, ENDPOINTS AND ESTIMANDS

Table 3 Study 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: ○ 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
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
● To investigate the safety and tolerability of locally administered ASP5354 in participants undergoing SLN biopsy	● Vital signs (blood pressure, pulse and respiratory rate) ● Standard 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
Table continued on next page	

Objectives	Endpoints
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.

Estimands

Estimand is not defined for any endpoint.

4 STUDY DESIGN AND DOSE RATIONALE

4.1 Overall 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 Standard of Care (SoC).

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 in each indication and then the Visualization Review Committee (VRC) will review the data for the breast cancer group and the melanoma group separately. If LN visualization with histopathologic confirmation occurs in the first 3 participants in each indication, then dose level expansion will occur and an additional 3 participants in each indication will be enrolled first with the 0.05 mg dose, followed by another 3 participants with the 0.6 mg dose. An interim analysis will be performed across initial dose levels (0.05, 0.2 and 0.6 mg) separately for breast cancer and melanoma by the VRC prior to further enrollment of participants.

If < 3 participants at 0.2 mg dose level have LN visualization with histopathologic confirmation on VRC review, 3 participants will be enrolled sequentially in the 0.6 mg and 1 mg dose levels. An interim analysis will then be performed across initial dose levels (0.2, 0.6 and 1 mg) separately for breast cancer and melanoma by the VRC prior to further enrollment of participants.

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

Conc: concentration

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

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

Participants will receive Tc-99mSC or Lymphoseek as part of SoC for SLN biopsy. In case the participant needs other surgery (e.g., lumpectomy, mastectomy etc.) on the same day of study drug administration, the other surgery is allowed once the SLN biopsy has completed. In the operating room, participants will receive ASP5354. After administration of ASP5354, visualization of the LN(s) will be assessed using a NIR-F imaging system followed by confirmation with the gamma probe. Once lymphatic mapping is completed, gamma probe will be used per SoC. For all the LNs observed by ASP5354, gamma probe will be attempted to measure radioactivity. If the LN is not observed with NIR-F after approximately 15 minutes following injection for breast cancer participants and approximately 15 to 30 minutes for melanoma participants depending on the location of the tumor and LNs, the investigator can proceed to use the gamma probe for LN detection. When the LN is detected with the gamma probe first, radioactivity will be measured and then NIR-F imaging should be attempted again for LN visualization. For participants with melanoma, usage of lymphoscintigraphy or SPECT as part of SoC is also allowed.

The visualization data to be collected will consist of the following:

- The visualization of the LN(s) will be assessed by the investigator intraoperatively using a binary "Yes or No" question.
  - If "Yes," the time of surgical identification of LN(s) will be collected by the investigator.
  - If "Yes," the number of LNs visualized and the number of LNs visualized with histopathology confirmed LN will be also assessed by the investigator.
    Note: Histopathology review will be conducted per standard practice at each site.
- Transdermal visualization of lymphatic spread of ASP5354 after injection of the drug will also be assessed using a binary "Yes or No" question.
- The fluorescence intensity of the LN will be qualitatively assessed using a Likert Scale (0 = None; 1 = Mild; 2 = Moderate; and 3 = Strong).
- The radioactivity count will be collected for all the LNs identified by ASP5354 and/or SoC.
- The number of LN(s) with metastatic spread will be assessed based on histopathologic review.
  Note: Histopathology review will be conducted per standard practice at each site.

During the surgery, video and/or image will be recorded to capture the lymphatic flow of ASP5354 and the signal intensity of each LN visualized. These images are used for Signal Background Ratio Analysis done by external reviewer (see [Section 7.1.2]).

The visualization data of the LNs will be assessed by the VRC and will be compared with the performance of SoC. For further details of the VRC, refer to [Section 10.1.5 Committee(s) Structure and the VRC Charter]. The VRC will be held at the following time points for each dose:

- Initial 3 participants at 0.2 mg in each tumor type have completed the SLN biopsies;
- Initial 3 participants at each subsequent dose levels (0.05 and 0.6 mg or 0.6 and 1 mg) in each tumor type have completed the SLN biopsies (total of 6 participants); and
- Additional 3 participants have completed SLN biopsies at the expanded or additional dose levels. The review of an additional 3 participants will continue until the optimal dose is defined. Maximum participants for each cohort (i.e., 12) has not been reached.

The data for the participants, at each subsequent dose level, who complete the SLN biopsy will be assessed by the VRC to decide whether to:

- Expand a dose level if additional participants are needed to increase confidence for the optimal dose selection;
- Stop a dose level when 100% of participants lack visualization or all participants have a fluorescence intensity of 0 or 1 on the qualitative Likert Scale, in which case additional participants would not be added to the dose level; or
- Define an optimal dose (in the case of 2 doses performing equally, the lower dose will be selected).

The VRC assessment for optimal dose determination will be based on the totality of the collected data, including but not limited to:

- Cummulative LN visualization (Yes/No)
- Histopathologic confirmation of LN tissue visualized with ASP5354
- Fluorescence intensity based on the Likert Scale (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
- Safety data

The optimal dose is defined as the dose that provides 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 of the LNs from all treated participants will be used for optimal dose determination. The optimal dose for breast cancer and melanoma will be selected separately.

Based on VRC review of the initial 3 dose levels, if none of the first 9 participants have LN visualization, then all 3 initial dose levels are stopped.The 1 and 2 mg dose levels or the 2 and 4 mg (breast cancer only) dose levels will be added dependant on the highest dose used in the 3 prior dose levels. If 2 of the initial 3 doses are stopped due to lack of visualization in the 6 participants in the dose levels, then the dose level will be expanded to the 1 and/or 2 mg dose level. The 4 mg dose level for breast cancer will only be added if the 2 mg dose level remained open. For further details of the VRC, refer to the VRC Charter.

If the investigator determines visualization of ASP5354 is not sufficient for LN imaging after local administration, the investigator can proceed with the use of other NIR-F imaging modalities or Blue dye for LN detection if indicated.

Safety and tolerability will be assessed by recording TEAEs and adverse reactions associated with the use of an IP, clinical laboratory evaluations (hematology, serum chemistry and urinalysis), 12-lead ECGs, vital signs measurements and physical examination. Blood samples for pharmacokinetic assessment will be collected pre- and post-administration of ASP5354 at defined time points up to the end of surgery.

4.2 Scientific Rationale for Study Design

This is an open label dose finding study to determine the optimal dose for lymphatic mapping in participants undergoing lymphatic mapping and SLN biopsy with a radioisotope as part of SoC. Radioisotope is used routinely for lymphatic mapping in localized breast cancer (blue dye may also be used) and melanoma and provides a unique opportunity to investigate ASP5354 as a lymphatic mapping agent in participants already undergoing this procedure.

Based on the nonclinical data of fastest transit time and strength of fluorescence of 0.1 mg in minipigs, the starting dose is 0.2 mg was selected. This will decrease the possibility of starting with a dose that may not lead to visualization in human participants. Treatment will be staggered so the data in the first 3 participants treated can be analyzed to determine whether to dose above and below 0.2 mg or use 2 higher doses. Optimal dose determination will require an inferior dose and a comparable higher dose. Important characteristics of an

optimal dose will include fluorescence of lymphatic tissue based on histopathologic confirmation and intensity of fluorescence.

The current study design will allow sequential assessment for safety, tolerability and ability for a given dose to provide LN visualization before exposing additional subjects to ASP5354. LN identification is binomial (Yes or No), as such descriptive statistics will be used for analysis.

4.3 Dose Rationale

The starting dose is 0.2 mg (0.2 mL of a 1 mg/mL ASP5354 solution) per participant, which will be locally administered as subcutaneous (breast cancer) or intradermal (melanoma) injection. The rationale for the starting dose is based on the results of the toxicology studies in rabbits and the phase 1 study after intravenous administration (Study 5354-CL-0001) and the estimated clinical efficacious dose in humans. ASP5354 solution at up to 5 mg/mL showed no local irritation after subcutaneous and intramuscular injection in rabbits (Studies 5354-TX-0101 and 5354-TX-0102), indicating 1 mg/mL ASP5354 solution can be used in this study. ASP5354 is confirmed safe and well tolerated up to 24 mg after intravenous administration in the phase 1 study (Study 5354-CL-0001). So, the starting dose of 0.2 mg is considered to be safe. The estimated clinical efficacious dose of 0.2 mg (0.2 mL of a 1 mg/mL ASP5354 solution) per participant is proposed because 0.1 mL of a 1 mg/mL ASP5354 solution provided comparable LN visualization after injection as compared with 0.1 mL of a 2.5 or 5 mg/mL ICG solution in the nonclinical study in Göttingen minipigs (Study 5354-PH-0004).

The potential highest dose tested in this study will be 4 mg (4 mL of a 1 mg/mL ASP5354 solution) per participant, which is well below the highest intravenous dose of 24 mg tested in the phase 1 study (Study 5354-CL-0001).

4.4 End of Study Definition

The study start is defined as the date the first participant signs informed consent. The end of the study is defined as the last visit or scheduled procedure shown in the Schedule of Assessments for the last participant in the study.

5 STUDY POPULATION

All screening assessments must be completed and reviewed to confirm the potential participant meets all eligibility criteria. Prospective approval of protocol deviations to eligibility criteria (also known as protocol waivers or exemptions) is not permitted.

5.1 Inclusion Criteria

Participant is eligible for participation in the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization for US study sites) must be

obtained from the participant prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).

2. Participant has been diagnosed with localized breast cancer (female only, stage 0 to 2, N0 and M0) or melanoma (stage 1 to 2, N0 and M0) and is scheduled to undergo surgical intervention for SLN detection and removal using Tc-99mSC or Lymphoseek as part of SoC.
3. Participant is considered an adult (≥ 18 years of age) according to local regulation at the time of signing the informed consent form (ICF).
4. Female participant is not pregnant (see [Section 10.2 Appendix 2 Contraception Requirements]) and at least 1 of the following conditions apply:
    a. Not a woman of childbearing potential (WOCBP) (see [Section 10.2 Appendix 2 Contraception Requirements])
    b. WOCBP who agrees to follow the contraceptive guidance (see [Section 10.2 Appendix 2 Contraception Requirements]) from the time of informed consent through at least 30 days after final study treatment administration.
5. Female participant must agree not to breastfeed starting at screening and throughout the study period and for 30 days after final study treatment administration.
6. Female participant must not donate ova starting at first dose of IP and throughout the study period and for 30 days after final study treatment administration.
7. Male participant with female partner(s) of childbearing potential (including breastfeeding partner) must agree to use contraception (see [Section 10.2 Appendix 2 Contraception Requirements]) throughout the treatment period and for 30 days after final study treatment administration.
8. Male participant must not donate sperm during the treatment period and for 30 days after final study treatment administration.
9. Male participant with pregnant partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy throughout the study period and for 30 days after final study treatment administration.
10. Participant agrees not to participate in another interventional study while participating in the present study.

Waivers to the inclusion criteria will **NOT** be allowed.

5.2 Exclusion Criteria

Participant will be excluded from participation in the study if any of the following apply:

1. Participant has had prior LN surgery in the area where LN detection is needed.
2. Participant has had prior LN radiation in the area where LN detection is needed.
3. Participant has had prior neo-adjuvant chemotherapy.
4. Participant has definitive LN metastases.
5. Participant has metastatic cancer.
6. Participant has received any investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to screening.

7. Participant has any condition, which, in the investigator's opinion, makes the participant unsuitable for study participation.
8. Participant has a known or suspected hypersensitivity to ASP5354, ICG or any components of the formulation used.
9. Participant has had previous exposure to ASP5354.
10. Participant has moderate to severe cardiac disease that limits daily functioning (New York Heart Association Class III-IV) or other medical conditions that the investigator feels would impact safety or study compliance.
11. Participant has a resting heart rate ≤ 45 bpm or ≥ 115 bpm, systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg within 1 week prior to IP administration. If the blood pressure exceeds the limits above, repeat readings can be taken. Participant who has adequately controlled blood pressure is eligible.
12. Participant has any of the following screening laboratory values:
    - Hemoglobin ≤ 9 g/dL
    - Absolute neutrophil count ≤ 1500/μL
    - Platelet count ≤ 100000/μL
    - Estimated glomerular filtration rate < 60 mL/min/1.73 m^2 based on the Chronic Kidney Disease Epidemiology Collaboration equation*
    - Serum bilirubin ≥ 1.5 × upper limit of normal (ULN)
    - Aspartate aminotransferase (AST) ≥ 2.5 × ULN
    - Alanine aminotransferase (ALT) ≥ 2.5 × ULN

    *eGFR = 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] × 1.159 [if African American]

    where:
    Scr is serum creatinine in mg/dL,
    κ is 0.7 for females and 0.9 for males,
    α is -0.329 for females and -0.411 for males,
    min indicates the minimum of Scr/κ or 1, and
    max indicates the maximum of Scr/κ or 1
13. Participant has received ICG, other NIR-F imaging agents, Blue dye (e.g., methylene blue or isosulfan blue) or Magtrace within 2 weeks prior to IP administration.
14. Participant has active alcohol abuse or illicit drug abuse (non-prescription or over-the-counter medications). Participant should not have consumed any alcohol or illicit drug (excluding cannabidiol products) within 24 hours of surgery.
15. Participant has been diagnosed with bilateral breast cancer

Waivers to the exclusion criteria will **NOT** be allowed.

5.3 Lifestyle Considerations

Not applicable.

5.4 Screen Failures

A screen failure is defined as a potential participant who signed the ICF but did not meet 1 or more criteria required for participation in the study.

For screen failures, the demographic data, date of signing the ICF, inclusion and exclusion criteria, AEs up to the time of screen failure and reason for screen failure will be collected in the electronic case report form (eCRF).

5.4.1 Rescreening

Rescreening is not allowed. However, in case that the results of screening assessments do not meet the parameters required by the eligibility criteria (e.g., clinical laboratory tests, vital signs, physical examination, ECGs, etc.) screening assessments may be repeated once within the screening period without the need to register the participant as a screen failure.

6 INVESTIGATIONAL PRODUCT

6.1 Investigational Product Administered

The assigned doses of ASP5354 will be administered as a single dose intraoperatively.

Table 4 Investigational Product

Name	ASP5354
Use	Test product
Dosage Formulation	Sterile powder for solution
Physical Description	Green cake
Unit Dose Strength	Each vial contains 1.3 mg of ASP5354 and has to be reconstituted with 1.2 mL sterile water for injection, to a concentration of 1 mg/mL.
Packaging and Labeling	ASP5354 sterile lyophilized powder will be provided in 2R amber glass vials and stored according to the instructions on the label
Route of Administration	Subcutaneous (breast cancer) or intradermal (melanoma) administration
Frequency/Duration	Single dose
Administration Instruction	Administered under fasting conditions intraoperatively
IMP or Non-IMP	IMP
Sourcing	Provided by sponsor

IMP: Investigational Medicinal Product

Refer to the pharmacy manual for detailed information regarding preparation, handling and storage of the IP.

6.1.1 Investigational Product Administration

ASP5354 sterile lyophilized powder for solution will be supplied by the sponsor or designee for subcutaneous (breast cancer) or intradermal (melanoma) administration.

Each participant will be administered a single dose of the assigned dose level of ASP5354. ASP5354 will be injected at a minimum of 2 and up to 4 sites of equally divided injections.

The exact date and time and location (i.e., 3 and 9 o'clock position of tumor) of IP administration will be documented.

Breast Cancer

Arm/ IP Name	Breast Cancer 0.05 mg/ ASP5354	Breast Cancer 0.2 mg/ ASP5354	Breast Cancer 0.6 mg/ ASP5354
Use	test product	test product	test product
Dose	0.05 mg	0.2 mg	0.6 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	0.05 mL in total	0.2 mL in total	0.6 mL in total
Frequency	single dose	single dose	single dose
Route	subcutaneous injection	subcutaneous injection	subcutaneous injection
Location	subareolar	subareolar	subareolar
Injection site	Minimum of 2, up to 4, sites equally divided injections	Minimum of 2, up to 4, sites equally divided injections	Minimum of 2, up to 4, sites equally dividedinjections
Duration	single dose	single dose	single dose

Arm/ IP Name	Breast Cancer 1 mg/ ASP5354	Breast Cancer 2 mg/ ASP5354	Breast Cancer 4 mg/ ASP5354
Use	test product	test product	test product
Dose	1 mg	2 mg	4 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	1 mL in total	2 mL in total	4 mL in total
Frequency	single dose	single dose	single dose
Route	subcutaneous injection	subcutaneous injection	subcutaneous injection
Location	subareolar	subareolar	subareolar
Injection site	Minimum of 2, up to 4 sites equally divided injections	Minimum of 2, up to 4 sites equally divided injections	Minimum of 2, up to 4 sites equally divided injections
Duration	single dose	single dose	single dose

ASP5354 will be injected subcutaneously at a minimum of 2, and up to 4. sites of equally divided subareolar injections. Please refrain from massaging the area of drug administration. After approximately 5 to 10 minutes, lymphatic mapping will then occur based on intraoperative fluorescence visualization using ASP5354. LNs will be identified by NIR-F imaging, followed by identification with the gamma probe. In the event that NIR-F imaging does not detect LN after approximately 15 minutes, the gamma probe should be used for LN identification. When the LN is detected with the gamma probe first, NIR-F imaging should be attempted again for LN visualization. Dissection of the LN will be conducted following identification by NIR-F imaging and/or the gamma probe. Before completing the surgery, NIR-F imaging and the gamma probe will be used to confirm further presence of other LNs, with further dissection and LN removal if indicated.

Melanoma

Arm/ IP Name	Melanoma 0.05 mg/ ASP5354	Melanoma 0.2 mg/ ASP5354	Melanoma 0.6 mg/ ASP5354
Use	test product	test product	test product
Dose	0.05 mg	0.2 mg	0.6 mg
Concentration	1.0 mg/mL	1.0 mg/mL	1.0 mg/mL
Volume	0.05 ml in total	0.2 ml in total	0.6 ml in total
Frequency	single dose	single dose	single dose
Route	intradermal injection	intradermal injection	intradermal injection
Location	peritumoral	peritumoral	peritumoral
Injection site	Minimum of 2, up to 4, sites equally divided injections	Minimum of 2, up to 4, sites equally divided injections	Minimum of 2, up to 4, sites equally divided injections
Duration	single dose	single dose	single dose

Arm/ IP Name	Melanoma 1 mg/ ASP5354	Melanoma 2 mg/ ASP5354
Use	test product	test product
Dose	1 mg	2 mg
Frequency	single dose	single dose
Concentration	1.0 mg/mL	1.0 mg/mL
Volume	1.0 mL in Total	2.0 mL in total
Frequency	single dose	single dose
Route	intradermal injection	intradermal injection
Location	peritumoral	peritumoral
Injection site	Minimum of 2, up to 4, sites equally divided injections	Minimum of 2, up to 4, sites equally divided injections
Duration	single dose	single dose

ASP5354 will be injected intradermally at a minimum of 2, and up to 4, sites of equally divided peritumoral injections. Please refrain from massaging the area of drug administration. After approximately 5 to 10 minutes, lymphatic mapping will occur based on intraoperative fluorescence visualization using ASP5354. LNs will be identified by NIR-F imaging, followed by identification with the gamma probe. Please note that the time from injection of IP to the first visualization may vary due to tumor and LN location. In the event that NIR-F imaging does not detect LN after approximately 15 to 30 minutes, the gamma probe should be used for LN identification. When the LN is detected with the gamma probe first, NIR-F imaging should be attempted again for LN visualization. Dissection of the LN will be conducted following identification by NIR-F imaging and/or the gamma probe. Before completing the surgery, NIR-F imaging and the gamma probe will be used to confirm further presence of other LNs with further dissection and LN removal if indicated.

6.1.2 Other Therapy

Tc-99mSC or Lymphoseek will be administered per SoC. Blue dye or Magtrace may be used for breast cancer only if ASP5354 does not detect LN and an additional modality is indicated per the surgeon's judgement.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and Labeling

All study drug(s) used in this study will be prepared, packaged and labeled under the responsibility of qualified staff at Astellas Pharma Inc. (API), or sponsor's designee in accordance with API or sponsor's designee standard operating procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Each carton and vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as an investigational drug.

A qualified person of Astellas Pharma Europe BV or sponsor's designee will perform the final release of the IP according to the requirements of the EU Directive 2003/94/EC Annex 13. Refer to the pharmacy manual for detailed information regarding packaging and labeling of the IP.

6.2.2 Handling, Storage and Accountability

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all IP received and any discrepancies are reported and resolved before use of the IP.
- Only participants enrolled in the study may receive IP and only authorized study site personnel may supply or administer IP. Only IP with appropriate expiry/retest dating may be dispensed.
- All IP must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions and access must be limited to the investigator and authorized study site personnel.
- The investigator, institution or the head of the medical institution (where applicable) is responsible for accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records).
- Further guidance and instruction on final disposition of used and unused IP is provided in the pharmacy manual or other document.

Refer to the pharmacy manual for detailed information regarding handling, storage and accountability of the IP.

6.3 Randomization and Blinding

This is an open-label study, and no randomization is planned. 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.

6.4 Investigational Product Compliance

Not applicable, as ASP5354 is a single dose imaging agent.

6.5 Dose Modification

Since this is a single dose study, dose modification is not applicable.

6.6 Continued Access to Investigational Product After the End of the Study

Not applicable, as ASP5354 is a single dose anatomical imaging agent and has no therapeutic indications.

6.7 Treatment of Overdose

In the event of suspected ASP5354 overdose, the participant should receive supportive care and monitoring. The medical monitor/expert should be contacted as applicable.

Refer to [Section 10.3.8.2 Medication Error, Overdose and "Off-label Use"] for reporting requirements for suspected overdose or other medication error.

6.8 Concomitant Therapy

6.8.1 Previous Treatment (Medication)

Investigate the following survey items and record in eCRF.

Previous treatment	Investigational period	Investigational items
ICG Blue Dye Magtrace Other NIR-F imaging agent Tc-99mSC Lymphoseek (generic name tilmanocept)	2 weeks prior to study treatment administration to end of screening period	Name of drug, dose, administration route, administration period, administration timing, and reason for use

ICG: indocyanine green; NIR-F: near-infrared fluorescence; Tc-99mSC: technetium-99m sulfur colloid

6.8.2 Concomitant Treatment (Medication and Non-Medication Therapy)

Investigate the following survey items and record in the eCRF.

Concomitant treatments		Investigational period	Investigational items
Concomitant medication	ICG Blue Dye Magtrace Other NIR-F imaging agent Tc-99mSC Lymphoseek (generic name tilmanocept)	Start of treatment period to end of follow up period	Name of drug, dose, administration route, administration period, administration timing, and reason for use
	All medication other than the above†		Name of drug, administration route, administration period, administration timing, and reason for use
Concomitant therapy	All non-medication therapy including dietary therapy		Name of therapy, treatment period, treatment timing, and reason for treatment

ICG: indocyanine green; NIR-F: near-infrared fluorescence; Tc-99mSC: technetium-99m sulfur colloid

† The following items are not the target of this investigation:

Dissolution/dilution solution (injection water, physiological saline, etc.), treatment prior to assessment, sterilization solution, washing solution and topical anesthesia (xylocaine gel, etc.).

6.8.3 Prohibited Medication

The following concomitant medications will not be allowed within 2 weeks prior to study treatment administration until the completion of the surgical procedure unless it is determined that LN detection with ASP5354, and radioisotope, is not sufficient.

- ICG*
- Other NIR-F imaging agent
- Blue dye

* ICG administration for other perspectives than LN visualization (e.g., vascular perfusion) is allowed after the completion of SLN biopsy.

A list of excluded concomitant medications is provided in [Section 10.5 Appendix 5 List of Excluded Concomitant Medications].

7 STUDY PROCEDURES AND ASSESSMENTS

- Study procedures and their timing are summarized in the Schedule of Assessments [Table 1]. Adherence to the study design requirements, including those specified in the schedule of assessments, is essential and required for study conduct. Prospective protocol waivers or exemptions are not allowed.
- Refer to the Alternative Schedule of Assessments [Table 10] in [Section 10.7 Appendix 7: Clinical Study Continuity] for acceptable alternate methods to assess safety and efficacy parameters in the event the study is interrupted due to a crisis (e.g., natural disaster, pandemic).

- Any change, divergence or departure from the study design or procedures identified in the protocol is considered a protocol deviation. All deviations from the protocol are to be recorded.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., imaging, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the Schedule of Assessments [Table 1].
- Refer to the Alternative Schedule of Assessments in Response to a Crisis [Table 10] for acceptable alternate methods to assess safety and efficacy parameters in the event the study is interrupted due to a crisis (e.g., natural disaster, pandemic).

7.1 Efficacy Assessments

The efficacy endpoint for this study is described in Study Objectives and Endpoints [Table 3].

7.1.1 Primary and Secondary Efficacy Assessments

Either during or post-operatively, the investigator will evaluate the details of all the LNs identified either by ASP5354 or Gamma Probe. These data will be captured to eCRF.

Following data is captured in eCRF per participant.

- Identification of at least 1 LN detected by ASP5354 and/or Tc-99mSC or Lymphoseek
- Times of injection of ASP5354 and Tc-99mSC or Lymphoseek
- Location (i.e., 3 and 9 o'clock) of ASP5354 administration
- Time of start of surgery
- Time of surgical identification of the LN(s) by ASP5354
- Time of incision for lymphatic mapping
- Time of removal of first LN
- Time of end of surgery
- Transdermal visualization of lymphatic spread of ASP5354 after injection

Following data is captured in eCRF per LN.

- "Sentinel LN" or "Non-sentinel LN"
- Method of identification (ASP5354 and/or Tc-99mSC or Lymphoseek)
- Dissection of LN tissue identified*
- Histopathologic confirmation of LN tissue by pathologist*

- Histopathologic confirmation of metastatic spread by pathologist*
- Likert scale:
  The intensity of fluorescence will be qualitatively assessed during surgery by the investigator, by direct examination of the LN. Likert Scale (0 = None, 1 = Mild, 2 = Moderate and 3 = Strong) will be used to determine the intensity of fluorescence at the time point of the LN identification.
- Radioactivity count:
  The radioactivity count of all the LNs identified by ASP5354 and /or Tc-99mSC or Lymphoseek will be counted with gamma probe.
  * may not be collected in case of Non-Sentinel LN

7.1.2 Signal Background Ratio

Video recording by NIR-F device is also required to evaluate the visualization of LN by ASP5354. Start recording a video at the time of injection of ASP5354 and continue until the first lymph node is visualized. For the remaining lymph nodes visualized, it is recommended to use video recording, but capturing images with the device is also acceptable. For further details, please refer to the Imaging Charter.

SBR is calculated by external reviewer based on the video recording by NIR-F device. Fluorescence images will be captured and recorded from injection time of ASP5354 until last LN is dissected. Images will be captured on a NIR-F and overlay view (if available) for all the LNs identified by ASP5354. SBR will be calculated as follows [Hoogstins et al, 2019]:

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

Additional detail will be provided in the Imaging Charter.

7.2 Safety Assessments

Study procedures and their timing are summarized in the Schedule of Assessments [Table 1]. Protocol waivers or exemptions are not allowed.

7.2.1 Laboratory Assessments

- See [Section 10.6 Appendix 6 Laboratory Assessments] for the list of clinical laboratory tests to be performed and refer to Schedule of Assessments [Table 1] for timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents.
- Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or subinvestigator who is a qualified physician. Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the investigator to be more severe than expected for the participant's condition.

7.2.2 Vital Signs

- Blood pressure, pulse rate and respiratory rate will be assessed.
- Blood pressure and pulse measurements will be assessed with the participant in the sitting or supine position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).
- Vital signs will be taken before blood collection for laboratory tests.

7.2.3 Physical Examination

Physical examinations will be performed as indicated in the Schedule of Assessments [Table 1] and whenever there is a medical indication.

A full physical examination will be performed at screening (visit 1). Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular. At visit 2 and follow-up (visit 3), a full physical examination is not mandatory, but a symptom-directed physical examination will be performed.

Height and weight will be measured using standard institution practice and equipment.

7.2.4 Electrocardiogram

Standard 12-lead ECGs will be collected per site's standard procedure at screening, preoperative and safety follow-up period. A single 12-lead ECGs will be collected after the participant has been resting in the supine position. Intraoperative ECG monitoring will occur at 30 minutes post dose (15 min ≤ and < 45 min).A 12-lead ECG is not mandatory for intraoperative and postoperative ECG, and assessment can be based on the rhythm strip or other site standard ECG as per the site's standard of care routine for cardiac monitoring.

The investigator will review, sign and date the ECG after recording to ensure participant safety. The time of the ECG, the interval measurements, as well as an overall conclusion, will be documented. This overall conclusion will be recorded as normal, abnormal not clinically significant or abnormal clinically significant in the eCRF. Any clinically significant ECG abnormalities should be recorded as an AE.

The ECG recordings will be printed, signed and saved in the source.

7.2.5 Order of Assessments

The following order should be followed when more than one assessment is required at a time point with blood sampling being collected nearest to the scheduled time point:

- 12-lead ECG
- Vital signs
- Blood collection for pharmacokinetics and clinical laboratory tests

7.2.6 Other Assessments

7.2.6.1 Green Coloration of Urine or Skin

The occurrence of green coloration of the urine or skin will be monitored throughout the study. If observed, any visual green coloration abnormalities will be recorded as an AE. If urine coloration occurs for participants who have a urethral catheter in place, the color of urine will be assessed during surgery and every 120 minutes (± 30 minutes window) after the end of surgery until it resolves or the participant is discharged, whichever is earlier. If no urethral catheter is in place, voided urine will be assessed for green coloration and the time collected until it resolves or the participant is discharged, whichever is earlier. The start and stop date and time will also be recorded. The occurrence of green coloration of the skin will be recorded as an AE any time after dose administration. The start and stop date and time will be recorded. Skin coloration will be followed up until it resolves or the participant is discharged, whichever is earlier.

7.3 Adverse Events and Other Safety Aspects

The definitions of an AE, SAE or ADE can be found in [Sections 10.3.1 Definition of Adverse Events, 10.3.2 Definition of Serious Adverse Events and 10.3.3 Adverse Device Effect], respectively.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative), investigator (surgeon) or anesthesiologist.

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE, SAE or ADE and remain responsible for following up AEs that are serious, considered related to the study, or that caused the participant to discontinue the IP and/or study [see Section 10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

The method of recording, evaluating, and assessing causality of AE, SAE and ADE and the procedures for completing and transmitting SAE/ADE reports are provided in [Section 10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

7.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All SAEs will be collected from the signing of the ICF until the follow-up visit at the time points specified in the Schedule of Assessments [Table 1] and reported on the eCRF.

All AEs will be collected from the signing of the ICF until the follow-up visit at the time points specified in the Schedule of Assessments [Table 1] and reported on the eCRF.

If the NCI CTCAE grade of an SAE/AE changes, the event should be relisted on the eCRF with the new NCI CTCAE grade and new onset date.

If the NCI CTCAE grade decreases, the SAE/AE should be relisted on the eCRF with the new NCI CTCAE grade and new onset date. The exception is ongoing pre-dose events that continue post-dose and improve post-dose. Such events should not be re-listed.

If the NCI CTCAE grade of an SAE reduces, the details of the AE should be provided on the SAE worksheet for the medical assessor to be able to assess the course of the event.

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in [Section 10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting]. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study IP or study participation, the investigator must promptly notify the sponsor.

The Reference Safety Information that will be used to determine expectedness of an SAE for this study is in the IB.

7.3.1.1 Adverse Device Effects

- ADEs are to be collected via the ADE worksheet and reported to the sponsor within 24 hours of awareness (see [Section 10.3.3.1]).
- Any complaint about a device must be reported regardless of whether the defect or deficiency had any effect on a participant or on study personnel (see [Section 10.3.3.3]).

7.3.2 Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

7.3.3 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in [Section 8.3 Lost to Follow-up]). Further information on follow-up procedures is provided in [Section 10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting].

If after the protocol-defined AE collection period (see [Section 7.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information]), an AE progresses to an SAE, or the investigator learns of any (S)AE (serious adverse event or adverse event) including death, where he/she considers there is reasonable possibility it is related to the IP or study participation, the investigator must promptly notify the sponsor.

7.3.4 Regulatory Reporting Requirements for Serious Adverse Events/Adverse Device Effects

- Prompt notification by the investigator to the sponsor of a SAE/ADE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study IP under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies and/or device manufacturer about the safety of a study IP under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.5 Disease-related Events and/or Disease-related Outcomes Not Qualifying as Adverse Events or Serious Adverse Events

Under this protocol, the following event will not be considered as an (S)AE:

- Preplanned and elective hospital/clinical procedures/interventions or procedures for diagnostic, therapeutic or surgical procedures for a preexisting condition that did not worsen during the course of the study. These procedures are collected in the eCRF.

7.3.6 Special Situations

Certain special situations observed in association with the IP, such as incorrect administration (e.g., wrong dose of IP or background therapy) are reported as protocol deviations and/or may require special reporting, as described below. These special situations are not considered AEs but do require to be communicated to Astellas as per the timelines defined below.

If a special situation is associated with, or results in, an AE, the AE is to be assessed separately from the special situation and captured as an AE in the eCRF. If the AE meets the definition of an SAE, the SAE is to be reported as described in [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects] and the details of the associated special situation are to be included in the clinical description on the special situation worksheet or pregnancy reporting form.

The special situations are:

- Pregnancy
- Medication error, overdose and use outside protocol
- Misuse/abuse
- Occupational exposure

- (Suspicion of) Transmission of infectious agent
- Suspected drug-drug interaction

Instructions and procedures for reporting special situations are provided in [Section 10.3.8 Reporting Procedures for Pregnancy and Special Situations].

7.4 Pharmacokinetics

7.4.1 Analysis of ASP5354 in Plasma

Blood samples for the analysis of ASP5354 in plasma will be collected as indicated in the Schedule of Assessments [Table 1] and Sample Collection and Timed Procedure Schedule [Table 2].

Blood samples will be collected via a peripherally placed intravenous cannula or by direct venipuncture in a suitable vein. Blood sampling, processing, storage and shipment instructions are provided in a Sample Handling and Processing Manual.

When deemed appropriate at a later date, plasma samples remaining after the pharmacokinetic analysis may be used for exploratory metabolic profiling after the study. This test will be described in a separate report and will not be incorporated in the integrated clinical study report (CSR).

7.5 Total Amount of Blood

The total amount of blood drawn for each participant will vary depending on the length of the surgery. At any time during the study, if any laboratory abnormalities are found for a participant, additional blood may be drawn for safety monitoring. The average expected blood draw is shown in Blood Sampling [Table 5].

Table 5 Blood Sampling

Sample Type	Number of Samples	Sample Volume (mL)	Total Volume (mL)
Serum chemistry	4	8.5	34.0
Hematology	4	4.0	16.0
ASP5354 pharmacokinetics	6	4.0	24.0
Total			**74.0**

7.6 Study Participant Feedback

This study will include an option for participants to complete an anonymized questionnaire in order to provide feedback on their clinical study experience. Individual participant responses will not be reviewed by investigators. Responses will be used by the sponsor to understand where improvements can be made in the clinical study process. This questionnaire will not collect data about the participant's condition, symptoms, treatment effect or AEs and therefore is not considered study data. Should any spontaneous information be collected about AEs, this would be reported and transferred to the safety database.

8 PARTICIPANT DISCONTINUATION

Refer to [Section 10.1.9 Study and Site Start and Closure] regarding discontinuation of study sites or of the study as a whole.

8.1 Discontinuation of Individual Participant(s) from Study Treatment

Since this is a single dose study, treatment discontinuation is not applicable.

8.2 Discontinuation of Individual Participant(s) from Study

The participant is free to withdraw from the study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to terminate a participant's involvement in the study at any time if the participant's clinical condition warrants it.

The reason for discontinuation from study must be documented in the participant's medical records.

A participant must discontinue the study for any of the following reasons:

- Participant requests to stop the study.
- Any clinical AE, laboratory abnormality or intercurrent illness that, in the opinion of the investigator, indicates to continue in the study is not in the best interest of the participant.
- Participant signs informed consent, but surgery is performed without administration of ASP5354. (Screening failure participants are excluded.)

8.3 Lost to Follow-up

Every reasonable effort is to be made to contact any participant lost to follow-up during the course of the study to complete study-related assessments and record outstanding data. These contact attempts should be documented in the participant's medical record.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

No statistical hypothesis is planned.

9.2 Analysis Sets

The allocation of participants to analysis sets will be determined prior to database hard-lock.

The following populations are defined:

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.

IP: investigational product

9.3 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 CSR.

9.3.1 General Considerations

In general, data will be summarized with descriptive statistics for continuous endpoints, and frequency and percentage for categorical endpoints, unless otherwise specified. Percentages by categories will be based on the number of participants with no missing data (i.e., will add up to 100%).

All the analyses will be performed by tumor type, unless otherwise specified.

Baseline will be defined as the last non missing observation prior to administration of IP, unless otherwise specified.

Demographics and baseline characteristics will be summarized by dose group and overall, for the full analysis set (FAS).

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 follow-up disposition will also be presented for the FAS by dose group and overall. All disposition details and dates of first and last evaluations for each participant will be listed.

Previous and concomitant treatment and medical history will be listed. IP exposure will be summarized by descriptive statistics and will be listed.

9.3.2 Primary Endpoint(s)/Estimand(s) Analysis

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

9.3.2.1 Primary Analysis

The optimal dose will be determined by VRC based on 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.

9.3.3 Secondary Endpoint(s)/Estimand(s) Analysis

The proportion of participants with at least 1 LN detected by visualization with histopathologic confirmation of LN tissue using ASP5354 will be estimated with exact 95% confidence interval (CI) by dose group.

The proportion of identified LNs with histopathologic confirmation of LN tissue will be estimated with exact 95% CI for ASP5354 by dose group and SoC treatment with either Tc-99mSC or Lymphoseek.

The secondary endpoint analysis will be performed using the FAS.

9.3.4 Exploratory Endpoint(s)/Estimand(s) Analysis

The Likert Scale of the intensity of the fluorescence will be summarized by frequency and percentage by dose group.

The SBR will be summarized using descriptive statistics by dose group.

The transdermal visualization of lymphatic spread of ASP5354 after injection will be summarized by frequency and percentage by dose group.

The time from injection to surgical identification of LN(s) will be summarized by descriptive statistics by dose group.

The frequency and percentage of participants with visualization of LNs using ASP5354 will be summarized by dose group. In addition, the number of LNs visualized using ASP5354 will be summarized by descriptive statistics by dose group.

The exploratory analysis will be performed using the FAS.

9.3.5 Safety Analyses

The safety and tolerability will be summarized by descriptive statistics for the safety analysis set (SAF) population. The SAF population consists of all participants who received ASP5354.

9.3.5.1 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, IP-related serious TEAEs and green coloration of urine or skin will be summarized by system organ class, preferred term and dose group. The worst grade will be summarized if the same AE is recorded more than once for a participant.

AE data will be listed.

9.3.5.2 Laboratory Assessments

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

Shifts from baseline to the postbaseline worst grade based on NCI-CTCAE until the follow-up period in clinical laboratory tests will be tabulated.

Laboratory data will be listed.

9.3.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline by dose group and time point.

Vital signs data will be listed.

9.3.5.4 Electrocardiogram

The standard 12-lead ECG results will be summarized by dose group and time point.

12-lead ECG data and interpretations will be listed.

9.3.6 Pharmacokinetics Analysis

Descriptive statistics will be used to summarize the plasma concentrations of ASP5354 by dose group and time point. Individual plasma concentrations of ASP5354 and the sampling times will be listed.

The pharmacokinetics analysis will be performed using the SAF.

9.3.7 Other Analyses

Other analyses such as the relationship between LNs visualization and pharmacokinetics or other exploratory endpoints may be explored when deemed appropriate. All details will be described in a separate analysis plan and results will be described in a separate report and will not be incorporated in the integrated CSR.

9.4 Interim Analysis

VRC review will be held after 3 participants in 0.2 mg dose level 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. During the VRC review, enrollment at that indication will be closed until the determination of the next dose level to be made. 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.

9.5 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 of ASP5354 for LN visualization in breast cancer and melanoma. Approximately up to 12 participants will be assigned to each of the initial 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.

9.6 Additional Conventions

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.

See the SAP for details of the definitions for analysis windows to be used for analyses by time point.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1 Ethical, Regulatory and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines
  - Applicable ICH GCP guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of Title 21 of the US Code of Federal Regulations, ICH guidelines, the

IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2 Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent of Participants

10.1.3.1 Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.
- The information provided shall be provided in writing and shall:
  - enable the participant to understand:
    - the nature, objectives, benefits, implications, risks and inconveniences of the clinical study;
    - the conditions under which the clinical study is to be conducted, including the expected duration of the participant's participation in the clinical study;
    - and the possible treatment alternatives, including the follow-up measures if the participation of the participant in the clinical study is discontinued;
  - be kept comprehensive, concise, clear, relevant, and understandable to a layperson;
  - be provided in a prior interview with an appropriately qualified member of the study team. Special attention shall be paid to the information needs of specific patient populations and of individual participants, as well as to the methods used to give the information. Care should be taken to verify that the participant has understood the information;
  - include information about the applicable damage compensation system;
  - include the study ISN number and information about the future availability of the clinical study results in terms understandable to a layperson;
- Participants must be informed that their participation is voluntary and shall have their protective rights and guarantees explained. In particular their right to refuse to participate and the right to withdraw from the clinical study at any time without any resulting detriment and without having to provide any justification shall be explained.
- Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

- Participants must be reconsented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant.

10.1.3.2 Supply of New and Important Information Influencing the Participant's Consent and Revision of the Written Information

- The investigator or his/her representative will immediately inform the participant verbally whenever new information becomes available that may be relevant to the participant's consent or may influence the participant's willingness to continue participating in the study (e.g., report of serious adverse drug reaction). The communication must be documented in the participant's medical records and whether the participant is willing to remain in the study or not must be confirmed and documented.
- The investigator must update the participant's ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the participant on all updated ICFs throughout their participation in the study. The investigator or his/her designee must reconsent participants with the updated ICF even if relevant information was provided verbally. The investigator or his/her representative who obtained the written informed consent and the participant should sign and date the ICF. A copy of the signed ICF will be given to the participant and the original will be placed in the participant's medical record. An entry must be made in the participant's records documenting the reconsent process.

10.1.4 Data Protection

The sponsor will use the personal data collected from participants in order to run the study and to use and publish the results of the study.

Astellas relies on the permission (or "consent") in order to use the data of participants and their permission is obtained by signing the ICF.

The investigator and/or the site personnel will record information from the medical file of study participants in eCRF and in an external (electronic) data file (e.g., central laboratory data). These records will identify the participants with a code instead of their name or other personal data. Only the investigator and the site personnel can match the code with the name of the study participant.

The sponsor collects information from the participants during the study that may be used for:

- submission to government regulatory authorities and IRB/IEC
- use in reports or public scientific presentations, and
- use in research, now or in the future.

However, the identity of participants will not be revealed if study participants' personal data is shared for these purposes.

The personal data of participants may be shared with:

- Astellas and its present or future affiliates,
- research, collaboration and licensing partners and/or service providers (such as laboratories conducting tests on behalf of Astellas) and authorized representatives of Astellas,
- study monitors appointed by Astellas or Astellas' service providers, and
- auditors/inspectors appointed by Astellas or Astellas' service providers or by health and regulatory authorities for GCP inspections.

The sponsor will inform the participants about their privacy rights and how to exercise them under the ICF which participants will sign in order to participate in the study. The sponsor will provide the investigator and study staff with a privacy notice explaining how their personal data will be used and how to exercise their privacy rights.

The sponsor will comply and process personal data in accordance with all applicable privacy laws and regulations.

The Sponsor has performed a privacy impact assessment to describe the data processing activities, assess their necessity and proportionality and manage the risks to the rights and freedoms of data participants resulting from the processing of personal information by assessing them and determining the measures or mitigation actions to address them. The Sponsor has assessed, among others, how the data is collected and the purposes they are used, the impact on data participant rights, the security mechanisms including the safeguards of potential transfer of data outside the European Union and the access rights to data. The Sponsor has taken actions to mitigate the risks to the rights and freedoms of data participants resulting from the processing of their data as part of their participation in the study.

10.1.5 Committee Structure

10.1.5.1 Visualization Review Committee

The visualization data of the LNs will be assessed by the VRC and will be compared with the performance of SoC. Members of the VRC include the sponsor's medical monitor, pharmacovigilance physician, and the investigator(s) who have treated participants. The VRC will be held at the following time points, but ad-hoc meetings may occur:

- Initial 3 participants at 0.2 mg in each tumor type have completed the SLN biopsies;
- Initial 3 participants at each subsequent dose levels (0.05 mg and 0.6 mg or 0.6 mg and 1 mg) in each tumor type have completed the SLN biopsies (total of 6 participants; and
- Additional 3 participants have completed SLN biopsies at the expanded or additional dose levels. The review of an additional 3 participants will continue until the optimal dose is defined.Maximum participants for each cohort (i.e., 12) has not been reached.

The data for the participants, at each subsequent dose level, who completed the SLN biopsy will be assessed by the VRC to decide whether to:

- Expand a dose level if additional participants are needed to increase confidence for the optimal dose selection;

- Stop a dose level when 100% of participants lack visualization or have a fluorescence intensity of 0 or 1 on the qualitative Likert Scale), in which case additional participants would not be added to the dose level; or
- Define an optimal dose (in the case of 2 doses performing equally, the lower dose will be selected).

For further details on the VRC, refer to the VRC charter.

10.1.6 Dissemination of Clinical Study Data

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final CSR that forms part of a marketing authorization application, be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator(s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for the coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database lock.

10.1.7 Data Quality Assurance

- All participant data relating to the study will be recorded on the eCRF unless transmitted to the sponsor or designee electronically in an external data file. The investigator is responsible for verifying that data entries on the eCRF are accurate and correct by physically or electronically signing the eCRF.
- Guidance on completion of CRFs will be provided in a separate eCRF Completion Guideline.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy, including definition of study critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organizations [CROs]).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator according to ICH or applicable local regulatory requirements, whichever is longer, after study completion. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

1. Source data must be available at the study site to document the existence of the participants and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.
2. The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
3. The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are handwritten on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved or transmitted electronically via computerized systems (and/or other kind of electronic devices) as part of regulated study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol-related assessments, AE tracking, electronic clinical outcome assessment and/or drug accountability.
4. Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments, and audit trail information, if applicable). All printed records must be kept in the participant file and be available for archiving.
5. Study monitors will perform ongoing source data review to confirm that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.9 Study and Site Start and Closure

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further investigational study intervention development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures or GCP guidelines
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants included earlier than expected

If the study is prematurely terminated or suspended, the sponsor or designee shall promptly inform the investigators, the IRBs/ IECs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

10.1.10 Arrangement for Use of Information and Publication of the Study

Information concerning the test product, patent applications, processes, unpublished scientific data, the IB and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the study in connection with the development of the product and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this study, the investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

Publication of the study results is discussed in the study agreement.

10.1.11 Quality Assurance

The sponsor is implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the QA and QC systems and written SOPs of the CRO will be applied.

The sponsor or sponsor’s designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, CRFs and source documents. Direct access to these documents will be required by the auditors.

To support quality around participant safety and reliability of study results, quality tolerance limits (QTLs) are defined and monitored. QTLs represent the acceptable variation of study data, taking into consideration the current state of medical and statistical knowledge about the variables to be analyzed, as well as the statistical design of the study. It is a level, point, or value associated with a parameter that should trigger an evaluation if a deviation is detected to determine if there is a possible systematic issue (i.e., a trend has occurred). The QTLs defined for this study are provided below.

Table 6 Quality Tolerance Limits

QTL #: Name and Parameter	Definition	Parameter Justification
QTL1: Sufficient data for assessment to decide optimal dose of ASP5354 for LN visualization: % missing at least 1 of all below data of anatomical visualization of LN(s) after dosing ASP5354. • LN tissue visualized (Y/N) • Visualized tissue is lymphatic in origin based on pathologic confirmation	Number of participants who missed at least 1 of all below data of anatomical visualization of LN(s) after dosing ASP5354 per total number of enrolled participants (%). • LN tissue visualized (Y/N) • Visualized tissue is lymphatic in origin based on pathologic confirmation	Since all of the data of anatomical visualization of the LN(s) after dosing ASP5354 is the primary endpoint to determine the optimal dose of ASP5354, missing data points would hinder evaluation of efficacy.
QTL2: Reliable data for assessment of LN visualization by ASP5354: % receiving excluded concomitant medications, listed in [Section 10.5 Appendix 5 List of Excluded Concomitant Medications] in protocol deviated manner	Number of participants who received excluded concomitant medications, listed in [Section 10.5 Appendix 5 List of Excluded Concomitant Medications] in protocol deviated manner per total number of enrolled participants (%).	Since excluded concomitant medication might affect visualization of the LN(s) by ASP5354.

QTL: quality tolerance limit, LN: lymph node

QTL Management Activities:

- For control of risks associated with QTL1: Sufficient data for assessment of anatomical visualization of the LN, refer to [Section 7.1 Efficacy Assessments].
- For control of risks associated with QTL2: Reliable data for assessment of LN visualization by ASP5354, refer to [Section 10.5 Appendix 5 List of Excluded Concomitant Medications].

10.2 Appendix 2 Contraception Requirements

WOCBP, who are eligible for participation in the study, including those who choose complete abstinence, must have pregnancy tests as specified in the Schedule of Assessments [Table 1]. Pregnancy test results must confirm that the participant is not pregnant.

WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A female is considered fertile (i.e., WOCBP) following menarche and until becoming postmenopausal unless permanently sterile.

Females in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal with one of the following (i.e., permanently sterile):
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
- Postmenopausal

A postmenopausal state is defined as at least 12 months after last menstrual bleeding without an alternative medical cause.

In case the last menstrual bleeding cannot be clearly determined, confirmation with more than 1 follicle-stimulating hormone (FSH) measurement of at least > 40 IU/L (or higher per local institutional guidelines) is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

Documentation of any of these categories can come from the study site personnel's review of the female participant's medical records, medical examination or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILDBEARING POTENTIAL

Female participants of childbearing potential are eligible for participation in the study if they agree to use one of the highly effective methods of contraception listed below from the time of signing the ICF and until the end of relevant systemic exposure, defined as at least 30 days after final study treatment administration.[a]

Highly effective methods of contraception (failure rate of < 1% per year when used consistently and correctly)[b]:

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
  - Oral

  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
  - Oral
  - Injectable
  - Implantable
- Other combined (estrogen- and progesterone-containing) methods
  - Vaginal ring
  - Injectable
  - Implantable
  - Intrauterine hormone-releasing system or intrauterine device
  - Bilateral tubal occlusion
- Vasectomized partner
  A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
- Sexual abstinence
  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the test product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant. It is not necessary to use any other method of contraception when complete abstinence is elected.

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

[b] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILDBEARING POTENTIAL

Male participants with female partners of childbearing potential are eligible for participation in the study if they agree to the following during treatment and until the end of relevant systemic exposure defined as at least 30 days after final study treatment administration.[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator
- Use a condom
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

10.3.1 Definition of Adverse Events

AE Definition:

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study IP, whether or not considered related to the study IP.

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study IP. This includes events related to the comparator and events related to the (study) procedures.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study IP administration even though it may have been present before the start of the study.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, biochemistry or urinalysis) or other safety assessment (e.g., vital signs, physical examination or ECGs), including those that worsen from baseline, that is considered to be clinically significant in the medical and

scientific judgment of the investigator and not related to underlying disease, is to be reported as an (S)AE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment, which is associated with the underlying disease, does not require reporting as an (S)AE, unless judged by the investigator to be more severe than expected for the participant's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

10.3.1.2 Potential Cases of Drug-induced Liver Injury

Refer to [Section 10.4 Appendix 4 Liver Safety Monitoring and Assessment] for detailed instructions on drug induced liver injury (DILI). Abnormal values in AST and/or ALT concurrent or with abnormal elevations in total bilirubin (TBL) that meet the criteria outlined in [Section 10.4 Appendix 4 Liver Safety Monitoring and Assessment] in the absence of other causes of liver injury, are considered potential cases of DILI (potential Hy's Law cases; [Temple, 2006]) and are always to be considered important medical events and reported per [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects].

10.3.2 Definition of Serious Adverse Events

An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization
  - In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity
  - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza,

and accidental trauma (e.g., sprained ankle), which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect
- Other situations:
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
  - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

If an event is not an AE per definition in [Section 10.3.1 Definition of Adverse Events], then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

10.3.3 Adverse Device Effect (ADE)

10.3.3.1 Definition of ADE

- Any adverse event related to the use of device (includes all hardware components).
  - Includes any event that is a result of a user error or intentional misuse.

10.3.3.2 Definition of Unanticipated adverse device effect (UADE)

- UADE is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants. Note the reporting of UADEs should follow reporting requirements for SAEs/SADEs

10.3.3.3 Definitions of Technical Complaints and Device Deficiencies

- Device Complaint: A quality complaint received in writing, electronically, or orally that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a device product. (In this definition, "effectiveness" refers to the actual function of the device, not to how the participant responds to the action of the device
- Device Deficiency: Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling.

10.3.3.4 Risks/Precautions

Refer to the SPY Portable Handheld Imager (SPY-PHI) and 1688 Advanced Imaging Modalities (AIM) 4k Platform Manuals for a full description of the risks and precautions

associated with all components of the SPY-PHI and 1688 AIM systems, respectively. The entirety of the Operator Manuals should be read before using the device(s). Failure to follow the instructions and warnings in the manual may result in unsafe operation of the system and/or injury to the subject or operator.

10.3.4 Assessment of Causality

- The investigator is obligated to assess the relationship between study IP, imaging device(s), study procedures and each occurrence of each AE/SAE/ADE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study IP administration will be considered and investigated.
- The investigator will also consult the IB and/or product information, for marketed products, in his/her assessment.
- For each AE/SAE/ADE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/ADE and has provided an assessment of causality.
- There may be situations in which an SAE/ADE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE/ADE data to the sponsor.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE/ADE follow-up report with the updated causality assessment.
- The causality assessment between study IP is one of the criteria used when determining regulatory reporting requirements.

Following a review of the relevant data, the causal relationship between the IP and each (S)AE/ADE will be assessed by answering "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the IP?"

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a "reasonable possibility" that an (S)AE/ADE may have been caused by the IP (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Has the participant been administered IP?
- Plausibility (i.e., could the event have been caused by the suspect IP? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)
- Dechallenge/dose reduction/rechallenge:
    - Dechallenge: Did the (S)AE/ADE resolve or improve after only stopping the dose of the suspect drug without any treatment?

  - Dose reduction: Did the (S)AE/ADE resolve or improve after reducing the dose of the suspect drug?
  - Rechallenge: Did the (S)AE/ADE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory or other test results: a specific lab investigation supports the assessment of the relationship between the (S)AE/ADE and the IP (e.g., based on values pre-, during and post-treatment)
- Available alternative explanations independent of IP exposure; such as other concomitant drugs, device malfunctions, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation
- Temporal relationship between exposure to the IP and (S)AE/ADE onset and/or resolution. Did the (S)AE/ADE occur in a reasonable temporal relationship to the administration of the IP?
- Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausible will be provided.

There may be situations in which an SAE/ADE has occurred and the investigator has minimal information to include in the initial report to the sponsor. While it is very important that the investigator always assesses causality for every event before the initial transmission of the SAE/ADE data to the sponsor, the initial report should be submitted without delay (i.e., within 24 hours of awareness). With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of "no" is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new information regarding the SAE/ADE and shall send an SAE/ADE follow-up report and update the eCRF with the new information and updated causality assessment.

Device Relatedness

Events will be classified as device related if they meet the following criteria:

- Not Related**:** Any reaction that does not follow a reasonable temporal sequence from administration of the device or surgical procedure, or study intervention AND that is likely to have been produced by the participant's clinical state or other modes of therapy administered to the participant.
- Related**:** A reaction that follows or may follow a reasonable temporal sequence from administration of the device, surgical procedure, or study intervention AND that follows a known response pattern to the suspected device/surgical procedure/study intervention.

10.3.5 Assessment of Severity

AEs, including abnormal clinical laboratory values, will be graded using the NCI-CTCAE guidelines (version 6.0). The items that are not stipulated in the NCI-CTCAE version 6.0 will be assessed according to the criteria below and entered into the eCRF:

Table 7 Grading Scale Defining the Severity of an Adverse Event

Grade	Assessment Standard
1 - Mild	Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
2 - Moderate	Minimal local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL†
3 - Severe	Medically significant but not immediately life threatening, hospitalization or prolonged hospitalization indicated; disabling; limiting self-care ADL‡
4 - Life-threatening	Life threatening consequences, urgent intervention indicated
5 - Death	Death related to AE

ADL: activities of daily living; AE: adverse event

† Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

‡ Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

10.3.6 Recording and Follow-up of AEs, SAEs and/or ADEs

AE, SAE and ADE Recording

- When an AE/SAE/ADE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/ADE information in the eCRF.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to the sponsor in lieu of completion of the eCRF.
- There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE/ADE.

Follow-up of AEs, SAEs and ADEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE, SAE or ADE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any postmortem findings including histopathology. New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE/ADE data to the sponsor within 24 hours of receipt of the information.

10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects

The investigator must complete and submit an SAE or ADE worksheet containing all information that is required by local and/or regional regulations to the sponsor by fax or email immediately (within 24 hours of awareness).

The SAE or ADE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE/ADE data, as well as the investigator causality assessment including the explanation for the causality assessment.

For contact details, see [Contact Details of Sponsor's Key Personnel]. Fax or email the SAE/special situations/ADE worksheet to:

Astellas Pharma Global Development Inc.
Global Pharmacovigilance
North America fax number: +1-888-396-3750
North America alternate fax number: +1-847-317-1241
Email: safety-us@astellas.com

If there are any questions, or if clarification is needed regarding the SAE/ADE, please contact the sponsor’s medical monitor/study physician or their designee [Contact Details of Sponsor’s Key Personnel].

Follow-up information for the event should be sent promptly (as soon as available, but no longer than within 7 days of the initial notification).

Full details of the SAE/ADE should be recorded on the medical records, SAE/special situation or ADE worksheet and on the eCRF.

The following minimum information is **required**:

- International study number/study number
- Participant number, sex and age
- Date of report
- Description of the SAE/ADE (event and seriousness criteria)
- Causal relationship to the IP/device (including reason)
- Drug provided (if any)

The sponsor or sponsor’s designee will medically evaluate the SAE/ADE and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., SUSAR reporting) according to current local/regional regulatory requirements. The sponsor or sponsor's designee will submit expedited safety

reports to competent authorities (CAs) and concerned ethics committee per current local regulations, and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB/IEC within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB/IEC of expedited safety reports should be retained by the study site. In the US, FDA expedited IND reporting guidelines will be followed.

The sponsor or sponsor's designee will also evaluate any ADE and determine if the report meets the requirements for expedited reporting.

The sponsor will notify all investigators responsible for ongoing clinical studies with the test product of all SUSARs, which require submission per local requirements to the IRB.

The investigators should provide written documentation of IRB notification for each report to the sponsor.

The investigator may contact the sponsor's medical monitor/study physician for any other problem related to the rights, safety or well-being of the participant.

10.3.8 Reporting Procedures for Pregnancy and Special Situations

10.3.8.1 Contraceptive Guidance and Collection of Pregnancy Information

If a female participant becomes pregnant during the study dosing period or within 30 days from the final study treatment administration, the investigator is to report the information to the sponsor according to the timelines in [Section 10.3.7 Reporting Procedures for Serious Adverse Events] using the pregnancy form and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male participant who becomes pregnant during the study dosing period or within 30 days from the final study treatment administration and report the information to the sponsor according to the timelines in [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects] using the pregnancy form.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data, etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female participant as an AE in the eCRF or SAE per [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects]. Participant pregnancy outcomes listed below are to be reported as SAEs:

- Spontaneous abortion/miscarriage, abortion and missed abortion
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the IP.

- If an infant dies more than 1 month after the birth, it is to be reported if a relationship between the death and intrauterine exposure to the IP is judged as *"*possible*"* by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus)
- Benign hydatidiform mole
- Blighted ovum

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination or other means as appropriate. (S)AEs experienced by the newborn/infant should be reported via the pregnancy form. Pregnancy needs to be followed up until final pregnancy outcome is reported.

10.3.8.2 Medication Error, Overdose and "Off-label Use"

If a medication error (defined as an unintended failure in the treatment process that leads to, or has the potential to lead to, harm to the participant), overdose or "off-label use" (i.e., use outside of the target disease defined in the protocol) is suspected, refer to [Section 6.7 Treatment of Overdose]. Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects] together with the details of the medication error, overdose and/or "off-label use."

10.3.8.3 Misuse/Abuse

Definition of misuse: Situations where the IP is/are intentionally and inappropriately used not in accordance with the intended use as defined in the protocol.

Definition of abuse: Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects.

If misuse or abuse of the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects] together with details of the misuse or abuse of the IP.

10.3.8.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the IP of study site personnel while preparing it for administration to the participant) to the IP occurs, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

10.3.8.5 Suspected Drug-drug Interaction

If a drug-drug interaction associated with the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7 Reporting Procedures for Serious Adverse Events/Adverse Device Effects] together with details of the suspected drug-drug interaction.

10.3.8.6 (Suspicion of) Transmission of Infectious Agent

If transmission of an infectious agent associated with the study intervention is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness) and any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7] together with the details of the suspected transmission of infectious agent.

10.3.9 Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary for conducting the study properly, the sponsor will inform all investigators involved in the study, as well as the appropriate regulatory authorities. Investigators should inform the IRB of such information when needed.

The investigator will also inform the participants, who will be required to sign an updated ICF in order to continue in the study.

10.3.10 Urgent Safety Measures

An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant CA, IRB/IEC, where applicable, in order to protect participants from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate a USM. The cause of a USM can be safety-, product- or procedure-related.

10.3.11 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the study physician (within 24 hours of awareness). Full details of the potential USM are to be recorded in the participant's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be a USM, the sponsor will take appropriate action to ensure the safety and welfare of the participants. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify the relevant CAs and concerned ethics committee within the timelines required per current local regulations, and will inform the investigators, as required. When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

10.4 Appendix 4 Liver Safety Monitoring and Assessment

The purpose of this appendix is to provide guidance for the monitoring of DILI during the course of the study. It should be noted that this section does not specify the end-of-study analyses of liver enzymes. The end-of-study liver enzymes analyses will be described in the SAP. Any participant enrolled in a study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN or bilirubin > 2 × ULN should undergo detailed testing for liver enzymes (including at least alkaline phosphatase (ALP), ALT, AST and TBL). Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Participants should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN is as shown below.

Table 8 Moderate and Severe Liver Abnormalities

	ALT or AST		TBL
Moderate	> 3 × ULN	or	> 2 × ULN
Severe	> 3 × ULN	and†	> 2 × ULN

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal

†Samples taken simultaneously or within maximum 24 hours.

In addition, the participant should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and† TBL > 2 × ULN or international normalized ratio (INR) > 1.5 (if INR testing is applicable/evaluated)
- ALT or AST > 5 × ULN and† (TBL > 2 × ULN in participants with liver metastases)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and clinical laboratory tests. The study site personnel are to complete

the liver abnormality case report form (LA-CRF). Participants with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly, and then weekly or less if abnormalities stabilize or the IP has been discontinued and the participant is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The sponsor should be contacted and informed of all participants for whom severe hepatic liver function abnormalities possibly attributable to IP are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases are to be recorded as "AEs" within the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic participants, and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications are to be entered in the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the participant's history, other testing may be appropriate including:
    - Acute viral hepatitis (A, B, C, D, E or other infectious agents)
    - Ultrasound or other imaging to assess biliary tract disease
    - Other clinical laboratory tests, including INR and direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

10.5 Appendix 5 List of Excluded Concomitant Medications

The following concomitant medications will not be allowed from 2 weeks prior to study treatment administration until the completion of the surgical procedure unless it is determined that LN detection is not sufficient.

Please note that this is not an exhaustive list. Investigators should verify the participant's concomitant medications for all prohibited and restricted drug classes.

Prohibited Medications

Class	Drug
ICG†	Indocyanine green
Other NIR-F imaging agent	Other agents which emit fluorescent when excited by NIR-F spectrum
Blue Dye	Indigocarmine
	Isosulfan blue
	Methylene blue
	Sulfan blue
Magtrace	Magtrace

† ICG administration for reasons other than LN visualization (e.g., vascular perfusion) is allowed after the completion of SLN biopsy.

10.6 Appendix 6 Clinical Laboratory Assessments

Laboratory tests will be performed according to the Schedule of Assessments [Table 1] and sent to the local laboratory for analysis.

Table 9 Clinical Laboratory Tests

Panel/Assessments	Parameters to be Analyzed
Hematology	Hemoglobin Hematocrit Erythrocytes Leukocytes Differential leukocytes Platelets
Biochemistry	Albumin Alanine aminotransferase Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Creatinine Glucose Inorganic phosphorus Lactate dehydrogenase Magnesium Potassium Sodium Total bilirubin Total protein
	Histamine and tryptase (only in participants with hypersensitivity reaction)
Urinalysis Urine, dipstick as applicable (if protein, blood, leukocytes or nitrites are abnormal, microscopy will be performed)	Protein Glucose pH Blood Leukocytes Urobilinogen Bilirubin Ketones Nitrite Color
	Microscopy (optional) Casts Crystals Epithelial cells Leucocytes Erythrocytes Bacteria
Pregnancy – Serum or Urine	Human chorionic gonadotropin (female participants only)

10.7 Appendix 7 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated Schedule of Assessments [Table 1] unless the site principal investigator discusses the need with the Astellas medical monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in [Section 7 Study Procedures and Assessments] due to a crisis.

INFORMED CONSENT

Participants who need to follow any or all of the alternate measures outlined in this Appendix will be required to provide informed consent, which explicitly informs them of the nature of and rationale for these changes, and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the participant followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the crisis:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible.
- Site facilities have been closed for clinical study conduct.
- Site has been restricted to treating patients with conditions outside of the scope of the study.
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel.
- Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel.

- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel.
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety.

Adherence to the original protocol as reflected in the Schedule of Assessments [Table 1] is expected, where plausible, in the case of a crisis. The alternate measures as noted in Alternative Schedule of Assessments in Response to a Crisis [Table 10] below are only permissible in the event of a crisis, and after discussing the need with the Astellas medical monitor to implement the alternate measures. This is to allow for continuity of receiving investigational medicinal product and maintaining critical safety and efficacy assessments for participants in the study at a time of crisis.

If one or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

Table 10 Alternative Schedule of Assessments in Response to a Crisis

Assessments	Alternate Approaches	Safety Follow-up Period
Day		**10 (Outpatient)** [1]
Window		**±4**
Visit Number		**3**
Physical Examination [2]	Can be obtained at local clinic.	X
Vital Signs [3]	Can be obtained at local clinic.	X
Clinical Laboratory Tests [4]	Visit collection of samples at local facility acceptable if results can be made available to investigative site.	X
Body Weight [3]	Can be obtained at local clinic.	X
Serum or Urine Pregnancy Test [5]	Visit collection of samples at local facility acceptable if results can be made available to investigative site.	X
ECG [6]	Can be obtained at local clinic.	X
Confirmation of Urine or Skin Coloration [7]	Visit collection of samples at local facility acceptable if results can be made available to investigative site.	→
Previous/Concomitant Medications	Remote/Virtual/Telemedicine Visits allowed.	X [8]
AE Assessment	Remote/Virtual/Telemedicine Visits allowed.	X [8]
Study Participant Feedback Questionnaire	Not need to be collected.	X

AE: adverse event, ECG: electrocardiogram; LN: lymph node

1. In the event of crisis, the alternate measures may be implemented for study procedures and assessments only for visits 3 after discussion with the Astellas Medical Monitor and/or designee.
2. A full physical examination will be performed at screening (visit 1). Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular. At visit 2 and follow-up (visit 3), a full physical examination is not mandatory, but a symptom-directed physical examination will be performed.
3. Vital signs include blood pressure, pulse and respiratory rate. All vital signs will be measured with the participant in the sitting or supine position. Height and weight will be measured using standard institution practice and equipment.
4. Clinical laboratory tests include blood collection for hematology (complete blood count) and serum chemistry and urine samples for urinalysis and urine color evaluation. These assessments will be performed by a local laboratory
5. This assessment will be required only for participants who are women of childbearing potential (see [Section 10.2, Appendix 2 Contraception Requirements]).
6. Standard 12-lead ECGs will be taken per site's standard of care for cardiac monitoring at safety follow-up period.
7. The occurrence of green coloration of the urine or skin will be monitored throughout the study. If observed, any visual green coloration abnormalities will be recorded as an AE. If it occurs for participants who have an urethral catheter in place, the color of urine will be assessed during surgery and every 120 minutes (± 30 minutes window) after the end of surgery until it resolves or the participant is discharged, whichever is earlier. If no urethral catheter is in place, voided urine will be assessed for green coloration and the time collected until it resolves or the participant is discharged, whichever is earlier. The start and stop date and time will also be recorded. The occurrence of green coloration of the skin will be recorded as an AE any time after dose administration. The start and stop date and time will be recorded. Skin coloration will be followed up until it resolves or the participant is discharged, whichever is earlier.

Footnotes continued on next page

8. If a participant experiences an AE or change in concomitant medications during the follow up period, the participant should call the study site to inform any of these changes. Should the participant develop a hypersensitivity reaction, an additional blood sample for determination of histamine and tryptase concentrations should be taken as soon as possible after the onset of the hypersensitivity reaction.

INVESTIGATIONAL PRODUCT SUPPLY

If any of the conditions outlined above in the Participants Procedures Assessment are met, one or all of the following mitigating strategies will be employed, as needed, to ensure continuity of IP supply to the participants:

- Increase stock of IP on site to reduce number of shipments required, if site space will allow.

DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

- Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window or other modifications) due to the crisis.

10.8 List of Abbreviations and Definition of Key Study Terms

List of Abbreviations

Abbreviations	Description of abbreviations
ADE	adverse device effect
ADL	activities of daily living
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	Astellas Pharma Inc.
AST	aspartate aminotransferase
AT	aminotransferases
BCRP	breast cancer resistance protein
CA	Competent Authorities
CBD	cannabidiol
CFR	Code of Federal Regulations
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CL_R	renal clearance
CRO	contract research organization
CSR	clinical study report
CYP	cytochrome P450
DILI	drug-induced liver injury
ECG	electrocardiogram
eCRF	electronic case report form
EMA	European Medicines Agency
FAS	full analysis set
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GI	gastrointestinal
GMP	Good Manufacturing Practices
HRT	hormone replacement therapy
IB	Investigator's Brochure
ICF	informed consent form
ICG	indocyanine green
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
INR	international normalized ratio
IP	investigational product
IRB	Institutional Review Board
IRT	interactive response technology
ISN	international study number

Abbreviations	Description of abbreviations
LA-CRF	liver abnormality case report form
LN	lymph node
MATE	multidrug and toxin extrusion
NCI-CTCAE	National Cancer Institute - Common Terminology Criteria for Adverse Events
NIR-F	near-infrared fluorescence
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT2	organic cation transporter 2
P-gp	P-glycoprotein
QA	quality assurance
QC	quality control
QTL	quality tolerance limit
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SBR	signal background ratio
SLN	sentinel lymph node
SoC	Standard of Care
SOP	standard operating procedure
SPECT	single photon emission computed tomography
SUSAR	suspected unexpected serious adverse reaction
TBL	total bilirubin
Tc-99mSC	technetium-99m sulfur colloid
TEAE	treatment-emergent adverse event
TNM	tumor, nodes and metastases
UADE	unanticipated adverse device effect
ULN	upper limit of normal
USM	urgent safety measure
VRC	Visualization Review Committee
WOCBP	women of childbearing potential

Definition of Key Study Terms

Terms	Definition of Terms
Baseline Assessment	Assessments of participants as they enter a study before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study. Note: Not all endpoints are themselves assessments since certain endpoints might apply to populations or emerge from analysis of results. That is, endpoints might be facts about assessments (e.g., prolongation of survival).
Enroll	To register or enter a participant into a study.
Screen failure	Potential participant who signed the ICF, but did not meet 1 or more criteria required for participation in the study and was not enrolled.
Screening	A process of active consideration of potential participants for enrollment in a study.
Screening period	Period of time before entering the investigational period, usually from the time when a participant signs the consent form until just before the test product or comparative drug (without randomization) is given to a participant.
Study period	Period of time from the first study site initiation date to the last study site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

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12 INVESTIGATOR'S SIGNATURE

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, Amendment 3 [Nonsubstantial]

11 MAY 2023

I have read all pages of this protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that subinvestigator(s) and other relevant members of my personnel have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: .....

Date (DD-MMM-YYYY)

Printed Name: .....

Insert name and qualification of the investigator

Address of study site: .....

.....

13 PROTOCOL ATTACHMENTS

Attachment 1	Electronic Sponsor Signatures