A Phase 1/2, Dose Escalation Study of Intravenous TK216 in Patients with Relapsed or Refractory Ewing Sarcoma

Protocol Number TK216-01

Version 7.0 (Amendment 6): 20 July 2021

1. Objectives

1.1 Part 1 Dose Escalation Segment

The primary objective of the Part 1 dose escalation segment is to determine the first cycle dose-limiting toxicities (DLTs) and the maximum tolerated dose (MTD) of TK216 administered intravenously via continuous infusion through a central venous catheter over seven days in patients with relapsed or refractory Ewing sarcoma (ES). (includes patients with Ewing sarcoma family of tumors-ESFT).

Secondary objectives of the dose escalation segment of the study are to assess:

- Safety profile of TK216 as characterized by Adverse Event (AE) type, severity, timing and relationship to study drug, as well as laboratory abnormalities in the first and subsequent treatment cycles
- Pharmacokinetics (PK) of TK216 in plasma
- Antitumor activity of TK216 as measured by Overall Response Rate (ORR), Duration of Response (DOR) as well as Progression-Free Survival (PFS) and Overall Survival (OS)
- Duration of Disease Control defined as first date of disease control identified (either CR, PR or SD) until the date of progression using RECIST, version 1.1
- Assay methods to detect EWS-FLI1 (or EWS-ERG and EWS-ets) molecular alterations and identify appropriate analytical cutoffs, and other relevant biomarker parameters
- Pharmacodynamics of TK216

1.2 Part 2 Schedule Escalation Segment

The primary objective of the Part 2 schedule escalation segment is to determine the first cycle dose-limiting toxicities (DLTs), maximum tolerated dose (MTD), and a biologically effective and recommended Phase 2 dose regimen (RP2D) of TK216 administered intravenously via continuous infusion through a central venous catheter in patients with relapsed or refractory Ewing sarcoma (ES). (includes patients with Ewing sarcoma family of tumors-ESFT).

Secondary objectives of the dose escalation segment of the study are to assess:

- Safety profile of TK216 alone and combined with vincristine as characterized by Adverse Event (AE) type, severity, timing and relationship to study drug, as well as laboratory abnormalities in the first and subsequent treatment cycles
- Pharmacokinetics (PK) of TK216 in plasma alone and when combined with vincristine

- Antitumor activity of TK216 as measured by Overall Response Rate (ORR), Duration of Response (DOR) as well as Progression-Free Survival (PFS) and Overall Survival (OS)
- Duration of Disease Control defined as first date of disease control identified (either CR, PR or SD) until the date of progression using RECIST, version 1.1
- Pharmacodynamics of TK216

1.3 Part 3 Expansion Segment

The primary objective of the Part 3 expansion segment is ORR defined as Complete Response (CR) and Partial Response (PR) of the RP2D regimen of TK216 administered intravenously via continuous infusion through a central venous catheter in patients with relapsed or refractory ES. (includes patients with Ewing sarcoma family of tumors-ESFT). The RP2D regimen may include use of vincristine, a standard therapy for Ewing sarcoma.

Secondary objectives of the Expansion Cohort segment of the study are to assess:

- Progression-Free Survival (PFS) defined as time from first dose of TK216 to tumor progression or death due to any cause
- Overall Survival (OS) defined as time from first dose of TK216 to death due to any cause
- Duration of response (DOR) as defined from the first date a response is identified (either CR or PR) until the date of disease progression
- Disease Control (DC) defined as the proportion of patients with a confirmed Complete Response (CR), Partial Response (PR) or Stable Disease (SD)
- Duration of Disease Control defined as first date of disease control identified (either CR, PR or SD) until the date of progression
- Safety and tolerability of TK216 alone and combined with vincristine as characterized by Adverse Event type, severity, timing and relationship to study drug, as well as laboratory abnormalities
- Exploratory biomarker development to enable prediction of drug toxicity, tumor response and the mechanism(s) of acquired study drug resistance.
- Pharmacodynamics of TK216 on molecular targets in surrogate tissue
- Pharmacokinetics (PK) of TK216 alone and when combined with vincristine

1.4 Part 4: Dose and Schedule Evaluation Segment

Primary Objective:

The primary objective of the Part 4 dose and schedule evaluations segment is o assess ORR defined as Complete Response (CR) and Partial Response (PR), per RECIST v1.1, at a starting dose of 175 mg/m^2/day of TK216 administered intravenously via continuous infusion over 28-days in patients with relapsed or refractory Ewing Sarcoma.

Secondary Objectives:

- Duration of response (DOR) per RECIST v1.1
- Disease control rate (DCR) per RECIST v1.1
- Progression free survival (PFS) per RECIST v1.1
- Overall survival (OS)
- Safety and tolerability of TK216 alone as characterized by AE type, severity, timing and relationship to study drug, as well as laboratory abnormalities

- Pharmacodynamics of TK216; Exploratory biomarker development to enable prediction of drug toxicity, tumor response and the mechanism(s) of acquired study drug resistance
- Pharmacokinetics of TK216 alone

2. Study Design

TK216-01 is a multicenter, open-label, Phase 1/2 study in which the safety and efficacy of TK216 will be evaluated in patients with relapsed or refractory ES (including patients with ESFT).

Once an appropriate patient has been identified, a 30-day screening period will begin to evaluate eligibility using the defined study inclusion and exclusion criteria.

The study will be executed in several Parts:

- **Part 1:** Dose Escalation Segment (Cohorts 1-6)
- **Part 2:** Schedule Escalation Segment (Cohorts 7-9)
- **Part 3:** Expansion Segment (Cohort 10)
- **Part 4:** Dose and Schedule Evaluation Segment (Cohort 11)

All patients will be treated until objective tumor progression per RECIST v1.1., withdrawal of consent, unacceptable toxicity, study termination by sponsor, or a maximum of 2-years. Patients may receive supportive care for bone marrow and/or gastrointestinal adverse events (e.g., with growth factors, antidiarrheals, antiemetics) and prophylaxis for established toxicities that are recurrent.

2.1 Drug Administration

TK216 will be administered intravenously by continuous infusion via a central venous catheter. Instructions on calculating the correct dose, as well as instructions on preparing the dosing solution will be provided in the Pharmacy Manual.

Vincristine is administered intravenously at a dose of 0.75 to 1.5 mg/m^2 up to a maximum dose of 2 mg.

2.2 Part 1 Dose Escalation Segment

The starting daily dose level in the dose escalation segment will be 18 mg/m^2/day.

A "3+3" patient enrollment scheme will be followed during the dose escalation. This segment will be performed in sequential cohorts of patients receiving TK216 intravenously by continuous infusion via a central venous catheter. Cycles will consist of treatment for 7 consecutive days followed by a 14-day rest period (Cycle = 21 days). If 2 of 3 patients experience a first-cycle DLT then accrual to the cohort will cease. If a first-cycle DLT is seen in one of the 3 patients in a cohort, that cohort will enroll an additional 3 patients. The dose escalation will continue until a first cycle DLT has been observed in 2 of 6 or 2 of 3 patients. DLT is defined as an adverse event occurring during the first cycle that is at least possibly related to TK216 and meets the DLT definition. When 0 of 3, or 1 of 6 patients in a cohort experience DLT, the dose will be escalated in the subsequent cohort.

Dose escalation will begin with an accelerated phase in which the dose will be doubled in successive cohorts of 3 patients until 1 patient experiences a first-cycle DLT (as defined in Table 1); or 2 patients experience similar AEs that are greater than or equal to grade 2 severity (greater than or equal to grade 3 severity for hematological AEs) which occur during the first cycle. A toxicity that is clearly and incontrovertibly unrelated to TK216 may be excluded in consultation with the medical monitor.

Once this predetermined toxicity level has been encountered, subsequent cohorts will dose escalate in 50% increments following review of AEs and discussion with the investigators. The dose level for newly enrolled patients will be assigned by Oncternal Therapeutics, Inc. at the time of registration.

Table 1 Dose Limiting Toxicity – Part 1 Dose Escalation Segment

Category	Criteria
Hematology toxicities	• Grade 4 neutropenia (absolute neutrophil count [ANC] < 500/mm^3) lasting >7 days; • Grade 4 anemia; • Febrile neutropenia (ANC <1000/mm^3 with a single temperature of > 38.3 ºC or a sustained temperature of ≥ 38 ºC for more than one hour); • Grade ≥ 3 neutropenic infection (i.e., infection documented clinically or microbiologically with grade ≥ 3 neutropenia); • Grade 4 thrombocytopenia (platelet count < 25,000/mm^3); • Grade 3 thrombocytopenia (platelet count < 50,000-25,000/mm^3) lasting >7 days or associated with clinically significant bleeding.
Gastrointestinal toxicities	• All ≥ Grade 4 vomiting or diarrhea; • Grade 3 nausea or vomiting despite optimal antiemetic therapy that fails to recover to at least Grade 2 within 72 hours; • Grade 3 diarrhea despite optimal management of the event that fails to recover to at least Grade 2 within 72 hours.
CNS toxicities	• Grade ≥ 3
Other non-hematological toxicities	• Grade ≥ 3; • Grade 2 increase in AST/ALT in combination with a grade 2 increase in bilirubin; • For patients with liver metastases with elevated liver transaminases at baseline (2.5-5x ULN), DLT shall be defined as a doubling of the baseline liver transaminase value(s); • Toxicity of any grade that interrupts the first seven day infusion for ≥24 hours.
Failure to recover (except alopecia)	• Failure to recover to Grade ≤ 2 toxicity or to baseline values after delaying the initiation of next cycle by a maximum of 21 days

Patients are eligible for DLT evaluation if they experience a DLT after receiving any amount of study drug or do not experience a DLT having taken a minimum of 85% of dose expected during the first cycle. Patients who do not fulfill these requirements and those who discontinue study participation prior to completing the first cycle will be replaced.

The MTD is the dose level at which 0 of 6 or 1 of 6 patients experience first-cycle DLT, and at least 2 of 3 or 2 of 6 patients experience first-cycle DLT at the next higher dose level. Effectively, the MTD is the highest dose associated with first-cycle DLT in < 33% of patients.

It is anticipated that 6 to 8 cohorts will be completed during the dose escalation segment of this trial. With the concurrence of the investigators and the study sponsor, further testing may be performed in up to 12 additional patients per dose level to refine the estimation of the MTD and RP2D at intermediate dose levels or to define a higher MTD and RP2D while using Cycle 1 primary supportive care prophylaxis (e.g., with growth factors, antidiarrheals, antiemetics) for bone marrow and/or gastrointestinal toxicities.

Additional cycles of therapy may be administered provided that the patient meets the following criteria on Day 1 of each cycle:

- ANC $\geq$ 1,000/mm^3
- Hemoglobin $\geq$ 8.0 gm/dL (Blood transfusions are permitted.)
- Platelets $\geq$ 50,000/mm^3
- Non-hematologic toxicity recovered to $\leq$ Grade 1 (or tolerable Grade 2)

Intra-patient dose escalation will be permitted during the dose escalation segment of this trial. Patients may have their dose of TK216 increased after they have completed 2 cycles of treatment at their assigned dose, assuming their initial dose was well tolerated and the subsequent cohort (at the next higher dose level) has been well tolerated through Cycle 1. At the investigator's discretion, the dose escalation can either take the form of an increase in daily dose with 7 days of dosing and 14 days of rest, or an increase of duration, with 14 days of dosing and 7 days of rest, but not both.

Other schedules of administration may be investigated after tolerability and PK data are acquired with this schedule.

2.3 Part 2 Schedule Escalation Segment

Once the MTD for patients receiving TK216 by continuous infusion for seven days has been identified, the tolerability of longer infusion times will be determined.

This segment will also be performed in sequential cohorts of patients receiving TK216 intravenously by continuous infusion via a central venous catheter. A "3+3" patient enrollment scheme will be followed during the schedule escalation. If 2 of 3 patients experience a first-cycle DLT then accrual to the cohort will cease, and the subsequent cohort will receive TK216 at the next lower dose. If a first-cycle DLT is seen in one of the 3 patients in a cohort, that cohort will enroll an additional 3 patients. DLT is defined as an adverse event occurring during the first cycle that is at least possibly related to TK216 and meets the DLT definition for the schedule escalation segment (Table 2). When 0 of 3, or 1 of 6 patients in a cohort experience first cycle DLT, the dose will be escalated in the subsequent cohort.

The starting daily dose level in the schedule escalation segment will be the MTD determined in the Part 1 dose escalation segment as described in the previous section.

The first schedule escalation cohort will receive TK216 by continuous IV infusion for 10 days.

The selection of dose and schedule for subsequent cohorts will be made by the Investigators and Sponsor after evaluating the first cycle results of the previous cohort. The dose level for newly enrolled patients will be assigned by Oncternal Therapeutics, Inc. at the time of registration.

By way of illustration:

- If 0 of 3 or 1 of 6 patients in the first dose escalation experience first cycle DLT, the second schedule escalation cohort will receive TK216 by continuous IV infusion at the same dose for 14 days.
- If 2 or more patients in the first dose escalation experience first cycle DLT, the second schedule escalation cohort will receive TK216 by continuous IV infusion at the next lower dose for 10 days.

The same decision algorithm will be used to select the dose and schedule for the third and subsequent schedule escalation cohorts.

It is anticipated that 4 to 6 cohorts will be completed during the schedule escalation segment of this trial. With the concurrence of the investigators and the study sponsor, further testing may be performed in up to 12 additional patients per dose level to refine the estimation of the MTD and RP2D at intermediate dose levels or to define a higher MTD and RP2D while using Cycle 1 primary supportive care prophylaxis (e.g., with growth factors, antidiarrheals, antiemetics) for bone marrow and/or gastrointestinal toxicities.

The duration of cycles will be variable. Following the scheduled infusions of TK216, a rest period will begin. The next cycle for a patient may begin as soon as all the patient's toxicities attributed to TK216 have resolved to less than Grade 3, or are clinically tolerable Grade 3 toxicities.

Once a patient completes Cycle 2 of any schedule escalation cohort, vincristine can be administered on Day 1 of a subsequent cycle and on Day 1 of each subsequent cycle. The initial dose of vincristine on Day 1 of the starting cycle will be 0.75 mg/m^2 given via intravenous infusion prior to the initiation of the TK216 infusion. Subsequent doses beginning on Day 1 of the next cycle and beyond will be 1.5 mg/m^2 up to a maximum dose of 2 mg.

Additional cycles of therapy may be administered provided that the patient meets the following criteria on Day 1 of each cycle:

- ANC $\geq$ 1,000/mm^3
- Hemoglobin $\geq$ 8.0 gm/dL (Blood transfusions are permitted.)
- Platelets $\geq$ 50,000/mm^3
- Non-hematologic toxicity(s) recovered to less than Grade 3, or are clinically tolerable Grade 3 toxicities
- In patients receiving vincristine, clinically tolerable neurotoxicity, and lack of seizure activity

A rest period of 14 days is required following the first cycle, but the rest period may be reduced following subsequent treatments if the above criteria are met.

If TK216 treatment has been well tolerated in Cycle 1 and 2, starting in Cycle 3 the duration of the TK216 infusion may be increased (e.g. from 14 to 17 or 14 to 21 days). If the longer infusion is well tolerated, the duration of the TK216 infusion may be increased further in subsequent cycles. There is no maximum treatment duration. If a longer infusion time is not well tolerated, the patient may remain on study, but receive a shorter duration of TK216 infusion in the next treatment cycle. The assessments noted for Days 8+/-1 and 15+/-1 in the Section 16.3 Schedule of Events (Schedule Escalation and Expansion Cohorts) should be performed every 7-8 days during longer infusions (Adverse events, Concomitant medications, Vital signs, Symptom driven physical exam, Hematology, Clinical chemistry, Coagulation, Urinalysis, and Plasma for PK assessment). A complete blood count (CBC with differential) should be obtained approximately half way between the weekly assessment at the study center or local laboratory.

Patients may have their dose increased to the next dose level at the next cycle if any cycle of TK216 is

well-tolerated with no clinically significant toxicity greater than Grade 2 attributed to TK216. If TK216 treatment has been well tolerated in Cycle 1 and 2, starting in Cycle 3 the duration of the TK216 infusion may be increased, in consultation with the Sponsor.

Intra-patient dose increases will be permitted during the schedule escalation segment of this trial. If any cycle of TK216 is well tolerated with no clinically significant toxicity greater than Grade 2 attributed to TK216, then the daily dose of TK216 may be increased to the next dose level on the next cycle upon discussion with the sponsor. No cycle should initiate:

1. Both vincristine for the first time and have a dose increase, or
2. Both vincristine for the first time and have an increase in the length of TK216 infusion, or
3. Increase the length of infusion for the first time and have a dose increase.

After the RP2D has been determined (recommended dose and schedule), an expansion cohort will be enrolled.

Table 2 Dose Limiting Toxicity – Part 2 Schedule Escalation Segment

Category	Criteria
Hematology toxicities	• Grade 4 neutropenia (absolute neutrophil count [ANC] < 500/mm^3) lasting more than 7 days; • Grade 4 neutropenic fever: life-threatening consequences, urgent intervention indicated • Grade 4 thrombocytopenia (platelet count < 25,000/mm^3) lasting more than 7 days; • Clinically significant thrombocytopenic bleeding;
Non-hematological toxicities	• Any ≥ Grade 3 toxicity that is not clinically tolerable; • For patients with liver metastases with elevated liver transaminases at baseline (2.5-5x ULN), DLT shall be defined as a doubling of the baseline liver transaminase value(s).
Failure to recover (except alopecia)	• Failure to recover to a clinically tolerable level of any toxicity attributed to TK216

Patients are eligible for DLT evaluation if they experience a DLT after receiving any amount of study drug or do not experience a DLT having taken a minimum of 85% of dose expected during the first cycle. Patients who do not fulfill these requirements and those who discontinue study participation prior to completing the first cycle will be replaced.

The RP2D may be determined by the MTD or optimal target inhibition with an acceptable safety profile.

2.4 Part 3 Expansion Cohort

The expansion cohort segment of this study will consist of approximately 18 ES patients.

All patients in the expansion cohort will receive the RP2D of TK216 in conjunction with primary supportive care prophylaxis (e.g., with growth factors, antidiarrheals, antiemetics) for bone marrow and/or gastrointestinal toxicities. The first three patients will also receive vincristine intravenously on the first day of each treatment cycle, at a dose of 0.75 mg/m^2. If DLT (Table 2) is observed in 1 of 3 patients, an additional three patients will receive the same doses of TK216 and vincristine. If fewer than 1 of 6 patients experience DLT, then the remaining patients in the expansion cohort will receive the RP2D of TK216 plus vincristine given intravenously on the first day of each treatment cycle, at a dose of 1.5 mg/m^2 to a maximum of 2 mg/m^2 per dose. If DLT is observed in ≥2 of the first six

patients, then the dosing of vincristine and/or TK216 will be revised in consultation between the Investigators and the Sponsor.

If TK216 treatment has been well tolerated in Cycle 1 and 2, starting in Cycle 3 the duration of the TK216 infusion may be increased (e.g., from 14 to 17 or 14 to 24 days). If the longer infusion is well tolerated, the duration of the TK216 infusion may be increased further in subsequent cycles. There is no maximum treatment duration. If a longer infusion time is not well tolerated, the patient may remain on study, but receive a shorter duration of TK216 infusion in the next treatment cycle. The assessments noted for Days 8+/-1 and 15+/-1 in the Section 16.3 Schedule of Events (Schedule Escalation and Expansion Cohorts) should be performed every 7-8 days during longer infusions (Adverse events, Concomitant medications, Vital signs, Symptom driven physical exam, Hematology, Clinical chemistry, Coagulation, Urinalysis, and Plasma for PK assessment). A complete blood count (CBC with differential) should be obtained approximately half way between the weekly assessment at the study center or local laboratory.

In Part 3 dosing will follow the schedule for the RP2D selected. If TK216 treatment has been well tolerated in Cycles 1 and 2, starting in Cycle 3 the duration of the TK216 infusion may be increased, in consultation with the Sponsor.

2.5 Part 4: Dose and Schedule Evaluation

The dose and schedule evaluation segment will consist of approximately 21 evaluable ES patients.

All patients in the dose and schedule evaluation segment will receive a starting dose of 175 mg/m2/day of TK216 intravenously by continuous infusion for 28 days per cycle.

If the patient's tumor response as determined by the Investigator is inadequate after at least one protocol-specified post-baseline assessment and they are not experiencing toxicities at this dose level, the Investigator may increase the dose to 200 mg/m^2/day after discussion with the Sponsor.

Vincristine may be administered in parallel with the TK216 infusion following progressive disease after TK216-01 monotherapy but only after consultation with the Sponsor.

2.6 Dose modification:

TK216 dose or schedule reduction for patients may be warranted after Cycle 1 as a consequence of drug-related toxicities. Dose reduction will be documented in the CRF along with reason for reduction. The decision to reduce the dose will be made by the Investigator, who may consult with the Sponsor if indicated.

Vincristine dosing will be interrupted for any clinically intolerable neuropathy (e.g., grade 4 foot drop, severe paresis, disabling paresthesias or ileus), and may be resumed if the neuropathy becomes clinically tolerable. If seizures occur the vincristine dosing should be interrupted until seizure activity is controlled and may be resumed if antitumor responses warrant in the opinion of the Investigator. In either case, the subsequent vincristine dose should be 0.75 mg/m^2. If the total bilirubin increases to greater than 1.9 mg/dL, vincristine dosing should be stopped. If the total bilirubin is between 1.5 – 1.9 mg/dL then vincristine should be administered at 0.75 mg/m^2.

2.6.1 Dose modifications

2.6.1.1 Dose reductions (Parts 1-3)

Dose or schedule reduction for patients may be warranted after Cycle 1 as a consequence of drug-related toxicities. Dose reduction will be documented in the CRF along with reason for reduction. The decision to reduce the dose will be made by the Investigator, who may consult with the Sponsor if indicated.

At a starting dose level of 18 mg/m^2/day, no dose reduction is anticipated for patients in the first cohort. If unacceptable toxicity occurs in a patient, then that patient will be instructed to stop treatment.

Patients with toxicities that are manageable with supportive therapy may not require dose reductions (*e.g.*, nausea/vomiting may be treated with antiemetics). Patients with toxicities up to Grade 3 that are clinically tolerable in the Investigator's opinion may not require dose reductions (e.g. severe diarrhea of short duration).

For adverse events not specified below, doses may be reduced or held at the discretion of the investigator for the patient's safety. The sponsor should be made aware of such reductions.

Suggested dose modifications based on type of AE or laboratory findings:

Grade 4 neutropenia or clinically intolerable Grade 3 neutropenia:

- First occurrence- hold TK216 until ANC $\geq$ 1,000/mm^3, then resume TK216 at same dose and schedule.
- Second or subsequent occurrence- hold TK216 until ANC $\geq$ 1,000/mm^3, then reduce TK216 daily dose to one dose level lower than the current dose.

Patients experiencing $\geq$ Grade 2 neutropenia may receive G-CSF or other myeloid growth factors after the first cycle or as defined in the dose and schedule escalation plan.

Grade 4 thrombocytopenia or any clinically significant thrombocytopenic bleeding:

- First occurrence- hold TK216 until platelets $\geqq$ 50,000/mm^3, then resume TK216 at same dose and schedule.
- Second or subsequent occurrence- hold TK216 until platelets $\geqq$ 50,000/mm^3, then reduce TK216 dose to one daily dose level lower than the current dose.
- Use of platelet growth factors or platelet transfusions is permissible.

Hemoglobin $\leq$ 8.0 gm/dL

- Any occurrence- hold TK216 until hemoglobin $\geq$ 8.0 gm/dL, then resume TK216 at same dose. Blood transfusions are permitted.

Grade 3 or greater non-hematologic toxicity (unless clearly and incontrovertibly unrelated to TK216):

- Any occurrence- hold TK216 until toxicities have resolved or improved to Grade 2 severity levels, then resume TK216 at same dose if event was a tolerable Grade 3 (at PI's discretion). Reduce TK216 dose to one dose cohort level lower than the current dose if event is Grade 3 or 4 or intolerable Grade 2 in severity.

Vincristine dosing will be interrupted for any clinically intolerable neuropathy (e.g. grade 4 foot drop, severe paresis, disabling paresthesias or ileus), and may be resumed if the neuropathy becomes clinically tolerable. If seizures occur the vincristine dosing should be interrupted until seizure activity is controlled, and may be resumed if antitumor responses warrant in the opinion of the Investigator. In either case, the subsequent vincristine dose should be 0.75 mg/m^2. If the total bilirubin increases to greater than 1.9 mg/dL, vincristine dosing should be stopped. If the total bilirubin is between 1.5 – 1.9 mg/dL then vincristine should be

administered at 0.75 mg/m2.

2.6.2 Dose Reductions (Part 4)

Dose reduction for patients may be warranted as a consequence of drug-related toxicities. Dose reduction will be documented in the CRF along with reason for reduction. The decision to reduce the dose will be made by the Investigator, who may consult with the Sponsor if indicated.

- Cohort 11 dose is 175 mg/m^2/day for 28 days.
    - First occurrence, wait for the AE to decrease to ≤ Grade 2 and then resume TK216 at same dose and schedule.
    - Second or subsequent occurrence of AE- hold the dose until AE ≤Grade 2, and then reduce TK216 daily dose to 145 mg/m^2/day. If a subsequent dose reduction is required, reduce TK216 daily dose to 130 mg/m^2/day.
    - If a further dose reduction below TK216 daily dose to 130 mg/m^2/day is required, discuss with Sponsor, as the patient may require discontinuation of TK216

Patients with toxicities that are manageable with supportive therapy may not require dose reductions (e.g., nausea/vomiting may be treated with antiemetics). Patients with toxicities up to Grade 3 that are clinically tolerable in the Investigator's opinion may not require dose reductions (e.g., severe diarrhea of short duration). If unacceptable toxicity occurs in a patient, then that patient will be instructed to stop treatment.

For adverse events not specified, doses may be reduced or held at the discretion of the investigator for the patient's safety. The Sponsor should be made aware of such reductions. If toxicities resolve at a lower dose level after one cycle, the investigator may adjust the dose upwards at their discretion.

Grade 3 or greater non-hematologic toxicity (unless clearly and incontrovertibly unrelated to TK216)

- Following any occurrence: hold TK216 until toxicities have resolved or improved to ≤ Grade 2 severity levels, then resume TK216 at same dose if event was a tolerable Grade 3 (at PI's discretion). Reduce TK216 dose to one dose level lower (145 mg/m^2/day, 130 mg/m^2/day) than the current dose if event is Grade 3 or 4 or intolerable Grade 2 in severity.

2.6.3 Dose Increase (Part 4)

The Cohort 11 starting dose is 175 mg/m^2/day for 28 days. If the patient's response is inadequate after at least one post-baseline assessment and they are not experiencing toxicities at this dose level, the Investigator may increase the dose to 200 mg/m^2/day after discussion with the Sponsor.

2.6.4 Management of Neutropenia (Part 4)

Patients in Cohort 11 of Part 4 may receive supportive care for bone marrow and/or gastrointestinal adverse events (e.g., with growth factors, antidiarrheals, antiemetics) and prophylaxis following institution's standard of care.

- A short-acting GCSF should be given daily if the ANC drops below 1,000/mm^3. CBCs should be done twice weekly and TK216 dosing can continue.
- GCSF should be continued until ANC is above 2,000/mm^3.
- If ANC ≤ 500/mm^3 TK216 should be held until ANC > 1000/mm^3.

3. Patient Eligibility

3.1 Inclusion criteria

Patients who meet the following inclusion criteria will be eligible to participate in this study:

1. Willing and able to provide written IRB/IEC-approved Informed Consent. For patients < 18 years of age, their parents or legal guardians must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.
2. Have histologically or cytologically confirmed diagnosis of Ewing sarcoma (including ESFT) with relapsed or refractory disease
    a. who have failed standard therapy and for whom no known curative therapy exists (For Parts 1-3), OR
    b. patients with metastatic disease who had standard chemotherapy at the time of diagnosis (Part 4)

    (NOTE: as of Protocol version 7, pathology reports and slides or blocks should be available for review or additional testing. If not available, site must discuss with Sponsor.)
3. Measurable disease according to RECIST version 1.1. Measurable disease can be verified from a previously documented CT scan or MRI as long as no anti-cancer treatments have been administered in the interim.
4. Must have a central venous catheter in place prior to initiating infusion of study drug.
5. Prior cancer therapy:
    a. Patients may have received any number of prior therapy regimens. In the Investigator's opinion, patients must have tolerated prior cytotoxic therapies well and have adequate bone marrow reserve. At the time of treatment initiation, at least 3 weeks must have elapsed after prior cytotoxic chemotherapy. At least 7 days must have elapsed since completion of any prior non-cytotoxic cancer therapy and any associated AEs must have resolved.(For Parts 1-3) OR
    b. Patients may have received no more than five prior systemic regimens. At the time of treatment initiation, at least 3 weeks must have elapsed since prior cytotoxic chemotherapy. At least 7 days must have elapsed since completion of any prior non-cytotoxic cancer therapy. (For Part 4)
6. Prior radiotherapy is allowed
    a. If ≥ 2 weeks have elapsed for local palliative XRT (small port); ≥ 6 months must have elapsed if prior total body irradiation, craniospinal XRT or if > 50% radiation of the pelvis; ≥ 6 weeks must have elapsed if other substantial bone marrow radiation. Patients who have received brain irradiation must have completed whole brain radiotherapy and/or gamma knife at least 4 weeks prior to enrollment (Parts 1-3) OR
    b. If ≥ 4 weeks has elapsed for radiation therapy (RT); ≥ 6 months must have elapsed if prior total body irradiation, craniospinal RT or if > 50% radiation of the pelvis; ≥ 6 weeks must have elapsed if other substantial bone marrow radiation. Patients who have received brain irradiation must have completed whole brain radiotherapy and/or gamma knife at least 4 weeks prior to enrollment. (Part 4)
7. Stem Cell Transplant or Rescue without TBI: No evidence of active graft vs. host disease and ≥ 3 months must have elapsed since transplant.

8. Patients with controlled asymptomatic CNS involvement are allowed (see Concomitant Medications). Patients not requiring steroids or requiring steroids at stable dose (≤ 4 mg/day dexamethasone or equivalent) for at least 2 weeks are eligible.
9. Resolution of all acute toxic effects (excluding alopecia) of any prior anti-cancer therapy to NCI CTCAE (Version 4.03) Grade ≤ 1 or to the baseline laboratory values as defined in the table below.
10. Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 in patients ≥17 years old; or Karnofsky/Lansky >50 in patients ≤16 years old.
11. Age
    a. Patients age ≥18 years old (Cohort 1)
    b. Patients must be ≥ 12 years old (Cohorts 2-5)
    c. Patients must be ≥ 10 years old (Cohorts 6-10)
    d. Patients must be ≥ 8 years old (Cohort 11)
12. Life expectancy of at least 3 months.

13. Baseline laboratory values fulfilling the following requirements:

Absolute Neutrophil Count (ANC)	$\geq 1000/mm^3$ ($\geq 1.0 \times 10^9/L$)
Platelets (PLT)	$\geq 100{,}000/mm^3$ ($\geq 100 \times 10^9/L$) (transfusion independent, defined as not receiving platelet transfusions within a 7 day period prior to screening)
Hemoglobin	> 9.0 g/dL (transfusions are allowed)
Serum Creatinine or Creatinine Clearance	≤ 1.5 x ULN > 60 mL/min
Total Serum Bilirubin	≤ 1.5 x ULN
Liver Transaminases (AST/ALT)	≤ 2.5 x ULN; ≤ 5 x ULN if liver metastases are present
Alkaline Phosphatase (ALP)	≤ 2.5 x ULN; ≤ 5 x ULN if liver and/or bone metastases are present
Serum calcium and potassium	Normal or ≤ CTCAE Grade 1 with or without supplementation.
Pregnancy test if female of child-bearing potential	Negative within 7 days of starting treatment
AST/ALT = aspartate aminotransferase/alanine aminotransferase, ULN = upper limit of normal Growth factor(s): Growth factors that support platelet or white cell number or function must not have been administered within the 7 days prior to screening.	

14. Cardiac ejection fraction ≥ 50% or shortening fraction ≥ 28%.
15. Females of child-bearing potential must have a negative pregnancy test during screening and be neither breastfeeding nor intending to become pregnant during study participation. Females of childbearing potential must agree to avoid pregnancy during the study and commit to abstinence from heterosexual intercourse or agree to use two methods of birth control (one highly effective method and one additional effective method) at least 4 weeks before the start of protocol therapy, for the duration of study participation, and for 6 months after the last dose of TK216
16. Males with partner(s) of childbearing potential must take appropriate precautions to avoid fathering a child from the screening period until 90 days after receiving the last dose of TK216. They must commit to abstinence from heterosexual intercourse or agree to use appropriate barrier contraception.
17. Prior to enrollment of females or males of reproductive potential, the investigator must document confirmation of the patient's understanding of the possible teratogenic effects of TK216.
18. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.
19. If the patient is to receive vincristine, no contraindication to vincristine administration.

3.2 Exclusion criteria

Patients will not be enrolled if they meet any of the following exclusion criteria:

1. Current participation in another therapeutic clinical trial.
2. Symptomatic brain metastases.
3. History of previous cancer (non ES), except squamous cell or basal-cell carcinoma of the skin or any in situ carcinoma that has been completely resected, which required therapy within the previous 3 years.
4. Incomplete recovery from any surgery (other than central venous catheter or port placement) prior to treatment.
5. Any of the following in the past 6 months: symptomatic congestive heart failure, cerebrovascular accident or transient ischemic attack, pulmonary embolism, deep vein thrombosis, symptomatic bradycardia, requirement for anti-arrhythmic medication.
6. History of prolonged QTc interval (e.g., repeated demonstration of a QTc interval > 450 milliseconds, unless associated with the use of medications known to prolong the QTc interval).

- (NOTE: for Part 4 repeated demonstration of a QTc interval > 470 milliseconds)

7. History of additional risk factors for torsade de pointes (e.g., heart failure, family history of long QT syndrome).
8. Use of concomitant medications that increase or possibly increase the risk to prolong the QTc interval and/or induce torsades de pointes ventricular arrhythmia.
9. Females who are breastfeeding/lactating.
10. Known active infections (e.g., bacterial, fungal, viral including hepatitis and HIV positivity).
11. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient inappropriate for entry into this study or compromise protocol objectives in the opinion of the Investigator and/or the Sponsor.

4. Study Plan

4.1 Study assessments

4.1.1 Physical examination

A complete physical examination will include the following: HEENT (head, ears, eyes, nose and throat), chest, lungs, heart, lymph nodes, abdomen, skin, musculoskeletal and neurological systems. Symptom-driven physical exams will be done as needed, based on observed adverse events.

4.1.2 Height and weight

Height and weight will be measured during screening. Weight only will be measured at subsequent visits. For pediatric patients, height should be measured periodically as determined by the Investigator

4.1.3 Electrocardiogram

An electrocardiogram (ECG) will be obtained using a 12-lead electrocardiograph will be done during the screening period to determine cardiac function. Each ECG is to be evaluated by the study

investigator for the presence of abnormalities at the time the ECG is recorded. The evaluating physician is to write his/her diagnosis on the ECG recording and sign and date. In some cases, it may be useful to repeat abnormal ECG's to rule out improper lead placement as contributing to the ECG abnormality.

4.1.4 Vital signs

Vital signs will include:

- blood pressure, respiratory rate, heart rate (with the patient in the sitting position following an approximate 5-minute rest); two measurements of blood pressure will be taken at each determination, 1 to 5 minutes apart.
- temperature (°C)

4.1.5 Clinical laboratory tests

All screening clinical laboratory test results are to be reviewed and assessed by the investigator, or designee, prior to study enrollment. Any screening laboratory result that is required for eligibility that is outside the reference range as allowed by entry criteria may be repeated, as deemed necessary by the Principal Investigator. If any repeat screening values continue to be outside the reference range, the patient will be excluded from the study, unless there is agreement between the sponsor and investigator that the laboratory deviation is not clinically significant. After enrollment into the study, the CBC, serum electrolytes, BUN, creatinine, liver function tests and urinalysis are to be reviewed and assessed for safety prior to drug administration on Day 1 of each treatment cycle.

Blood samples for the following tests will be collected:

- Hematology: complete blood count (CBC) including WBC with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) RBC, hemoglobin, hematocrit, and platelet count.
- Clinical chemistry: serum sodium, potassium, chloride, bicarbonate, calcium, magnesium, BUN, creatinine, eGFR, uric acid, total bilirubin, direct (conjugated) bilirubin, indirect (unconjugated) bilirubin (calculated), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, lactate dehydrogenase (LDH), amylase, lipase, triglycerides, total cholesterol, inorganic phosphate, total protein, albumin, creatine phosphokinase (CPK), glucose.
- Coagulation: prothrombin time (PT), and activated partial thromboplastin time (APTT). An international normalized ratio (INR) may be assessed only if the PT assessment is unavailable.

Urine will be collected for the following tests:

- Urinalysis: color, protein, glucose, bilirubin, urobilinogen, ketones, blood, pH, specific gravity, and leukocytes.

4.1.6 Other Assessments

4.1.6.1 Urine pregnancy test

Urine pregnancy tests will be conducted for all females of child bearing potential.

4.1.6.2 Demographics

Information regarding the patient's gender, age, and racial or ethnic origin will be collected.

4.1.6.3 Pallative Radiation Therapy (Part 4)

Patients may rapidly develop tumor-related symptoms even in the face of stable or minimally progressive disease. Therefore, as of Protocol version 7.0, palliative local radiation therapy may be allowed if the following criteria are met:

1. Treatment is required for a symptomatic, non-target lesion(s) (example: pain due to compression of nerve in a closed space).
2. Planned radiation therapy is focal and the field does not involve the treatment of target lesion(s) directly or indirectly by an inability to fully shield adjacent target lesions from any radiation effect.
3. There is a reasonable possibility that the patient will benefit from additional systemic therapy with TK216.
4. Approval for this treatment has been granted by Oncternal.

4.1.6.4 Medical History

The patient's medical history will be taken with particular attention to questions related to 1) a thorough review of body systems including any past or current conditions; 2) previous and current pharmacotherapy or chronic use of any medication within 14 days prior to screening; and 3) history of allergies or hypersensitivity to drugs.

4.1.6.5 Performance status

The ECOG performance status (in patients ≥17 years old) or the Karnofsky /Lansky status (in patients ≤16 years old) will be assessed at Screening and at the beginning of each cycle.

4.1.6.6 Tumor assessments

For the Part 1 dose escalation segment, tumor assessments will be performed from evaluation of a CT or an MRI scan obtained within 10 days of beginning Cycle 1 at and after completing the infusions of Cycles 2, 4, 6, etc (i.e., every 6 weeks).

For the Part 2 schedule escalation segment, tumor assessments will be performed soon after the end of the infusion during each even-numbered cycle but prior to initiating the infusion for the next cycle.

For the Part 3 expansion segment, the scan schedule will be at the end of every even-numbered cycle's rest period, and prior to starting the next infusion. The same methodology should be used for all assessments throughout the study. Assessments will be done using RECIST, version 1.1. Tumor

assessments will continue to be done after the patient discontinues from the study, until the criteria for progressive disease have been met, or the patient begins another cancer therapy, or withdraws consent.

For the Part 4 dose and schedule evaluation segment, tumor assessments will be performed from evaluation of a CT (chest/abdomen/pelvis) or an MRI scan obtained within 10 days of beginning Cycle 1 and approximately every 8 weeks thereafter (e.g. after completing the infusions of Cycles 2, 4, 6, etc).

4.1.7 **Adverse events**

An adverse event (AE) is any untoward medical occurrence in a patient administered a study drug, and that does not necessarily have a causal relationship with the study drug. (An AE can be any unfavorable and unintended sign or symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.) Adverse events include any symptom, physical sign, syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen during the course of a clinical trial, after starting treatment, whether considered treatment related or not.

Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of pre-existing illnesses should be recorded. Exacerbation of a pre-existing illness is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the study. It may include a worsening or increase in severity of signs or symptoms of the illness, increase in the frequency of signs and symptoms of an intermittent illness, or the appearance of a new manifestation/complication. Exacerbation of a pre-existing illness should be considered when a patient requires new or additional concomitant therapy for the treatment of that illness during the study. Lack of, or insufficient clinical response, benefit, efficacy, therapeutic effect, or pharmacologic action, should not be recorded as an adverse event. The investigator must make the distinction between exacerbation of a pre-existing illness and lack of therapeutic effect.

For all adverse events, the investigator must pursue and obtain information adequate to determine both the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to the sponsor or its designated representative (see Appendix 1). For all adverse events, the investigator is required to obtain sufficient information to assess the causality of the adverse event (i.e., study drug or other illness).

Follow-up of the adverse event, after study drug has been discontinued, is required if the adverse event or its sequelae persist. Follow-up of all adverse events is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and the sponsor or its designated representative.

Adverse events may be volunteered spontaneously by the patient, or be discovered as a result of general questioning by the investigator or by physical examination or laboratory tests. Patients will be continuously monitored for adverse events (AEs) during the study. At each visit during the study or at each telephone contact after the first dose, patients will be asked to specifically describe any signs, symptoms, or AEs occurring since the previous visit. Questions will be phrased so that they do not "lead" the patient into giving information that is not valid. All adverse events regardless of treatment group or suspected causal relationship to study drug will be recorded in source documentation and on the adverse event page(s) of the case report form (CRF).

Conditions that the patient experienced before treatment with study drug (Pre-dose symptoms) and any new signs, symptoms, or AEs that occur since starting treatment with study drug (regardless of causality) are to be assessed and recorded. Pre-dose baseline assessments must be performed prior to treatment with TK216. Any baseline symptoms or adverse events noted prior to treatment with TK216 should be recorded as part of the Medical History. The assessment and recording of each symptom or AE must also be described by its duration (start date, time and duration), its severity (mild, moderate, severe, very severe), its relationship to the study medication (unrelated, unlikely or possibly related), whether it influenced the course of the study medication, and whether it required specific therapy.

The severity of signs, symptoms, or AEs is to be determined by using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v 4.03. If a sign, symptom or AE is not included in the toxicity severity grading scale, the intensity of the event will be graded as shown below.

- **mild (grade 1):** Symptoms causing no or minimal interference with usual social and functional activities. Symptoms are usually transient and require no special treatment.
- **moderate (grade 2):** Symptoms causing greater than minimal interference with usual social and functional activities. Symptoms are usually ameliorated by simple therapeutic measures
- **severe (grade 3):** Symptoms causing inability to perform usual social and functional activities. Symptoms traditionally require systemic drug therapy or other treatment
- **very severe (grade 4, life-threatening):** Symptoms causing inability to perform basic self-care functions or require medical or operative intervention to prevent permanent impairment, persistent disability, or death.
- **death related to AE (grade 5)**

4.1.7.1 Assessment of causal relationship

A medically-qualified investigator must assess the relationship of any AE to the use of study drug, based on available information, using the following guidelines:

- **Not related**: There is not a reasonable causal relationship to the investigational product and the adverse event.
- **Unlikely related**: No temporal association or the cause of the event has been identified, or the drug or biologic cannot be implicated.
- **Possibly related**: There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
- **Related**: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

The investigator reports causality, but the sponsor retains the final decision on causality when filing to the FDA.

4.1.7.2 Expectedness of an adverse event

The expectedness of an adverse event or suspected adverse reaction shall be determined according to the specified reference document containing safety information (e.g., most current investigator's brochure or product label). Any AE that is not identified in nature, severity, or specificity in the current study drug reference document(s) (e.g., investigator's brochure) is considered unexpected. Events that are mentioned in the investigator's brochure as occurring with a class of drugs or as anticipated from the pharmacological

properties of the drug, but not specifically mentioned as occurring with the particular drug under investigation are considered unexpected.

4.1.7.3 Abnormal clinical test findings

Any clinically significant changes in physical examination findings and abnormal objective test findings (e.g., laboratory, x-ray, ECG) should also be recorded as adverse events. The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

1. test result is associated with accompanying symptoms, and/or
2. test result requires additional diagnostic testing or medical/surgical intervention, and/or
3. test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
4. test result leads to any of the outcomes included in the definition of a serious adverse event, and/or
5. test result is considered to be an adverse event by the investigator or sponsor

Merely repeating an abnormal test, in the absence of any of the above conditions, does not meet condition #2 above for reporting as an adverse event. If additional diagnostic testing (condition #2) is performed to rule out a potential problem/abnormality, and if the test does not confirm the problem/abnormality, the abnormal laboratory result for which diagnostic testing was performed would not be considered an adverse event.

All clinically important abnormal test results occurring during the study will be repeated at appropriate intervals until the abnormal result returns either to baseline or to a level deemed acceptable by the investigator and the sponsor (or its designated representative), or until a diagnosis that explains the abnormal result is made.

Any abnormal test result that is determined to be an error does not require reporting as an adverse event, even if it did meet 1 of the above conditions except for condition #4.

4.1.7.4 Serious adverse events

All serious adverse events (SAEs) (defined below) regardless of treatment group or suspected relationship to study drug must be reported immediately (within 1 working day) by telephone to the sponsor or its designated representative (see Appendix 1).

An SAE is defined as an adverse event that:

- A **life-threatening** event is any AE that places the patient at immediate risk of death from the reaction/event as it occurred (i.e., it does not refer to an AE that, had it occurred in a more severe form, might have caused death).
- **Disability** is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Inpatient hospitalization** is defined as any inpatient admission, regardless of duration. For chronic or long-term inpatients, inpatient admission also includes transfer within the hospital to an acute/intensive care inpatient unit. Inpatient admission in the absence of a precipitating, treatment-emergent, adverse event may meet criteria for "seriousness", but should not be considered or reported as a serious adverse event (e.g., admission for treatment of a pre-

existing condition not associated with the development of a new adverse event or with a worsening of a pre-existing condition; social admission (e.g., patient has no place to sleep); administrative admission (e.g., for an annual physical exam); protocol-specified admission during a clinical study (e.g., for a procedure required by protocol); optional admission not associated with a precipitating clinical adverse event (e.g., for elective cosmetic surgery). In addition, inpatient admission does not include any of the following: A visit to the emergency room or hospital clinic; out-patient, same day, ambulatory procedures; observation or short-stay units; rehabilitation facilities; hospice facilities, respite care (e.g., care-giver relief); skilled nursing facilities; nursing homes; custodial care facilities; or clinical research, Phase 1 units.

- **Prolongation of hospitalization** is defined as any extension of an inpatient hospitalization beyond the stay anticipated/required in relation to the original reason for the initial admission. For protocol-specified hospitalizations in clinical trials, prolongation is defined as any extension beyond the length of stay required by protocol. Prolongation in absence of a precipitating, treatment-emergent, adverse event may meet criteria for "seriousness", but should not be considered or reported as a serious adverse event.
- An **important medical event** is defined as any adverse event that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient and may require medical or surgical intervention in order to prevent the event from becoming a serious adverse event, as determined by appropriate medical judgment. Examples of such medical events include; allergic bronchospasm requiring intensive treatment in an emergency room or other setting, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or development of a drug dependency.
- Is a congenital anomaly/birth defect.
- Results in death.
- All SAEs must be reported by the investigator or qualified designee within 1 working day of first becoming aware of the event. SAEs that occur at any time on or after Cycle 1 Day 1 through 28 days after the last day of the final administration of study drug.
- **Suspected unexpected serious adverse reactions (SUSARs)** are serious events that are not listed in the IB and that the investigator identifies as related to investigational product or procedure. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. Oncternal Therapeutics, Inc. has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

4.1.7.5 Non-serious adverse events

For this study, all non-serious adverse events occurring from the start of infusion on Cycle 1 Day 1 through the last follow-up visit required by protocol, or 28 days after the last administration of study drug, whichever comes later, will be collected regardless of treatment group or suspected relationship to study drug, and must be reported via eCRF.

4.1.7.6 Reporting a pregnancy

Pregnancy occurring during a clinical investigation, although not considered an SAE, must be reported within the same timelines as an SAE. The positive pregnancy test will be recorded in the eCRF and pregnancy will be reported on the paper pregnancy CRF, which will be provided by the CRO. The pregnancy will be followed until final outcome. Any associated AEs or SAEs that occur to the mother or fetus will be recorded in the AE or SAE eCRF. If a pregnancy occurs in the partner of a patient participating in the trial, the pregnancy will be reported on the paper pregnancy CRF provided by the CRO. The pregnancy will be followed until final outcome.

4.2 Schedule of Study Assessments

4.2.1 Screening Assessments : Parts 1-3

The screening assessments for all Cohorts in the study will be completed and the results evaluated by the study investigator within 30 days prior to the start of dosing. The procedures listed below will be performed:

- informed consent: must be obtained prior to performing any study related procedure and may be obtained within 30 days prior to starting treatment; for patients <18 years of age, their parent or legal guardian must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines;
- demographics;
- medical history (any events up to the start of infusion);
- tumor assessments: Measurable disease for eligibility purposes can be verified from a previously documented CT scan or MRI as long as no anti-cancer treatments have been administered in the interim. Baseline tumor assessments will be by CT or MRI performed within approximately 10 days of the first treatment.
- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- height and weight;
- complete physical examination;
- 12-lead ECG;
- Echocardiogram (or MUGA)
- tests for HBVsAg, HCV Ab, HIV Ab;
- serum or urine pregnancy test (female patients of child bearing potential only);
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication assessment.

4.2.2 Dose Escalation Cohorts 1, 2, 3; Day 1 though End of Cycle 1

4.2.2.1 Day 1, Cycle 1 Assessments, Admission to Hospital (Cohorts 1, 2, 3)

On Day 1 of dose escalation Cohorts 1, 2 and 3, patients will be admitted to the hospital to prepare for the initiation of dosing. The following procedures will be performed:

- ECOG or Karnofsky/Lansky performance status**;
- vital signs, pre-dose and 2 hours post-dose;
- weight**

- complete physical examination**;
- 12-lead ECG: predose**;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis)**;
- urine pregnancy test (women of childbearing potential only)**;
- the infusion via a central venous catheter should be initiated in the late afternoon on Day 1, in order to facilitate collection of pharmacokinetic samples (see below); this infusion will continue for 16 hours, and be stopped in the morning of Day 2;
- A 12-lead ECG between 1 and 3 hours after initiating the infusion;
- plasma PK samples: pre-dose, 1, 2, and 3 hours after initiation of the infusion on Day 1;
- pharmacodynamic samples: pre-dose on Day 1;
- concomitant medication assessment;
- adverse event assessment.

** If the screening assessments for ECOG or Karnofsky/Lansky performance status, weight, physical examination, ECG, clinical laboratory tests and urine pregnancy test were performed within approximately 72 hours prior to dosing on Day 1 of Cycle 1, these assessments do not need to be repeated except as required by institutional standards.

4.2.2.2 Day 2, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 2 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- a 12-lead ECG will be done on Day 2 between hours 14 and 16 hours after the start of the infusion on Day 1 (to coincide with the plasma PK samples done at the same time points).
- plasma PK samples at 14 hours, 15 hours and 16 hours after starting the infusion on Day 1;
- suspend infusion 16 hours after starting on Day 1 (immediately after drawing 16 hour PK sample), in the morning of Day 2;
- plasma PK samples at 0.5, 1, 2, 4 and 8 hours after discontinuing the infusion;
- restart infusion 8 hours later, in the late afternoon; infusion will then be continuous until Day 8;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

4.2.2.3 Day 3, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 3 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- pharmacodynamic samples (one set of samples on Day 3 or Day 4)

- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

4.2.2.4 Day 4, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 4 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- pharmacodynamic samples (one set of samples on Day 3 or Day 4)
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

4.2.2.5 Day 5, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 5 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

4.2.2.6 Day 6, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 6 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

4.2.2.7 Day 7, Cycle 1 Assessments, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 7 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;

- adverse event assessment.

4.2.2.8 Day 8, Cycle 1 Assessments, Discharge from Hospital (Cohorts 1, 2, 3)

On Day 8 of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed prior to the patient's discharge from the hospital:

- 12-lead ECG between 1 and 3 hours of discontinuing the infusion;
- pharmacodynamic samples immediately prior to discontinuing the infusion;
- plasma PK samples will also be collected on the last day of the first treatment cycle (C1D8) at three time-points prior to discontinuing the infusion, separated by approximately 1 hour, with the last of these collected immediately before stopping the infusion;
- discontinuation of infusion at in the morning;
- plasma PK samples at 0.5, 1, 2, 4 and 8 hours after discontinuing the infusion;
- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

4.2.2.9 Day 15, Cycle 1 assessments, Outpatient Visit (Cohorts 1, 2, 3)

On Day 15 (+/- 2 days) of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed during an outpatient visit:

- vital signs;
- weight;
- symptom-driven physical examination;
- clinical laboratory tests (hematology, clinical chemistries, coagulation);
- concomitant medication check;
- adverse event assessment.

4.2.2.10 Day 22, Cycle 1 assessments, Outpatient Visit for Patients not Continuing Treatment (Cohorts 1, 2, 3)

On Day 22 (+3 days) of Cycle 1 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed during an outpatient visit, if the patient is not continuing to Cycle 2 (the procedures to be performed are the same as for an early termination visit/End of Treatment visit, but the patient will be considered to have completed the study if the patient completes Cycle 1):

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;

- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- pharmacodynamic samples;
- concomitant medication check;
- adverse event assessment.

4.2.2.11 Day 1 of Cycle 2 and 3, Admission to Hospital (Cohorts 1, 2, 3)

This visit will also be considered Day 22 of the previous cycle of dose escalation Cohorts 1, 2 and 3 for those patients continuing treatment. The procedures listed below will be performed prior to the start of the infusion:

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG pre-dose;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- pharmacodynamic samples;
- concomitant medication check;
- adverse event assessment;
- initiation of continuous infusion via a central venous catheter.

4.2.2.12 Day 2 of Cycle 2 and 3, Continuation of Hospitalization (Cohorts 1, 2, 3)

On Day 2 of Cycle 2 and 3 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- symptom-driven physical examination;
- 12-lead ECG done between 20 and 24 hours after starting the infusion on Day 1 of cycles 2 and 3 (to coincide with the PK sample);
- plasma PK sample taken between 20 and 24 hours after starting the infusion on Day 1 of cycles 2 and 3;
- concomitant medication check;
- adverse event assessment.

4.2.2.13 Day 3 of Cycle 2 and 3, Discharge from Hospital (Cohorts 1, 2, 3)

On Day 3 of Cycle 2 and 3 of dose escalation Cohorts 1, 2 and 3, prior to discharge from the hospital, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;

- vital signs
- symptom-driven physical examination;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

4.2.2.14 Days 4 through 7 of Cycle 2 and 3 (Cohorts 1, 2, 3)

On Days 4 through 7 of Cycle 2 and 3 of dose escalation Cohorts 1, 2 and 3, the continuous infusion via a central venous catheter will continue as an outpatient. If the patient experiences any problems with the infusion, the site should be contacted.

4.2.2.15 Day 8 of Cycle 2 and 3, Outpatient Visit (Cohorts 1, 2, 3)

On Day 8 (+/- 2 days) of Cycle 2 and 3 of dose escalation Cohorts 1, 2 and 3, the patient will return for an outpatient visit, at which time the infusion will be discontinued and the following procedures will be performed:

- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG prior to discontinuing the infusion;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

4.2.2.16 Day 15 of Cycle 2 and 3, Outpatient Visit (Cohorts 1, 2, 3)

On Day 15 (+/- 2 days) of Cycle 2 and 3 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed during an outpatient visit:

- clinical laboratory tests (hematology, clinical chemistries, coagulation);
- concomitant medication check;
- adverse event assessment.

4.2.2.17 Day 22 of Cycle 2 and 3, Outpatient Visit (Cohorts 1, 2, 3)

On Day 22 (+/- 3 days) of Cycles 2 and 3 of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed during an outpatient visit, if the patient is not continuing to the next cycle (the procedures to be performed are the same as for an early termination/EOT visit:

- ECOG or Karnofsky/Lansky performance status;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);

- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment.

4.2.2.18 Day 1, Cycle 4 and all Subsequent Cycles, Outpatient Visit (Cohorts 1, 2, 3)

This visit will also be considered Day 22 (+/- 3 days) of the previous cycle of dose escalation Cohorts 1, 2 and 3 for those patients continuing treatment. The procedures listed below will be performed:

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment;
- initiation of continuous infusion via a central venous catheter; infusion will continue until Day 8.

4.2.2.19 Days 2 – 7, Cycle 4 and all Subsequent Cycles (Cohorts 1, 2, 3)

On Days 2 through 7 of Cycle 4 and all subsequent cycles, the continuous infusion via a central venous catheter will continue as an outpatient. If the patient experiences any problems with the infusion, the site should be contacted.

4.2.2.20 Day 8, Cycle 4 and all Subsequent Cycles, Outpatient Visit (Cohorts 1, 2, 3)

On Day 8 (+/- 2 days) of Cycle 4 and all subsequent cycles of dose escalation Cohorts 1, 2 and 3, the patient will return for an outpatient visit, at which time the infusion will be discontinued and the following procedures will be performed:

- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

4.2.2.21 Day 22, Cycle 4 and all Subsequent Cycles, Outpatient Visit (Cohorts 1, 2, 3)

On Day 22 (+/- 3 days) of Cycle 4 and all subsequent cycles of dose escalation Cohorts 1, 2 and 3, the procedures listed below will be performed if the patient is not continuing to the next cycle (the procedures to be performed are the same as for an early termination/EOT visit):

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment.

4.2.3 Cohort 4 and all Subsequent Dose Escalation Cohorts, Day 1 through end of Cycle 1 (Cohorts 4, 5, 6)

6.3.4.1 Day 1, Cycle 1 Admission to Hospital (Cohorts 4, 5, 6)

On Day 1 of Cycle 1 of Cohort 4 and all subsequent dose escalation cohorts, patients will be admitted to the hospital to prepare for the initiation of dosing. The following procedures will be performed:

- ECOG or Karnofsky/Lansky performance status**;
- weight;
- vital signs;
- 12-lead ECG: pre-dose**;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis)**;
- urine pregnancy test (women of childbearing potential only)**;
- complete physical examination**;
- the infusion via a central venous catheter should be initiated in the late afternoon on Day 1, in order to facilitate collection of pharmacokinetic samples (see below); this infusion will continue for 16 hours, and be stopped at in the morning of Day 2;
- 12-lead ECG: post-dose between 1 and 3 hours after initiation of the infusion on Day 1 (to coincide with the PK samples);
- plasma PK samples: pre-dose, 1, 2 and 3 hours after initiation of the infusion on Day 1;
- pharmacodynamic samples: pre-dose on Day 1;
- concomitant medication assessment;
- adverse event assessment.

** If the screening assessments for ECOG or Karnofsky/Lansky performance status, weight, physical examination, ECG, clinical laboratory tests and urine pregnancy test were performed within

approximately 72 hours prior to dosing on Day 1 of Cycle 1, these assessments do not need to be repeated, except as required by institutional standards.

6.3.4.2 Day 2, Cycle 1 Continuation of Hospitalization (Cohorts 4, 5, 6)

On Day 2 of Cycle 1 of Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed.

- 12-lead ECG between 14 and 16 hours after starting the infusion on Day 1;
- plasma PK samples at 14 hours, 15 hours and 16 hours after starting the infusion on Day 1;
- suspend infusion 16 hours after starting on Day 1 (immediately after drawing 16 hour PK sample), in the morning of Day 2;
- plasma PK samples at 0.5, 1, 2, 4 and 8 hours after discontinuing the infusion;
- restart infusion 8 hours later, immediately after collecting the 8-hour plasma PK sample, in the late afternoon; infusion will then be continuous until Day 8;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- 12-lead ECG;
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

6.3.4.3 Day 3, Cycle 1 Continuation of Hospitalization (Cohorts 4, 5, 6)

On Day 3 of Cycle 1 of Cycle 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- pharmacodynamic samples (one set of samples on Day 3 or Day 4);
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

6.3.4.4 Day 4, Cycle 1 Discharge from Hospital (Cohorts 4, 5, 6)

On Day 4 of Cycle 1 of Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed prior to discharge from the hospital:

- continuous infusion via a central venous catheter of study drug;
- vital signs;
- pharmacodynamic samples (one set of samples on Day 3 or Day 4);
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

6.3.4.5 Days 5 - 7, Cycle 1 (Cohorts 4, 5, 6)

On Days 5 through 7 of Cycle 1 in Cohort 4 and all subsequent dose escalation cohorts, the continuous infusion via a central venous catheter will continue as an outpatient. If the patient experiences any problems with the infusion, the site should be contacted.

6.3.4.6 Day 8, Cycle 1, Outpatient Visit (Cohorts 4, 5, 6)

On Day 8 (+/- 2 days) of Cycle 1 in Cohort 4 and all subsequent dose escalation cohorts, the patient will return for an outpatient visit, at which time the following procedures will be performed:

- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG between 1 and 3 hours prior to discontinuing the infusion;
- plasma PK samples will also be collected on the last day of the first treatment cycle (C1D8) at three time-points separated by approximately 1 hour, with the last of these collected immediately before stopping the infusion;
- pharmacodynamic samples immediately prior to discontinuing the infusions;
- discontinuation of infusion at in the morning;
- plasma PK samples at 0.5, 1, 2, 4 and 8 hours after discontinuing the infusion;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

6.3.4.7 Day 15, Cycle 1, Outpatient Visit (Cohorts 4, 5, 6)

On Day 15 (+/- 2 days) of Cycle 1 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed during an outpatient visit:

- vital signs;
- weight;
- symptom-driven physical examination;
- clinical laboratory tests (hematology, clinical chemistries, coagulation);
- concomitant medication check;
- adverse event assessment.

6.3.4.8 Day 22, Cycle 1, Outpatient Visit for those Patients not Continuing to Cycle 2 (Cohorts 4, 5, 6)

On Day 22 (+3 days) of Cycle 1 of dose escalation Cohorts 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed if the patient is not continuing to the next cycle (the procedures to be performed are the same as for an early termination/EOT visit):

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;

- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- pharmacodynamic samples;
- concomitant medication check;
- adverse event assessment.

6.3.4.9 Day 1, Cycles 2 and 3, Admission to Hospital (Cohorts 4, 5, 6)

This visit will also be considered Day 22 of the previous cycle of dose escalation Cohorts 4 and all subsequent dose escalation cohorts for those patients continuing treatment. The procedures listed below will be performed after the patient is admitted to the hospital:

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment;
- initiation of continuous infusion via a central venous catheter.

6.3.4.10 Day 2, Cycles 2 and 3, Continuation of Hospitalization (Cohorts 4, 5, 6)

On Day 2 of Cycle 2 and 3 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed:

- continuous infusion via a central venous catheter of study drug;
- vital signs
- symptom-driven physical examination;
- 12-lead ECG between 20 and 24 hours of the initiation of the infusion;
- plasma PK samples between 20 and 24 hours of the initiation of the infusion;
- concomitant medication check;
- adverse event assessment.

6.3.4.11 Day 3, Cycle 2 and 3, Discharge from Hospital (Cohorts 4, 5, 6)

On Day 3 of Cycle 2 and 3 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, prior to discharge from the hospital, the procedures listed below will be performed:

- continuous infusion via central venous catheter of study drug;
- vital signs
- symptom-driven physical examination;

- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

6.3.4.12 Days 4 – 7, Cycles 2 and 3 (Cohorts 4, 5, 6)

On Days 4 through 7 of Cycle 2 and 3 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the continuous infusion via a central venous catheter will continue as an outpatient. If the patient experiences any problems with the infusion, the site should be contacted.

6.3.4.13 Day 8, Cycles 2 and 3, Outpatient Visit (Cohorts 4, 5, 6)

On Day 8 (+/- 2 days) of Cycle 2 and 3 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the patient will return for an outpatient visit, at which time the infusion will be discontinued and the following procedures will be performed:

- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

6.3.4.14 Day 15, Cycles 2 and 3, Outpatient Visit (Cohorts 4, 5, 6)

On Day 15 (+/- 2 days) of Cycle 2 and 3 of dose escalation Cohort 4 and subsequent dose escalation cohorts, the procedures listed below will be performed during an outpatient visit:

- clinical laboratory tests (hematology, clinical chemistries, coagulation);
- concomitant medication check;
- adverse event assessment.

6.3.4.15 Day 22, Cycles 2 and 3, Outpatient Visit (Cohorts 4, 5, 6)

On Day 22 (+/- 3 days) of Cycle 2 and 3 of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed during an outpatient visit, if the patient is not continuing to the next cycle (the procedures to be performed are the same as for an early termination/EOT visit:

- ECOG or Karnofsky/Lansky performance status;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;

- concomitant medication check;
- adverse event assessment.

6.3.4.16 Day 1, Cycle 4 and all Subsequent Dose Escalation Cycles, Outpatient Visit (Cohorts 4, 5, 6)

This visit will also be considered Day 22 of the previous cycle of dose escalation Cohort 4 and all subsequent dose escalation cohorts for those patients continuing treatment. The procedures listed below will be performed:

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment;
- initiation of continuous infusion via a central venous catheter; infusion will continue until Day 8.

6.3.4.17 Days 2 – 7, Cycle 4 and all Subsequent Dose Escalation Cycles (Cohorts 4, 5, 6)

On Days 2 through 7 of Cycle 4 and all subsequent cycles of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the continuous infusion via a central venous catheter will continue as an outpatient. If the patient experiences any problems with the infusion, the site should be contacted.

6.3.4.18 Day 8, Cycle 4 and all Subsequent Dose Escalation Cycles, Outpatient Visit (Cohorts 4, 5, 6)

On Day 8 (+/- 2 days) of Cycle 4 and all subsequent cycles of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the patient will return for an outpatient visit, at which time the infusion will be discontinued and the following procedures will be performed:

- vital signs;
- weight;
- symptom-driven physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- concomitant medication check;
- adverse event assessment.

6.3.4.19 Day 22, Cycle 4 and all Subsequent Dose Escalation Cycles, Outpatient Visit (Cohorts 4, 5, 6)

On Day 22 (+/- 3 days) of Cycle 4 and all subsequent cycles of dose escalation Cohort 4 and all subsequent dose escalation cohorts, the procedures listed below will be performed if the patient is not continuing to the next cycle (the procedures to be performed are the same as for an early termination/EOT visit):

- ECOG or Karnofsky/Lansky performance status;
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a CT scan or MRI will be done every 6 weeks during the study (end of Cycles 2, 4, 6, etc.). The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment.

6.3.5 All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

No hospitalization is required for the schedule escalation cohorts.

6.3.5.1 Day 1, All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 1 of Cycle 1 for all schedule escalation cohorts, the following procedures will be performed.

- ECOG or Karnofsky/Lansky performance status**;
- Weight**; height Cycle 1 only
- vital signs;
- 12-lead ECG: pre-dose**; For Cycle 1 only, post-dose between 1 and 3 hours after initiation of infusion;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis)**;
- for Cycle 1 only, urine pregnancy test (women of childbearing potential only)**;
- complete physical examination**;
- for Cycles 1 and 2 only, pharmacodynamic samples: pre-dose on Day 1; Samples in subsequent cycles may be collected if clinically indicated, for example, to evaluate biomarkers of response
- a single pharmacokinetic sample (see section 6.3.9);
- a single sample for pharmacogenomics to assess CYP polymorphisms
- the TK216 infusion via a central venous catheter will be initiated; infusion will continue for the number of days assigned in the cohort;
- the vincristine infusion will be administered prior to the start of the TK216 infusion starting in cycle 3+ of schedule escalation cohorts, if applicable;
- the vincristine infusion will be administered prior to the start of the TK216 infusion starting in cycle 1 of expansion cohort, if applicable;

- concomitant medication assessment;
- adverse event assessment.

** If the assessments for ECOG or Karnofsky/Lansky performance status, weight, physical examination, ECG, clinical laboratory tests and urine pregnancy test were performed within approximately 72 hours prior to starting a new infusion cycle, these assessments do not need to be repeated.

6.3.5.2 Day 4 (+/-1), All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 4 of Cycle 1, the procedures listed below will be performed.

- continuous infusion via a central venous catheter of study drug;
- For Cycle 1 only, pharmacodynamic sample;
- a single pharmacokinetic sample (see section 6.3.9) if the patient is in the schedule escalation segment;

6.3.5.3 Day 8 (+/-1), Cycle 1 (Cohorts 7, 8, 9)

On Day 8 of Cycle 1, the procedures listed below will be performed.

- continuous infusion via a central venous catheter of TK216 study drug;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a single pharmacokinetic sample (see section 6.3.9);
-
- vital signs;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment.

6.3.5.4 Day 11 (+/-1), All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 11, the procedures listed below will be performed, as applicable to patients continuing the infusion or patients ending the scheduled infusion.

- continuous infusion via a central venous catheter of study drug, if applicable;
- a single pharmacokinetic sample, if the scheduled infusion is continuing (see section 6.3.9), or;
- pharmacokinetic samples, in Cycle 1 only, if the scheduled infusion is ending (see section 6.3.9);
- for Cycle 1 only, pharmacodynamic sample, if ending the scheduled infusion;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis), if ending the scheduled infusion;
- vital signs, if ending the scheduled infusion;
- 12-lead ECG, if ending the scheduled infusion;
- symptom-driven physical examination;
- concomitant medication assessment, if ending the scheduled infusion;
- adverse event assessment, if ending the scheduled infusion;

- tumor/disease evaluation soon after stopping scheduled infusion and prior to beginning next infusion, if applicable.

6.3.5.5 Day 15 (+/-1), All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 15, the procedures listed below will be performed, as applicable to patients continuing the infusion or patients ending the scheduled infusion.

- continuous infusion via a central venous catheter of study drug, if applicable;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- a single pharmacokinetic sample, if the scheduled infusion is continuing or the patient is in the expansion cohort (see section 6.3.9), or;
- pharmacokinetic samples, in Cycle 1 only, if the scheduled infusion is ending (see section 6.3.9);
- for Cycle 1 only, pharmacodynamic sample, if ending the scheduled infusion;
- vital signs;
- 12-lead ECG, if ending the scheduled infusion;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment;
- tumor/disease evaluation soon after stopping scheduled infusion and prior to beginning next infusion, if applicable.

6.3.5.6 Day 18 (+/-1), All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 18, the procedures listed below will be performed, as applicable to patients continuing the infusion or patients ending the scheduled infusion.

- continuous infusion via a central venous catheter of study drug, if applicable;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis), if ending the scheduled infusion;
- for cycle 1 only, pharmacodynamic sample, if ending the scheduled infusion;
- a single pharmacokinetic sample, if the scheduled infusion is continuing (see section 6.3.9), or;
- pharmacokinetic samples, during Cycle 1 only, if the scheduled infusion is ending (see section 6.3.9);
- vital signs, if ending the scheduled infusion;
- 12-lead ECG, if ending the scheduled infusion;
- symptom-driven physical examination;
- concomitant medication assessment, if ending the scheduled infusion;
- adverse event assessment, if ending the scheduled infusion;
- tumor/disease evaluation soon after stopping scheduled infusion and prior to beginning next infusion, if applicable.

6.3.5.7 Day 22 (+/-1), All Schedule Escalation Cohorts and Cycles (Cohorts 7, 8, 9)

On Day 22 the procedures listed below will be performed, as applicable to patients continuing the infusion or patients ending the scheduled infusion.

- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- for Cycle 1 only, pharmacodynamic sample;
- pharmacokinetic sample(s) (see section 6.3.9);
- vital signs;
- 12-lead ECG;
- symptom-driven physical examination;
- concomitant medication assessment;
- adverse event assessment;
- tumor/disease evaluation soon after stopping scheduled infusion and prior to beginning next infusion, if applicable.

6.3.5.8 Rest Period, All Schedule Escalation Cohorts, Cycle 1 and Subsequent Cycles (if applicable), Seven Days after ending Infusion (Cohorts 7, 8, 9)

- Cycle 1 of every schedule escalation cohort will have a 14-day rest period. Subsequent cohorts will have a rest period of varying duration (see section 4.3). Seven days (+/- 2 days) after the TK216 infusion is stopped in Cycle 1 (or subsequent cycles, if applicable), the procedures listed below will be performed. The clinical laboratory testing may be done at a local laboratory, and the interview may be conducted by telephone. If a patient is starting a new cycle before this visit (see sections 4.3 and 6.3.8.1), the day 1 clinical laboratory tests should only be repeated if they were not done within 72 hours of starting the infusion.
- clinical laboratory tests (hematology, clinical chemistries, coagulation);
- concomitant medication assessment;
- adverse event assessment.
- This visit may also be considered Day 1 of Cycle 2, or subsequent cycles, if the patient meets the criteria to resume (see Sections 4.3 and 6.3.8.1) at the end of Cycle 1.

6.3.5.9 Pharmacokinetic Assessments, Schedule Escalation Cohorts, Cycle 1 (Cohorts 7, 8, 9)

During Cycle 1 only of the schedule escalation cohorts, PK assessments will be collected at the end of the cycle infusion,+/- 10 minutes, at the following time points:

- 1 hour prior to stopping the infusion;
- immediately prior to stopping the infusion;
- .5 hours (30 minutes) after stopping the infusion;
- 1 hour after stopping the infusion;
- 2 hours after stopping the infusion;
- 4 hours after stopping the infusion;

- and 8 hours after stopping the infusion.

For all cycles during the schedule escalation and expansion cohorts, a single PK assessment will be done at each of the following days, if the scheduled infusion is continuing: Days (+/- 1) 4, 8, 11, 15, 18, and 22.

6.3.6 Expansion Cohort (Cohort 10)

4.2.3.1 Expansion Cohort: On study Schedule (Cohort 10)

Patients enrolled in the Expansion Cohort will be assessed and treated the same as described for the Schedule Escalation Cohorts in Section 6.3.5, as applicable to the selected regimen.

4.2.3.2 Early Termination/End of Treatment Visit, All Cohorts, All Cycles (Cohort 10)

The following procedures will be performed at the time a patient is discontinued from the study, if prior to completing a cycle. This visit should be performed within 2 days of discontinuation. The End of Treatment Visit will be done if a patient completes a cycle but does not continue to the next cycle. This visit should be performed either at the last visit in the cycle (Day 22) or within 7 days after the completion of the cycle. During the schedule escalation segment, this visit should be done within 2 days if the patient discontinues before the end of cycle 1 or if the patient discontinues during an infusion of any subsequent cycle, or within 7 days if the patient discontinues after the end of an infusion but prior to starting the next cycle.

- ECOG or Karnofsky/Lansky performance status
- vital signs;
- weight;
- complete physical examination;
- 12-lead ECG;
- clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- urine pregnancy test: women of child-bearing potential only;
- if a CT scan or MRI is due, it should be done at this visit. The same method of assessing disease must be used throughout the study;
- concomitant medication check;
- adverse event assessment.

4.2.4 Infusion Period, All Cycles (Cohort 11)

As of Protocol version 7.0, the regimen given is to be TK216 175 mg/m^2 continuous infusion IV for 28 days. The patient will be assessed for toxicities during the 28-day cycles and may take treatment rests, if needed. Treatment rests unrelated to toxicities are not encouraged but may occur at the PI's discretion after discussion with the Sponsor.

4.2.4.1 Day 1 (Cohort 11)

- ECOG or Karnofsky/Lansky performance status**;
- Vital signs and weight**;
- Symptom driven physical examination**;

- Clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis)**;
  - Woman of childbearing potential should have a pregnancy test.
- **Cycles 1 and 2 only**: Collect single pre-infusion PK blood sample.
- **Cycles 1 and 2 only:** Collect pre-infusion Pharmacodynamic samples: Biomarkers & PBMC.
- Collect Pharmacogenomic sample: Cycle 1 only
- TK216 infusion via a central venous catheter will be initiated; infusion will continue for 28 days
- Adverse event & concomitant medication assessment.

Additional cycles of therapy may be administered provided that the patient meets the following criteria on Day 1 of each subsequent cycle:

- ANC $\geq$ 1,000/mm^3
- Hemoglobin $\geq$ 8.0 gm/dL (Blood transfusions are permitted)
- Platelets $\geq$ 50,000/mm^3
- Non-hematologic toxicity recovered to $\leq$ Grade 1 (or tolerable Grade 2)

** If the assessments for ECOG performance status, weight, physical examination, clinical laboratory tests and urine pregnancy test were performed within approximately 72 hours prior to starting a new infusion cycle, these assessments do not need to be repeated. Periodic height check, as determined by the Investigator may be included in the procedures of any cycle.

4.2.4.2 Day 8 (±1) (Cohort 11)

The procedures listed below will be performed:

- Bag change for TK216
- Vital signs
- Symptom-driven physical examination
- Clinical laboratory tests (hematology, clinical chemistries)
- **Cycles 1 and 2**: Collect single blood samples from a peripheral vein for PK analysis
- Adverse events and concomitant medication assessment

4.2.4.3 Day 15 (±1), All Cycles (Cohort 11)

On Day 15, the procedures listed below will be performed:

- Vital signs: temperature, weight, blood pressure, heart rate and respiratory rate;
- Symptom-driven physical examination
- Clinical laboratory tests (hematology, clinical chemistries)
- **Cycles 1 and 2**: Collect PD specimens (biomarker & PBMC) at Day 15 bag change.
- **Cycle 1 only**: Collect a single PK at the Day 15 bag change.
- Adverse Event and concomitant medication assessment

4.2.4.4 Day 21 (±1) (Cohort 11)

The procedures listed below will be performed:

- Bag change for TK216
- Vital signs
- Symptom-driven physical examination
- Clinical laboratory tests (hematology, clinical chemistries)
- **Cycles 1 and 2**: Collect single PK only
- Adverse events and concomitant medication assessment

4.2.4.5 Day 28 (±1), All Cycles (End of Infusion) (Cohort 11)

On Day 28, the procedures listed below will be performed:

- Vital signs: temperature, weight, blood pressure, heart rate and respiratory rate;
- Symptom-driven physical examination
- Clinical laboratory tests (hematology, clinical chemistries)
- **Cycles 1 and 2**: Collect PD specimens (biomarker & PBMC) at the end of infusion.
- **Cycle 1 and 2**: Collect a single PK at the end of infusion.
- Adverse Event and concomitant medication assessment

If Day 28 and Day 1 of the next cycle occur on the same day, please add the assessments below:

- TK216 infusion via a central venous catheter will be initiated (preferred). TK216 infusion via a peripheral line is allowed; infusion will continue for 28 days
- ECOG or Karnofsky/Lansky performance status**;
- Additional clinical laboratory tests (coagulation, urinalysis)**;
- For woman of childbearing potential, a urine pregnancy test

4.2.4.6 Rest Period, All Cycles, if needed (Cohort 11)

There is no mandatory or pre-specified rest period. If TK216 administration is paused, every seven days (±1 day) after the TK216 infusion is stopped, the procedures listed below should be performed.

- Clinical laboratory tests (hematology);
- AE and concomitant medication assessment;
- The clinical laboratory testing may be done at a local laboratory, and the interview may be conducted by telephone.
- If the rest period is greater than 7 days (+1); an optional pre-dose PK sample may be requested.

When the patient re-starts, Day 1 assessments should be completed and continued eligibility should be assessed.

4.2.5 Early Termination/End of Treatment Visit, Cohort 11

For all patients in Cohort 11, an End of Treatment Visit will be conducted within 7 days after treatment discontinuation, and a Safety Follow-Up visit will be conducted approximately 28 days following the last day of TK216 infusion. The following procedures will be performed at the End of Treatment Visit:

- ECOG performance status
- Vital signs;
- Weight;
- For pediatric patients, height
- Complete physical examination;
- 12-lead ECG;
- Clinical laboratory tests (hematology, clinical chemistries, coagulation, urinalysis);
- Collect PD specimens (biomarker & PBMC);
- Urine pregnancy test: women of child-bearing potential only;
- If a CT scan or MRI has not been completed within 6 weeks, it should be done at this visit. The same method of assessing disease must be used throughout the study;
- Concomitant medication check;
- Adverse event assessment.

4.2.6 TK216 Therapy Following Disease Progression (Part 4)

Patients may rapidly develop tumor-related symptoms even in the face of stable or minimally progressive disease. Therefore, as of Protocol version 7.0, palliative local radiation therapy may be allowed if the following criteria are met:

1. Treatment is required for a symptomatic, non-target lesion(s) (example: pain due to compression of nerve in a closed space).
2. Planned radiation therapy is focal and the field does not involve the treatment of target lesion(s) directly or indirectly by an inability to fully shield adjacent target lesions from any radiation effect.
3. There is a reasonable possibility that the patient will benefit from additional systemic therapy with TK216.
4. Approval for this treatment has been granted by Oncternal.

4.2.7 Post-treatment follow-up

A Safety Follow-Up telephone call will be conducted approximately 28 days following the last day of TK216 infusion, if the infusion is terminated early; or approximately 28 days after the completion of the last cycle which the patient completes. Additionally, each patient will be contacted by telephone or email approximately every 3 months following study discontinuation until death, loss to follow-up, or withdrawal of consent in order to assess disease progression and survival status. A review of the medical record may also be performed if applicable to assess disease progression and survival status.

4.2.8 Patient withdrawal criteria and procedures

A patient will be permanently discontinued from study treatment if the patient develops a toxicity or concurrent illness that, in the investigator's judgment, precludes further treatment with the study drug.

A patient may be permanently discontinued from the study for any of the following reasons:

- Intolerable adverse event that does not improve with dose adjustments

- significant abnormal clinical laboratory values that are possibly, probably or certainly attributed to study drug. This includes Grade 3 or greater laboratory abnormalities that do not improve to ≤ Grade 1 or baseline severity within 4 weeks of the last dose received.
- pregnancy
- non-compliance (not completing all required assessments at required times, missing scheduled visits, high frequency of missed doses, refusing to complete duration of dosing) or protocol violation
- patient withdraws consent and refuses to participate in the study
- investigator's or sponsor's decision that withdrawal is in the patient's best interest
- termination of the study by the sponsor (see Section 10.7)
- RECIST, version 1.1-defined disease progression is observed

If for any reason, a patient is discontinued from study before the patient completes 1 cycle of treatment; all assessments outlined in the Flow Chart of Study Assessments in the Study Synopsis and listed in Section 6.3.10 are to be completed. The reason(s) for a patient's discontinuation of treatment or withdrawal from the study will be clearly documented in the source documents and on the CRF. Any patient who completes Cycle 1 will be considered to have completed the study.

7 Study Drugs

7.3 Pharmaceutical information

7.3.4 TK216

7.3.4.1 Description

TK216 is a small molecule inhibitor of the EWS-FLI1 fusion protein, and other related *ets*-family member proteins that are implicated in the pathogenesis of Ewing sarcoma.

7.3.5 Vincristine

7.3.5.1 Description

Vincristine is an alkaloid isolated from *Vinca rosea* Linn (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. The p450 cytochrome involved with vincristine metabolism is CYP3A4. There is poor CSF penetration.

Common toxicities include alopecia, constipation and loss of deep tendon reflexes. Occasional toxicities include jaw pain, headache, weakness, abdominal pain, mild myelosuppression, peripheral paresthesias, wrist drop, foot drop and abnormal gait.

Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.

A complete description is found in the prescribing information (Pfizer, 2013)

7.3.5.2 Dosage form, strength and route of administration

Vincristine Sulfate Injection, USP is a sterile, preservative–free, single use only solution available for intravenous use in 2 mL (1 mg and 2 mg) vials. Each mL contains 1 mg Vincristine Sulfate, USP, 100 mg mannitol and Water for Injection, USP. Q.S. Sulfuric acid or sodium hydroxide have been added for pH control. The pH of Vincristine Sulfate Injection, USP ranges from 4.0 to 5.0. At the time of manufacture, the air in the containers is replaced by nitrogen (Pfizer, 2013).

7.3.5.3 Supply and stability information

Vincristine will be obtained from the site pharmacy.

7.3.5.4 Administration guidelines

The intrathecal administration of vincristine sulfate injection usually results in death. To reduce the potential for fatal medication errors due to incorrect route of administration, Vincristine sulphate injection should be diluted in a flexible plastic container and prominently labeled as indicated for intravenous use only. Syringes containing this product must be labelled, using the auxiliary sticker provided, to state "FOR INTRAVENOUS USE ONLY – FATAL IF GIVEN BY OTHER ROUTES."

Vincristine Sulfate Injection, USP when diluted with 0.9% Sodium Chloride Injection in concentrations from 0.0015 mg/mL to 0.08 mg/mL is stable for up to 24 hours when protected from light or 8 hours under normal light at 25°C (Pfizer, 2013).

Injection of vincristine sulfate should be accomplished as per institutional policy. Vincristine sulfate must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken to ensure that the needle or catheter is securely within the vein to avoid extravasation during administration.

7.4 Procedures for monitoring patient compliance

Calculations of the dose administered will be documented in the source documents at the site and confirmed during monitoring visits by the clinical monitor. In addition, the dose administered will be documented in the electronic CRFs. Any interruptions or discontinuations of the infusion will be documented in both the source documents and the electronic CRFs.

7.5 Accountability

Master drug accountability forms will be used to maintain accurate records of receipt, distribution, and return of all drug supplies shipped to the site from the sponsor's representative. A drug accountability form will be maintained at each location where drug is stored for patient administration, i.e., main pharmacy, satellite pharmacy, physician's office or other dispensing areas. Individual patient drug accountability forms will be used to maintain accurate records of drug dispensed, returned and consumed by each patient.

When the clinical site receives supplies of TK216 and the custom diluent from the sponsor, or its representative, a visual inspection of the drug will be conducted and the condition of supplies will be recorded. Any damaged or missing supplies are to be reported on the accountability forms. The date, investigational drug lot numbers and/or vial numbers, and the amount of drug received will be documented on a master drug accountability form. During the course of the study, the initials and number of each patient to whom drug is dispensed, the date, quantity of drug dispensed, all transfers, returns, and disposal/destruction of drug are to be documented on the accountability forms.

Drug supplies will be stored in a secure, limited-access storage area under the recommended storage conditions. Regular periodic inventory of the investigational drug supply will be performed.

8 Ethical Considerations

8.3 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

The IRB/IEC is responsible for the review and approval of relevant study documentation to assure the protection of the rights and welfare of human patients. Relevant documents requiring review and approval by an IRB/IEC include but are not limited to; the study protocol and amendments, the patient written informed consent form and consent form updates, patient recruitment documentation (e.g., advertisements), written information provided to patients, Investigator's Brochure (IB) and any revisions or Addenda, available safety information, patient payment/compensation, investigator's current curriculum vitae (or other qualification documentation), and any other documents that the IRB/IEC may need to fulfill its responsibilities. The IRB/IEC is required to operate in compliance with current regulations of the local regulatory authorities, the International Conference on Harmonisation (ICH) guidelines and current Good Clinical Practice (cGCP) guidelines. Written approval from the IEC must be obtained before the study can be started (consent of the first patient) or before the investigational study drug is administered to a patient.

Changes to the study requiring an amendment to the protocol or changes to the informed consent form; must be approved in writing by the IRB/IEC. IRB/IEC approval must be obtained prior to the implementation of such changes.

The sponsor or representative will report promptly to the investigator any new information that may indicate an adverse effect on the safety of the patients or the conduct of the study. The investigator is responsible for informing the IRB/IEC of any new safety information (e.g., safety report presented as an IB Addendum), and for reporting the progress of the study. At the end of the study, defined as the last visit of the last patient, the investigator will provide a final report to the IRB/IEC (if required).

8.4 Ethical Conduct of the Study

The clinical study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki (in its revised edition, Tokyo 2004), and that are consistent with the guidelines for current Good Clinical Practice (cGCP) and applicable regulatory requirements.

8.5 Informed Consent

The principal investigator is responsible for ensuring that no patient undergoes any study-related examination or activity before that patient has given written informed consent to participate in the study. The patient must give the written consent only after detailed information about the study has been provided. The verbal explanation will cover all the elements specified in the written information provided to the patient.

An investigator or representative will inform the patient of the aims, methods and potential hazards of the study. The patient must be given every opportunity to clarify any points he/she does not understand and, if necessary, ask for more information. At the end of the informed consent discussion, the patient will be given time to consider the study information and to freely decide his/her participation. If the patient agrees to participate in the study, the informed consent document must be

signed by both the patient and by the person who conducted the informed consent discussion. A copy of the signed consent will be given to the patient and the original will be archived in the investigator site file.

It should be emphasized to the patient that he/she is free to withdraw from the study at any time. Patients who refuse to give or who withdraw written informed consent should not be included or continue in the study.

8.6 Confidentiality of records

The investigator must assure that the patients' anonymity will be maintained. Patients should not be identified by name on any documents submitted to the sponsor or during verbal communications. Patients will be identified with their initials and a protocol-assigned patient number.

The investigator will maintain all signed informed consent forms in strict confidence, and will maintain a separate log of patients' initials and hospital/clinic accession number.

All laboratory specimens and evaluation forms will be identified using only a coded number, patient number, patient initials and/or date of birth in order to maintain confidentiality. All records will be kept in a secured area in the clinical research unit. Computer entry and networking programs will be performed using coded numbers.

The patient will be informed that all clinical information is confidential and must consent to direct access to his/her original medical records and study data for study-related sponsor monitoring, audit, IRB/IEC review and regulatory inspection.

8.7 Discontinuation of study

The Sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons. Such a termination must be implemented by the investigator, if instructed to do so by the Sponsor, in a time frame that is compatible with the wellbeing of study patients.

8.8 Compliance statement

The study will be conducted in accordance with standards that meet regulations relating to current Good Clinical Practice. These standards respect the following guidelines: current Good Clinical Practice; Consolidated Guideline (International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996); United States (US) Code of Federal Regulations (CFR) dealing with clinical studies (21 CFR parts 50, 54, 56, 312, and 314), and the Declaration of Helsinki.

8.9 Quality control

The sponsor, or its representative, will be responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that the study is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, cGCP and the applicable regulatory requirements.

9 Statistical Considerations

The clinical outcomes, laboratory, PK, and other safety data from all segments of the study will be analyzed descriptively. In addition to determining DLT and RP2D, results will be analyzed to

determine if a sufficient response signal and safety profile justifies further study. Descriptive statistical summaries for demographic and patient baseline characteristics will be produced, as well as statistical summaries of safety, efficacy and pharmacokinetic/pharmacodynamic results, where categories for statistical summaries will consist of the dose level and duration initially assigned for Parts 1 and 2 dose and schedule escalation, and the initial dose and duration in the Expansion Cohort (RP2D). In addition, exploratory analyses of both toxicity, response (ORR), Stable Disease (SD) and pharmacodynamic data will be performed for both the assigned and actual daily dose and duration of infusions, the actual number of days of treatment, and for cumulative exposure to study drug as expressed by the product sum of dose over time (area under the dose-time curve).

Disease Control Rate (DCR) defined as the proportion of patients with a confirmed Complete Response (CR), Partial Response (PR) or Stable Disease (SD) per RECIST v1.1 Duration of Response (DOR) defined from the first date a response is identified (either CR or PR) until the date of objective tumor progression per RECIST v1.1 Progression-Free Survival (PFS) defined as time from first dose of TK216 to objective tumor progression per RECIST v1.1 or death due to any cause, whichever occurs first.

9.3 Sample size calculation

The sample size for the Part 1 dose-escalation portion and Part 2 schedule-escalation portion of the study will be determined by the number of escalation steps and the required sample within cohort (3 or 6 patients). The sample size for the expansion phase will be chosen to assist in determining whether sufficient preliminary evidence of efficacy is seen to justify further study. If at least 4 of the total of 18 patients enrolled at the RP2D achieve a response or have stable disease for at least 3 months, the statistical basis of evidence will be satisfied. This schema will use a promising ORR/SD of 30% and an uninteresting ORR/SD of 10%, with a one-sided α-level of 0.10 and power of 80%. Note that durable stable disease of 30% is also a promising outcome.

The following table gives the probability of dose escalation as a function of the true DLT probability:

True Probability of DLT	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Probability of Escalation	0.97	0.91	0.71	0.49	0.31	0.17	0.08	0.03

For Part 4 (Cohort 11), the sample size was chosen to support the primary objective of assessing clinical response in patients with relapsed or refractory ES (including patients with ESFT) to assist in determining whether sufficient preliminary evidence of efficacy is seen to justify further study.

With a hypothesized ORR of 25% and an unacceptable (null) ORR of 5%, a sample size of 21 patients achieves 80% power assuming a one-sided exact test and a one-sided α-level of 0.025. If at least 4 of the total 21 evaluable patients enrolled in the cohort achieve a response, the statistical basis of evidence will be satisfied.

9.4 Safety

All patients who receive any amount of study drug will be included in the safety analyses. All adverse events will be mapped to preferred terms and system organ classes using the MedDRA dictionary. Patient incidence of adverse events will be displayed by dose group and by system organ class. Adverse events will also be summarized by severity and relationship to study drug. Patient incidence of serious adverse events will also be summarized. The type and number of DLTs will be separately presented by dose group, as appropriate.

Laboratory parameters will be summarized using descriptive statistics at baseline and at each post-baseline time point. Changes from baseline will also be summarized.

9.5 Pharmacokinetics

Pharmacokinetic parameter values will be summarized by descriptive statistics at each dose level. Analysis of variance (ANOVA) for the enantiomer of TK216 only will be used to compare the dose normalized PK parameters (dnC_{max}, $dnAUC_{inf}$, $dnAUC_t$) and $t_{1/2}$ across dose levels. A non-parametric test for the enantiomer of TK216 only will be used to compare T_{max} across dose levels.

9.6 Pharmacodynamics

Pharmacodynamic variables will be summarized by dose group and time point. Correlations between pharmacodynamic variables and efficacy variables may also be performed.

9.7 Efficacy

A modified intent to treat (mITT) approach will be used for efficacy analysis, in which the mITT population will consist of all patients who receive at least a partial dose of study therapy (i.e., any portion of the continuous infusion). Tumor response rates will be summarized by dose group and for all patients who receive the RP2D, including those from the dose and schedule escalation and expansion phases. Responses will be classified as CR, PR, SD or PD according to RECISTv.1.1 criteria. Summaries will be based on the best response recorded up until disease progression. Patients who discontinue prior to the first protocol-scheduled response assessment post first infusion will be considered as non-responders in the primary efficacy analysis. Objective tumor response (CR or PR) will also be summarized, as will PFS, OS, DCR and duration of response and DCR. Time to event data will be summarized by Kaplan-Meier methods, including 25^{th}, 50^{th} (median) and 75^{th} percentiles with point estimates and two-sided 95% confidence intervals, as well as number and percent of censored observations.

10 Statistical analysis

The statistical analyses will be reported using summary tables, figures, and data listings. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums. Categorical variables will be summarized by counts and by percentage of patients in corresponding categories. All raw data obtained from the case report forms as well as any derived data will be included in data listings. All analyses will be based on the Safety Population, which will include all patients who receive any amount of study drug.

10.3 Safety analysis

All patients who receive any amount of study drug will be included in the safety analyses. All adverse events will be mapped to preferred terms and system organ classes using the MedDRA dictionary. Patient incidence of adverse events will be displayed by dose group and by system organ class. Adverse events will also be summarized by severity and relationship to study drug. Patient incidence of serious adverse events will also be summarized.

Laboratory parameters will be summarized using descriptive statistics at baseline and at each post-baseline time point. Changes from baseline will also be summarized. In addition, shift tables (i.e.,

CTCAE grade at baseline versus CTCAE grade at follow-up) will be provided to assess changes in laboratory values from baseline to follow-up.

10.4 Pharmacokinetics

Pharmacokinetic variables will be summarized by dose group and time point.

10.5 Pharmacodynamics

Pharmacodynamic variables will be summarized by dose group and time point.

10.6 Efficacy

Tumor response rates will be summarized by dose group and for all dose groups combined. Responses will be classified as CR, PR, SD or PD according to RECIST criteria. Summaries will be based on the best response recorded up until disease progression. Objective tumor response (CR or PR) will also be summarized.

11 References

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