Phase II study on Trabectedin in advanced rearranged Mesenchymal chondrosarcoma (MCS)

EudraCT:	2019-003733-41
Study code:	ISG-MSC
Clinicaltrial.gov NCT	NCT04305548
Study drug:	Trabectedin
Study Number/Version/Date:	Vers 3.0 09 January 2023
Sponsor's Name and Address:	I.S.G. Italian Sarcoma Group ETS Via Farini 31 – 40124, Bologna – Italy
Sponsor's Phone:	+39 333 5359192
Sponsor's email:	clinicaltrials@italiansarcomagroup.org
Coordinating Center:	Fondazione IRCCS Istituto Nazionale Tumori di Milano Via Venezian 1 Milano, Italy
Principal Coordinating investigators:	Silvia Stacchiotti (Principal) Giacomo Giulio Baldi (Co-coordinator) Department of Medical Oncology Nuovo Ospedale "Santo Stefano" Prato, Italy
Methodology:	Non randomized, single arm, phase II study
Type:	No Profit
Founding:	Pharma Mar

PROTOCOL SIGNATURES PAGE

Sponsor: I.S.G.Italian Sarcoma Group ETS

Study Title: Phase II study on trabectedin in adults and young adults HEY1-NOCA2 positive skeletal and extra-skeletal mesenchymal chondrosarcoma (MCS)

Sponsor Protocol Code: ISG-MSC

EudraCT Number: 2019-003733-41

Protocol Version: 3.0

Protocol Date: 09 January 2023

I have read the contents of this protocol and agree to abide by all provisions set for therein.
I agree to personally conduct or supervise this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with the Declaration of Helsinki and with the International Conference on Harmonisation guidelines on Good Clinical Practice (ICH E6), and applicable Italian regulatory requirements.
I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.
I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. I agree to make available to sponsor personnel, their representatives and relevant regulatory authorities, my subject's study records in order to verify the data that I have entered into the case report forms.

Sponsor Signature:
Silvia Stacchiotti, MD

Investigator Coordinator signature:
Silvia Stacchiotti, MD

Site…….

Principal Investigator……

Signature……...

Document	Version date	Summary of Changes and Rationale
1.0 (final)	16 Sep 2019	Not applicable (N/A)
2.0	12 Feb 2021	• Section 3.3 describing Risks and benefits added • Section 4.1 Inclusion Criteria: Criteria nr 14 erroneously entered as inclusion. This criteria have been moved to Exclusion Criteria section with the nr 17 • Section 4.1 Inclusion Criteria nr 10 updated to align the contraception indication according to the potential fetotoxic and genotoxic effects of Yondelis in line with recommendation in the Yondelis SmPC: effective contraception to be used during treatment and thereafter for 3 months in WOBCP and for 5 months in men in fertile age. • Section 7.3 Other Efficacy Variables : Clinical benefit definition aligned to those provided in Section 2 Objectives
3.0	09 Jan 2023	• Page 1: - Change of Logo - Sponsor's name, address and telephone number updated • Protocol signature page: - Sponsor's name and signature updated • Section 8.6: Sponsor's name updated • Section 8.7: brand name Yondelis® deleted

SUMMARY

1. Introduction

1.1 Disease background: mesenchymal chondrosarcoma

Mesenchymal chondrosarcoma (MCS) is an ultra-rare sarcoma that accounts for <3% of all primary chondrosarcomas [1,2], first described by Lichtenstein and Bernstein in 1959 [3]. Although occurring at any age, the peak incidence is in the second and the third decades of life, being males and females affected equally. MCS shows a widespread distribution, but the most common sites of origin are the craniofacial bones (especially the jawbones), the ribs, the ileum and the vertebrae. Additionally, one fifth to one third of the cases primarily affects the somatic soft tissues with the meninges being one of the most common sites of extra-skeletal involvement. Rarely it can occur in viscera.

A biphasic pattern of undifferentiated small blue round cells and islands of hyaline cartilage characterize MCS from the morphological point of view. Positivity for SOX9 with negative staining for FLI-1 in the small cell component may be helpful in distinguishing mesenchymal chondrosarcoma from Ewing sarcoma [4,5]. *HEY1-NCOA2* fusion has been identified in MCS, representing an intrachromosomal rearrangement of chromosome 8q that results in an in-frame fusion of *HEY1* exon 4 to *NCOA2* exon 13 at the mRNA level. It is an important diagnostic marker, since, so far, it has been reported only in MCS, both bone and soft tissue. [6].

MCS is an aggressive neoplasm and shows a strong tendency towards local and metastatic recurrences. The clinical course is frequently protracted and relentless, yet the outcome for these patients ultimately appears to be poor with reported 10-year survival rate in the range of 27% to 67% [1,7-11]. Distant metastases are observed even after a delay of >20 years and a long-term follow-up are mandatory. Median OS in metastatic patients in the series available is roughly 3 years [10,11]; the outcome of patients with metastases at presentations remains poor and most will ultimately died from the disease.
In primary disease, surgery is the mainstay of treatment usually combined to concomitant radiotherapy and anthracycline-based multidrug chemotherapy [2,8-11]; in case of locally advanced and/or metastatic disease therapeutic options are limited, with responses reported to chemotherapy with doxorubicin, cisplatin or ifosfamide [5,8,9,11]. Available data, although limited, suggests that MCS is more sensitive to chemotherapy than the other chondrosarcoma subtypes; on this basis a Ewing-like chemotherapy regimens is suggested [13].

Recently the outcome data of 112 patients with advanced chondrosarcoma were collected in four sarcoma reference centres [12]. All the 25 patients diagnosed with mesenchymal chondrosarcoma included in this series were treated with chemotherapy combination regimens, four of them achieving a partial response with doxorubicin-based regimens. The mean PFS for all chemotherapy regimens in MCS was 6.7 months with a mean PFS of 3.7 months for patients treated with a combination of doxorubicin and ifosfamide and a mean PFS of 7.7 months for patients treated with a combination of doxorubicin and cisplatin.

Considering the ultimately very poor prognosis and few options available, new agents are needed for patients with advanced disease.

1.2 Study rationale: trabectedin in MCS

Trabectedin is approved by the European Medicine Agency (EMA) for treatment of advanced soft tissue sarcoma from the second line, after failure of front-line chemotherapy containing anthracycline [14,15]. Of note, trabectedin is instead not approved for treatment of primary bone sarcoma, among which bone MCS, nor in pediatric patients.
Trabectedin is reported to be also particularly effective against myxoid liposarcoma and this has been related to its capacity to interfere with the transcriptional activity of the oncogenic fusion

proteins of translocation-related sarcoma (TRS) [19,20]. Available clinical data suggested a peculiar activity of trabectedin against TRS [16-18]; retrospective analysis of eight phase II clinical studies reported encouraging results of trabectedin in 81 TRS patients with disease control rate of 59%, median PFS of 4.1 months and median overall survival of 17.4 months [16]. These data support trabectedin use also in MCS, since they belong to the family of TRS.

The only clinical data available on trabectedin in MCS come from the sub-analysis of extra-skeletal myxoid chondrosarcoma and extra-skeletal MCS patients included in a Japanese randomized phase II trial comparing trabectedin versus best-supportive care and suggest a possible anti- tumour effect of this drug in the disease [17,21]. One of the three patients with MCS treated with trabectedin within this study achieved a partial response and was still receiving trabectedin at the final data cut-off (with a PFS of 22.2 months); the other two patients showed a prolonged stable disease.

Additional data on a larger number of patients are needed to define which can be the role of trabectedin in MCS.

On this basis, we decided to design an exploratory Italian phase II prospective multicentric clinical study on trabectedin in patients aged ≥16 years with *HEY1-NOCA2* positive skeletal or extra- skeletal advanced mesenchymal chondrosarcoma (MCS) from II line, aimed at evaluating the activity of trabectedin in 2nd to 4th line.

2. Objectives

2.1 Primary

The primary objective of this study is to explore the activity of trabectedin from 2nd to 4th line, in patients aged ≥ 16 years with advanced *HEY1-NOCA2* positive MCS pre-treated with anthracycline-based chemotherapy. Therefore, with reference to a study population of patients with progressive by RECIST v1.1, locally advanced or metastatic, *HEY1-NCOA2* positive MCS pre- treated with one, two or three lines of medical treatment, the **primary end-point** of the study will be to assess:

Overall tumour Response Rate, according to RECIST v 1.1 [22]

2.2 Secondary

The activity of trabectedin in advanced MCS will be also evaluated according to Choi criteria [23]. In addition, trabectedin efficacy will be investigated by means of progression-free survival (PFS), clinical benefit rate (RECIST CR + PR + SD > 6 months), overall survival (OS) and duration of response.
The toxicity profile of trabectedin will be also evaluated.

Therefore, secondary end-points of this study will be to assess:

1. Choi Response Rate
2. Overall Survival (OS)
3. Progression Free Survival (PFS)
4. Clinical Benefit Rate
5. Duration of response
6. Safety

2.3 Exploratory and Translational Objectives

As additional exploratory analyses, all the primary/secondary efficacy endpoints will be evaluated in the two subgroups obtained by separating skeletal MCS from extra-skeletal MCS; in addition, tumour transcriptional pattern and immunological profile of recruited patients will be evaluated and correlated with the response.

Molecular analyses will be carried out at SOC Oncogenetica and Oncogenomica of Centro di Riferimento Oncologico di Aviano to dissect the molecular bases of trabectedin activity in MCS.

Specific aims are:

- Exploration of transcriptomic and genomic profile of responsive versus unresponsive tumors. Genomic, mRNA, miRNA expression pattern of a sizable number of responsive and unresponsive tumors will be analyzed and compared.
- Evaluation of efficacy of liquid biopsy (measure of *HEY1-NCOA2* fusion in cell free DNA/RNA) in anticipating response/progression under treatment.
- Validation of *HEY1-NCOA2* transcriptional targets involved in MCS response to trabectedin by in vitro experiments.

A specific optional informed consent will be provided in order to collect the blood samples as described on Sect 7.5

3. Overall study design

This is an Italian, multicentre, prospective, uncontrolled, open-label, investigator-initiated, phase II clinical study to explore the activity of trabectedin in a population of patients aged ≥16 years with progressive by RECIST v1.1, advanced (locally advanced or metastatic), *HEY1-NCOA2* positive MCS, pre-treated with anthracycline-based chemotherapy.

The primary end-point of the study is the overall response rate by RECIST v1.1. Secondary end-points are PFS, OS, clinical benefit rate, response rate by Choi criteria, duration of response and safety of trabectedin.
Patients with advanced, histological and molecularly (presence of the *HEY1-NCOA2* fusion) centrally confirmed diagnosis of MCS, with an evidence of RECIST progression within the 6 months prior to starting the study treatment and pre-treated with anthracycline-based chemotherapy may enter the study. Study treatments will be administered until the evidence of progression or toxicity.

Subgroup analyses to correlate the activity of trabectedin with the site of origin of the tumour (i.e. skeletal versus extra-skeletal MCS), tumour gene expression and immunological profile will be also performed.

Subjects with evidence of progressive disease, already pre-treated with anthracycline-based chemotherapy receiving trabectedin in 2nd-line to 4th-line are eligible for inclusion in the study.
A maximum of 16 patients evaluable for the primary end-point will be enrolled in about 24-36 months.
To reach the target of 16 evaluable patients, a total number of 20 patients will be included in the study.
Patients will be evaluated for the primary end-point if they have completed at least one cycle of trabectedin and have at least one post-baseline radiologic disease assessment. Patients with early PD or who died due to PD before the first scheduled tumour assessment will also be included in the analysis.

3.1 Statistical analysis plan

A total of 16 patients will be required to detect a response rate of 30% or greater and to exclude a response rate of 5% or less, type-I (alfa) and type-II (beta) error levels being fixed at 10%. In case of positive results, a phase II study on a larger series of patients will be considered.
The response rate of 5% was taken as a reference, representing the expected response rate with standard second line chemotherapy in extraskeletal mesenchymal chondrosarcoma.

3.2 Feasibility

The Institutions involved in this study are referral centres for treatment of soft tissue sarcoma in Italy and are able to enrol 5-10 progressive metastatic/locally advanced MCS per year. Referral will be supported also by the Italian Network on Rare Tumours as well as by Italian Sarcoma Group centres, the national effort devoted to allowing distant patient sharing, patient rational referral across institutions, widespread compliance with clinical practice guidelines in the area of rare tumours, including sarcomas.

3.3 Experimental drug administration and study procedures potential risks and benefits

Risks of experimental drug administration

Toxicity associated with trabectedin exposure is commonly low grade and not clinically significant. Trabectedin must be withheld for drug-related Grade 3 toxicities, but may be resumed upon recovery to Grade ≤1; trabectedin will be permanently discontinued for any drug-related Grade 4 AE.

Risks of study procedures

Risks of blood sampling: Blood sampling is performed in the study which is also done in normal routine clinical practice for monitoring patients who receive anti-tumoral therapy. For blood sampling there is always a very minor risk of infection, bruising, or syncope during a blood draw.There is also the discomfort of having one's blood drawn.

Risks of Computerized Tomography and Nuclear Magnetic Resonance: Tumor evaluation with CT and/or MRI is performed in the study, but the amount of radiation used is considered minimal; therefore, the risk for radiation exposure is low. Since the study does not allow the inclusion of pregnant women and pregnancy test is performed at day1 of any cycle the risk associate to TC and MRI, for the pregnancy is not foresee Additional examinations are not invasive, are part of routine clinical practice in cancer treatment and do not put the subject at additional risk compared to the clinical practice.

During the study the patient will be regularly monitored for safety/toxicity so to allow to early detect any clinically significant event that could occur.

Benefits:

Mesenchymal chondrosarcoma is an aggressive tumor characterized by a very poor prognosis in the advanced phase; few therapeutic options are available in clinical practice and new agents are needed for patients with advanced disease (see Section 1.2: rationale for trabectedin use).
Tumor response and tumor growth arrest can result in symptomatic release and avoid /delay the onset of new symptoms. Tumor response could translate also in an improvement in patient outcome.

4. Study population

4.1 Inclusion Criteria

Patients who meet all the following criteria may participate in the study:

1. Age ≥ 16 years old
2. Histological centrally confirmed diagnosis of skeletal or extra-skeletal MCS with the documented presence of *HEY1-NCOA2* fusion (a paraffin embedded tumour block is required for centralized review)
3. Locally advanced disease (i.e. surgical resection of local disease unfeasible radically or unaccepted by the patient or amenable to become less demolitive or feasible or easier after cytoreduction) and/or metastatic disease
4. Measurable or evaluable disease with RECIST v1.1
5. Evidence of progression by RECIST v1.1 during the 6 months before study entry
6. Patients must be pre-treated with at least one prior chemotherapy treatment containing anthracyclines for the advanced phase of disease and with a maximum of 3 lines
7. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2
8. Adequate bone marrow function, defined as the following:

WBC	≥ 3.0 x 10^9/L
ANC	≥1.5 x 10^9/L
Platelets	≥ 100 x 10^9/L
Hb	≥ 9 g/dL

Blood transfusions to reach the baseline requested Hb level are not allowed

9. Adequate organ function, defined as the following:

Total Bilirubin	≤upper limit of normal (UNL)
AST (SGOT)	≤2.5 x UNL
ALT (SGPT)	≤2.5 x UNL
GGT	≤2.5 x UNL
Creatinine	≤ 1.5 x the ULN within normal institutional limits or Creatinine clearance ≥30 ml/min, serum creatinine ≤1.5mg/dl (≤132.6 µmol/l)
Alkaline phosphatase	≤2.5 x ULN
PT-INR/PTT	≤1.5 x upper limit of normal (Patients who are being therapeutically anti-coagulated with an agent such as warfarin or heparin will be allowed to participate provided that no prior evidence of underlying abnormality in these parameters exists).
CPK	≤2.5 x ULN
Albumin	≥25 g/L

10. Female patients of childbearing potential must have negative pregnancy test within 7 days before initiation each cycle of chemotherapy. Post-menopausal women must be amenorrhoeic for at least 12 months to be considered of non-childbearing potential. Male and female patients of reproductive potential must agree to employ an effective method of birth control throughout the study and thereafter, at the end of study treatment, for 3 months in female patients of childbearing potential and for 5 months in men in fertile age
11. Cardiac ejection fraction ≥50% as measured by echocardiogram

12. No history of arterial and/or venous thromboembolic event within the previous 12 months
13. The patient or legal representative must be able to read and understand the informed consent form (ICF) and must have been willing to give written informed consent and any locally required authorisation before any study-specific procedures, including screening evaluations, sampling, and analyses.

4.2 Exclusion criteria

1. Other primary malignancy with <5 years clinically assessed disease free interval, except basal cell skin cancer, cervical carcinoma in situ or other neoplasm judged to entail a low risk of relapse
2. Previous treatment with radiation therapy within 14 days of first day of study drug dosing, or patients who have not recovered from adverse events due to agents previously administered
3. Previous radiotherapy to 25% of the bone marrow
4. Major surgery within 2 weeks prior to study entry
5. Participation in another clinical study with an investigational product, which last dose was taken less than 4 weeks prior to the start of the treatment.
6. Persistent toxicities (≥ NCI CTCAE v5.0 grade 2) with the exception of alopecia, caused by previous anticancer therapies.
7. Pregnancy or breast feeding
8. Grade III/IV cardiac problems as defined by the New York Heart Association Criteria (i.e. congestive heart failure, myocardial infarction within 6 months of study)
9. Medical history of arterial thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), or pulmonary embolism within 6 months prior to the initiation of study treatment
10. Known brain metastasis
11. Known chronic liver disease (i.e. chronic active hepatitis and cirrhosis)
12. Known diagnosis of human deficiency virus (HIV) infection
13. Active or chronic hepatitis B or C requiring treatment with antiviral therapy
14. Medical history of hemorrhage or a bleeding event ≥ Grade 3 (NCI-CTCAE v 5.0) within 4 weeks prior to the initiation of study treatment
15. Evidence of any other serious or unstable illness, or medical, psychological, or social condition, that could jeopardize the safety of the subject and/or his/her compliance with study procedures, or may interfere with the subject's participation in the study or evaluation of the study results
16. Known hypersensitivity to any of the study drugs, study drug classes, or excipients in the formulation of the study drugs
17. Any other factors, that, at judgment of investigator, could affect the safety of the patients according to the available trabectedin safety data
18. Expected non-compliance to medical regimens

4.3 Removing patients from the study

Patients may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document patient outcome, if possible. The Investigator should inquire about the reason for withdrawal, request the patient to return all unused investigational product(s), request the patient to return for a final visit, if applicable, and follow-up with the patient regarding any unresolved adverse events. If the patient withdraws from the trial and also withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The coordinating center may retain and continue to use any data collected before such withdrawal of consent. A discontinuation occurs

when an enrolled patient ceases participation in the trial, regardless of the circumstances, prior to completion of the protocol.
The Investigator must determine the primary reason for discontinuation:

1. Withdrawal due to adverse event. When a discontinuation is due to a serious adverse event (SAE), the serious adverse event must be reported in accordance with the reporting requirements and the discontinuation must be reported immediately to the coordinating clinical monitor or his/her representative.
2. Patient decision. Patients may decide to withdraw from the trial at any time. Patients who withdraw from treatment should be followed for survival, and their subsequent treatments should be recorded. If a patient withdraws consent, they will be specifically asked if they are withdrawing consent to:
    - to further participation in the study including any further follow up (e.g., survival calls)
    - withdrawal of consent to the use of their study generated data
    - withdrawal to the use of any samples
3. Investigator decision. Patients must be discontinued if the Investigator believes it to be in the patient's best interest to begin different chemotherapy or biological therapy for his/her disease. Patients may also be discontinued from the trial for poor compliance at the discretion of the Investigator. The final evaluation required by the protocol will be performed at the time of trial discontinuation. The Investigator will record the reason for trial discontinuation and provide or arrange for appropriate follow-up (if required) for the patient.
4. Incorrectly enrolled patients i.e., the patient does not meet the required inclusion/exclusion criteria for the study.
5. Pregnancy. Patients who become pregnant must not receive further treatment in this trial. Pregnant patients should be followed for the duration of the pregnancy, and the outcome of the pregnancy should be recorded.
6. New treatment. Patients who begin a new investigational therapy, chemotherapy, cytokine therapy, immunotherapy or other anticancer treatments must not receive further treatment in this trial.
7. Patient lost to follow-up
8. Disease progression according to RECIST 1.1
9. Death

Any patient discontinuing investigational product should be seen at discontinuation for the evaluations outlined in the study schedule. The patient's tumour status should be assessed clinically and, if appropriate, disease progression should be confirmed by radiological assessment. After discontinuation of study medication, the principal Investigator/Sub-Investigator will perform the best possible observation(s), test(s) and evaluation(s) as well as give appropriate medication and all possible measures for the safety of the patient.
In addition, they will record on the eCRF the date of discontinuation, the reasons, manifestation and treatment at the time of discontinuation. Patients will be required to attend the End of Treatment visit.
After discontinuation of the study medication at any point in the study, all ongoing AEs or SAEs must be followed until resolution unless, in the Investigator's opinion the condition is unlikely to resolve due to the patients underlying disease, or the patient is lost to follow up. All new AEs and SAEs occurring during the 30 calendar days after the last dose of study medication must be reported (if SAEs, they must be reported within 24 hours as described in the dedicated section) and followed to resolution as above. Patients should be seen at least 30 days after discontinuing study medication to collect and / or complete AE information.
The status of ongoing, withdrawn (from the study) and "lost to follow-up" patients at the time of an overall survival analysis should be obtained by the site personnel by checking the patient notes, hospital records, contacting the patient's general practitioner and checking publicly available death registries. In the event that the patient has actively withdrawn consent to the processing of their personal data, the vital status of the patient can be obtained by site personnel from publicly available resources where it is possible to do so under applicable local laws.

5. Dosage and administration

5.1 Drug and treatment duration

This is a single arm study and the investigational drug used in this trial is trabectedin.
Trabectedin will be supplied free of charge by Pharma Mar for each patient treatment aged under 18 years, and for all patient with skeletal MCS, since trabectedin is off-label in paediatric patients and in patients with bone sarcomas.
For adult patients with extra-skeletal MSC where trabectedin is used according its Marketing Authorization, Trabectedin will be provided by SSN in compliance with the Art. 2 comma 1 of the Italian D.M. of 17 December 2004 for non-profit trials.
Study treatment will be administered until evidence of progression or unacceptable toxicity, patient's own willingness, non-compliance or according to clinical investigator's decision.

5.2 Treatment plan

Central venous catheter is mandatory.

Study treatment will consist in:

- trabectedin: 1.5 mg/m² - 1.3 mg/m² (at investigator's discretion, with a top-dose of 2.6 total mg per cycle), given in 24-hour continuous infusion [24]
- adequate antiemetic cover with anti–HT3 and steroids
- pre-medication with dexamethasone 4 mg per os BID the day before starting chemotherapy (day -1), dexametasone 20 mg IV, 30 min before trabectedin infusion (day 1) and dexamethasone 4 mg per os bid for the next 2 days after having completed trabectedin infusion (day 2 and 3).
- anticoagulant therapy with low molecular weight heparin (the day before starting chemotherapy and for the next eight days) for prophylaxis of central line thrombosis is optional and recommended in presence of risk factors for thrombosis.
- colony stimulating factors can be administered for hematologic toxicity according to local standard practice.

On Day 22 treatment will be postponed one week in the event of:

- neutrophils <1500/mmc
- platelets <100.000/mmc
- total bilirubin >ULN
- transaminases >2.5 x ULN
- creatine phosphokinase (CPK) >2.5 x ULN
- alkaline phosphatase >2.5 x ULN (consider hepatic isoenzymes 5-nucleotidase or GGT, if the elevation could be osseous in origin)
- haemoglobin <9 g/dl
- any other grade 3 or 4 adverse reactions (such as nausea, vomiting, fatigue)

If these conditions persist on day 29, treatment must be delayed for up to 3 weeks until the criteria are met. If these conditions still persist after 4 weeks from the last cycle, the patient will be withdrawn from the study program and will be treated at the discretion of the physician responsible.

Additional monitoring of haematological parameters, bilirubin, alkaline phosphatase, aminotransferases and CPK should occur weekly during the first two cycles of therapy, and at least once between treatments in subsequent cycles.

5.3 Dose reductions

Prior to re-treatment, patients must fulfil the baseline criteria defined above.
The chemotherapy doses of each cycle after the first will be modified on the basis of the toxicities induced by the preceding cycle according to the following scheme. The level 1 dose will be applied if the preceding cycle was carried out at 100% of the drug dose. The level 2 dose will be applied if the preceding cycle was carried out according to level 1 drug dose. The level 3 reduction, which will be applied if the preceding cycle was carried out according to level 2 drug dose, involves removal of the patient from the study and treatment at the discretion of the physician responsible.

Reduction levels	Parameter	Values	Trabectedin
Level 1	ANC **or** PLT **or** bilirubin **or** AST/ALT **or** ALP **or** any other toxicity	<500/mmc for >5 days or associated with fever or infection <25.000/mmc >ULN >G3 not recovered <G1 >2.5 x ULN G3-G4	1.1 mg/m2
Level 2	ANC **or** PLT **or** bilirubin **or** AST/ALT **or** ALP **or** any other toxicity	<500/mmc for >5 days or associated with fever or infection <25.000/mmc > ULN >G3 not recovered <G1 >2.5 x ULN G3-G4	0.9 mg/m2
Level 3	ANC **or** PLT **or** bilirubin **or** AST/ALT **or** ALP **or** any other toxicity	<500/mmc for >5 days or associated with fever or infection <25.000/mmc > ULN >G3 not recovered <G1 >2.5 x ULN G3-G4	**WITHDRAW FROM TRIAL**

Anemia does not require a dosage reduction.
Once a dose has been reduced because of toxicity, dose escalation in the subsequent cycles is not recommended.

5.4 Supporting Therapy

Febrile neutropenia lasting >3 days will be treated as a general rule with broad spectrum antibiotics given intravenously with the patient hospitalized.

5.5 Forbidden and permitted concomitant therapies

Aprepitant is forbidden during the study. In addition, in vitro studies have shown that trabectedin metabolism can be modified by concomitant administration of products that induce, inhibit or are metabolized by **cytochrome CYP3A4**, such as **cyclosporine, terfenadine, ketoconazole, erythromycin, troleandomycin, verapamil, rifampicin** and **nifedipine**. If necessary, the administration of such agents can be done with caution (Please refer to Annex 5 for the list of CYP3A4 inducers/inhibiors).
Drugs that diminish the hepatic blood flow could affect trabectedin elimination and should not be given until 72 hours after each trabectedin perfusion. The following medicines are known to reduce blood flow to the liver: **somatostatine** and its analogues octreotide and lanreotide, as well as **non cardioselective betablockers (carteolol, nadolol, penbutolol, propranolol, oxprenolol, sotalol, carvedilol, labetalol)**. Selective betablockers (atenolol, bisoprolol, celiprolol, metoprolol or

nebivolol) can be used with caution because decrease of cardiac output may be a contributing factor to the decrease in hepatic blood flow caused by betablockers.

All concomitant medications (including start / stop dates, total daily dose, and indication) must be recorded in the patient's source documentation and in the eCRF.

- Treatment with non-conventional therapies (e.g. herbs or acupuncture) and vitamin/mineral supplements is acceptable provided that they do not interfere with the study endpoints, in the opinion of the investigator. However, St John's Wort (herbal preparation based on the plant species hypericum) is not permitted.
- Patients may receive palliative or supportive care for any underlying illness.
- Palliative and supportive care for the other disease-related symptoms and for toxicity associated with treatment will be offered to all patients on this trial. The use of antidiarrheal or anti-emetics according to standard practice is strongly encouraged.
- Patients may receive bisphosphonates for bone metastases.
- Patients taking chronic erythropoietin are permitted.
- Major surgery is not permitted during the study period. Patient undergoing a major surgical procedure will interrupt the study treatment and will be withdrawn from the study.

5.6 Trabectedin guidelines

5.6.1 Pharmaceutical form

Powder for concentrate for solution for infusion.

5.6.2 Packaging

The powder has a white to off-white colour and comes in a glass vial. Each carton contains 1 vial of 1 mg of trabectedin. Each vial of powder contains 8 mg of potassium and 0.4 g of sucrose.

5.6.3 Labelling

The study drugs labels will contain all the information to comply with standing regulatory requirements for clinical trials.

5.5.4 Preparation, handling and storage

Vials with 1 mg must be reconstituted with 20ml sterile water for injection. The mixture should be shaken manually to assure a complete dissolution. The reconstituted solution results in a clear, colourless or slightly yellowish solution, essentially free of visible particles. The reconstituted solution contains 0.05mg/ml of trabectedin.
In clinical practice no incompatibilities of trabectedin and PVC bags, type I glass bottles or PVC or polyethylene tubing, polyisoprene reservoirs and titanium implantable vascular access systems has been observed.
It must be stored in a limited access area, refrigerated, at proper environmental conditions (temperature range between 2^0 C and 8^0 C). The unopened drug product vial has a 60-months expiration date. The dilution should be used before 24h if kept between temperature ranges.

5.6.5 After reconstitution

Chemical and physical stability has been demonstrated for 30 hours up to 25ºC.
From a microbiological point of view, the reconstituted solution should be diluted and used immediately. If not diluted and used immediately, in-use storage times and conditions prior to use of the reconstituted product are the responsibility of the user and would normally not be longer than

24 hours at 2ºC to 8ºC, unless reconstitution has taken place in controlled and validated aseptic conditions.

5.6.6 After dilution

Chemical and physical stability has been demonstrated for 30 hours up to 25ºC.
Trabectedin vials are for single use only. Trabectedin will be dispensed under supervision of the investigator, a qualified member of the investigation team or a Hospital pharmacist. Study drug must be dispensed only to patients participating in the study.
The remaining portion of study drug vial dispensed to a patient must be discarded immediately and should not be dispensed again, even for the same patient. The investigator formally agrees not to dispense study drug from any other centre, nor to store it in a site different from those agreed with the sponsor.

6. Study assessment

6.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject or each subject's legally acceptable representative (parents in case of minors) prior to participating in a clinical trial or future biomedical research.
As the trial includes both paediatric and adult patients, a written absent will be obtained from the minors wherever it is possible to do so (according to age). For those children who are not able to read, write or understand regarding assent, the clinician will explain the study and obtain verbal assent.
The informed consent includes also the authorization for translational analysis on transcriptomic and genomic pattern and immunological profile of the tumour (as described in section 7.5).
Due to the rarity of the histology under evaluation, it is of extreme importance to acquire biological information of the disease in order to better define and understand its biology and find possible predictor markers of treatment response.

6.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial. The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the Ethics Committee approval/favourable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature. Specifics about a trial and the trial population will be added to the consent form template at the protocol level. The informed consent will adhere to Ethics Committee requirements, applicable laws and regulations and Sponsor requirements.

6.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

6.3 Screening Visit

The screening tests visit will be performed and completed within 28 days from the day 1 of the first cycle of the study treatment and are specified in the study flow-chart (APPENDIX 1).
Informed consent, eligibility criteria will be checked and a complete medical history will be obtained (including prior treatments). Physical examination will be performed. Laboratory evaluations and a urine pregnancy test will be done for women of childbearing potential. ECOG Performance Status will be assessed, disease histology and sites of involvement will be recorded.

6.3.1 Screening Test within 28 days prior to start the study drug

Patients who have signed the informed consent form will then undergo all the pre-treatment screening tests. Each subject will spend a maximum of 4 weeks in screening.

- **Signed informed consent:** enrolment in the study is defined as the signing of the Informed Consent. All the below reported procedures should be done only after signature of Informed

Consent (except for tumour radiological evaluations if performed before the signature of informed consent and within 28 days prior to start of study drugs)

- The day the patient signs the Informed Consent the site must send the **Screening Form** to the Sponsor Clinical Trial Unit. The Sponsor will return the document completed with the screening ID, that will be used through the study.
- **Demographic data:** age, sex
- **Medical History and Physical Examination**: complete physical exam (including body weight and height, pain assessment, neurological examination) with vital signs (pulse, temperature, blood pressure) and ECOG performance status assessment should be performed. Cancer signs and symptoms will be collected during screening
- **Record all Concomitant prescribed and over-the-counter Medications.**
- **Central Revision Diagnosis:** after the subject screening ID has been assigned, at least one pre-treatment representative formalin-fixed paraffin-embedded tumour block will be collected for central pathology review. First diagnosis sample or another more recent available sample obtained during the routine care previous to study entry will be acceptable. Patients cannot be included in the study unless a tumour block is available. Tumour biopsy at the start of the study is not compulsory but recommended if clinically acceptable.

  The tumour sample is to be shipped by courier to the central pathology review laboratory along with (1) the trial-specific pathology review form and (2) the center's anonymized pathology report. Diagnosis confirmation from the central pathology reviewer will be available in approximately one week. The central pathology review is a compulsory requirement for trial entry in all cases without exception. The study treatment should not be initiated unless the diagnosis has been confirmed by this means.

  The centralized review will be performed at

  - Padova Pathology Unit and
  - Fondazione IRCCS Istituto Nazionale dei Tumori, Milan,

  Anatomia Patologica
  via A Gabelli, 61 35121 Padova
  35121 Padova
  ITALY

  Anatomia Patologica
  Fondazione IRCCS, Istituto Nazionale dei Tumori
  Via Giacomo Venezian 1,
  20133 Milano ITALY

  The patient could be enrolled in the study when inclusion and exclusion criteria compliance is confirmed and the MCS diagnosis is confirmed by central pathologists.

- **Blood samples for serum chemistry and haematology**
  Haematology: WBC, ANC, Haemoglobin, platelet count
  Serum Chemistry: sodium, potassium, phosphorus, calcium, creatinine, urea, total bilirubin, alkaline phosphatase, AST, ALT, lipase, creatin phosphokinase, albumin, total protein
  Coagulation: PT, PTT
  Thyroid exam: TSH, FT3, FT4

Urine exam pregnancy test for female subjects as alternative to plasmatic beta-HCG dosage, if applicable (test must be negative within 7 days from treatment start)

- **Cardiac function** evaluated with ECG and cardiac ultrasound (then every 3 cycles during treatment)

- **Tumour assessment radiological evaluation using RECIST 1.1**
  CT scan with contrast medium and/or conventional MRI with contrast medium of the tumour site(s) of disease
  All suspected sites of disease should be imaged.

  Brain-Chest-Abdomen and Pelvic CT scan (CAP-CT) with contrast should be performed for staging the disease (if not already done during for the tumour lesion(s) evaluation)

- **Total body bone scintigraphy**

- **Enrolment:** The patient could be enrolled in the study when inclusion and exclusion criteria compliance is confirmed and the diagnosis confirmation from the central pathologist reviewer is received at the site.
  The site must send the **Enrolment Form** to the Sponsor Clinical Trial Unit. The Sponsor will return the document completed with the patient's ID, that will be used through the study.

6.4 During treatment (day 1 of each cycle +/-3 days)

On day 1 of each cycle the following assessments/evaluation should be done prior to the scheduled dose of trabectedin:

- **Physical Examination**: complete physical exam (including body weight and height, pain assessment, neurological examination) with vital signs (pulse, temperature, blood pressure) and ECOG performance status assessment should be performed.
- Review of **concomitant medications**
- **Blood samples for serum chemistry and haematology** (within 24 hr prior dosing).
  Haematology: WBC, ANC, Haemoglobin, platelet count
  Serum Chemistry: sodium, potassium, phosphorus, calcium, creatinine, urea, total bilirubin, alkaline phosphatase, AST, ALT, lipase, creatin phosphokinase, albumin, total protein
  Coagulation: PT, PTT
  Thyroid exam: TSH, FT3, FT4
  Urine exam, pregnancy test for female subjects as alternative to plasmatic beta-HCG dosage, if applicable

- **Cardiac function** evaluated with ECG and cardiac ultrasound (every 3 Cycles)

- **Toxicity and safety will be evaluated according to CTCAE v.5.0. (**Adverse Event recording)

6.5 Staging procedures

- **Tumour assessment radiological evaluation using RECIST 1.1**

CT/MRI evaluation will be performed every 2 cycles (just before cycle 3 week 6, just before the cycle 5 week 12, just before the cycle 6 week 18) up to week 18 then every 12 weeks.

6.6 End of treatment

At the end of treatment, the following assessment will be done

- **Physical Examination**: complete physical exam (including body weight and height, pain assessment, neurological examination) with vital signs (pulse, temperature, blood pressure) and ECOG performance status assessment should be performed.
- Review of **concomitant medications**
- **Blood samples for serum chemistry and haematology.**
  Haematology: WBC, ANC, Haemoglobin, platelet count
  Serum Chemistry: sodium, potassium, phosphorus, calcium, creatinine, urea, total bilirubin, alkaline phosphatase, AST, ALT, lipase, creatin phosphokinase, albumin, total protein
  Coagulation: PT, PTT
  Thyroid exam: TSH, FT3, FT4
  Urine exam, Pregnancy test for female subjects as alternative to plasmatic beta-HCG dosage, if applicable
- **Cardiac function** evaluated with ECG and cardiac ultrasound
- **Toxicity and safety will be evaluated according to CTCAE v.5.0. (**Adverse Event recording)

6.7 Follow-up

All patients should be followed until death, if possible.
The date and cause of death must be evaluated and documented in the CRF.
Follow up visits should take place every 6 months after tumour progression to the study treatment
In these visits the patient status will be assessed (alive, dead, lost to follow up), as well as any new anti-cancer treatments.

6.8 How to perform MRI and CT scan

To perform the examination the following protocols will have to be applied:

Magnetic Resonance (MRI)

- Conventional MRI

  - Pre-contrast examination:

    Always: Axial T1 SE, and T2 FSE (with or without fat pre-saturation) or STIR, other sequences depending of local habits.

    Diffusion study: Axial b0, b50, b400, b800, b1000

  - Contrast examination:

    TSE T1 after contrast administration and GE T1 fat-sat after contrast administration

Computed Tomography

  - Pre-contrast examination

  - Contrast examination: to be performed using 120 ml of a conventional iodinated contrast agent, administered intravenously by an automated injector at a rate of 3 ml/sec; the

contrast examination will be done in: arterial phase (delay: bolus tracking), portal venous phase (60 sec).

- Coronal mpr in venous phase

- Tumour density will be determined by measuring CT attenuation coefficient in Hounsfield Unit (HU) by drawing a region of interest around the margin of the entire tumour, using section thickness of 5 mm in the arterial and venous phase. Two-dimensional regions of interest of the entire lesion will be drawn, and all axial sections encompassing the lesion will be included. Software will calculate semi-quantitatively the mean tumour attenuation in HU defined as the average of all the pixels enclosed in the volume of interest.

7. Evaluation criteria

7.1 Evaluation by RECIST 1.1

Tumour progression according RECIST v1.1 in the 6 months before entering this study will be centrally confirmed at each single participating center, in all cases.
An additional centralized review of all the scans to be performed at Fondazione IRCCS Istituto Nazionale dei Tumori (Milan) is foreseen at the end of the study.
Tumour assessments and determination of extent of disease will be performed within 28 days before the day 1 of the first cycle of chemotherapy. Complete tumour assessments will be performed after 2 cycles of treatment with imaging (CT scan and/or MRI), then every 2 cycles. Additional tumour assessments may be performed if clinically indicated. RECIST version 1.1 [22] (Table 1) will be applied for tumour assessment. If an objective response (CR or PR) is observed, the response must be confirmed at least 4 weeks later.
The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence. The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

7.2 Evaluation by modified Choi Criteria

Also Choi response criteria as defined for GIST and applied to both CT scan and MRI will be used for tumour assessment.
Considering the limitation of dimensional criteria for response assessment in sarcoma treated with trabectedin, tumour response will be recorded according to Choi Criteria as defined for GIST treated with imatinib [25]. In particular, Choi criteria are based on changes in tumour size and density following contrast administration on CT scan. Choi criteria will be applied even to MRI [23] assuming that changes in contrast enhancement on subtracted contrast enhanced T1 weighted sequences parallel changes in density on CT, both being markers of tumour vascularization. Therefore, according to Choi criteria a radiological partial response (PR) will be defined by the presence of a ≥10% decrease in tumour size or a ≥15% decrease in tumour density/contrast enhancement on CT/MRI, while progression will be qualified in case of new lesion or in case of
≥10% increase in tumour greatest maximal diameter without any criteria for PR by tumour density/contrast enhancement or ≥15% increase in tumour density/contrast enhancement without any criteria for PR by tumour size. Choi criteria applied even to MRI are summarized in Appendix 4. To allow the response assessment according to Choi criteria, both MRI and CT scan evaluations will be performed according to section 6.8.

7.3 Other Efficacy Variables

Progression Free Survival (PFS) is defined as the time between the treatment start and the evidence of progression according to RECIST, or death. Subjects who die, regardless of the cause of death, will be considered to have had an event.

Overall survival (OS) is defined as the time between the treatment start and death. Subjects who die, regardless of the cause of death, will be considered to have had an event. All subjects who are lost to follow-up prior to the end of the trial or who are withdrawn from the trial will be censored at the time of last contact. Subjects who are still being treated will be censored at the last available date where the subject is known to be alive.

Clinical Benefit is defined as the probability that the patient will remain alive, without disease progression, after 6 months of treatment (RECIST CR + PR + SD > 6 months, as defined in Section 2.2)

Duration of response (DOR) is defined as the duration of time between the date of first documented response (CR or PR) and the date of first documented progression or death due to any cause. If a subject has not had an event, DOR is censored at the date of last adequate tumour

assessment. Subjects who never achieved a BOR of CR or PR will be excluded from the analysis. Calculation will be done applying, alternatively, RECIST or Choi evaluation criteria. The distribution function of DOR will be estimated using the Kaplan-Meier method.

7.4 Exploratory Subgroup analysis

Response Rate by RECIST v1.1 and Choi, PFS and OS, clinical benefit rate and DOR will be evaluated according to the primary site of the disease, dividing in two groups: primary skeletal MCS (first group) and primary extra-skeletal MCS (second group).
Response rate will be also correlated to the tumour transcriptional pattern and immunological profile of the tumour of patients who will enter the study by analysing pre-treatment available paraffin embedded tumour-blocks.

7.5 Biological sample collection

Tumour block collection

Formalin-Fixed Paraffin-Embedded (FFPE) blocks will be collected during the screening period for central pathological review. First diagnosis sample or another more recent available sample obtained during the routine care previous to study entry will be acceptable. Tumour biopsy at study entry will not be compulsory but recommended if it is done for clinical practice and clinically acceptable (e.g. superficial or easily accessible locations).

A post-treatment tumour sample will be collected when possible (selected patients for whom post-treatment biopsy could be performed).

For molecular studies FFPE and/or frozen pre-treatment samples will be dissected in order to gain a tumuor cellularity greater than 70%. Please see the translational research section 10, for more details on tumour block collection.

Blood sample collection

For all the patients who have provided the optional biological informed consent, the blood sample collection will be done as below specified.
Blood drawing (10 ml in EDTA) for molecular analyses. Buffy coats (for genomic DNA) and plasma (for liquid biopsy) will be collected and snap-frozen in 3 aliquots.

Blood samples will be collected as follows:
- Two 5-mL tubes within 72 hours prior to starting the treatment (baseline).
- Two 5-mL tubes within 72 hours after radiological response is documented.
- Two 5-mL tubes within 72 hours after progressive disease is documented.

8. Safety

8.1 Reporting Adverse Events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE), as provided in this protocol. During the study when there is a safety evaluation, the Investigator or site staff will be responsible for detecting, documenting and reporting AEs and SAEs, as detailed in both this section of the protocol and in the AE/SAE section of the Clinical Record Form (CRF). AEs and SAEs must be documented into the source documents and reported into CRF.
All AEs must be collected and reported from the date of treatment start until 30 days after administration of the last dose of study drugs or until the start of a new antitumour therapy, whichever occurs first. All AEs suspected to be related to study drugs must be followed after the time of therapy discontinuation until the event or its sequelae resolve or until symptoms stabilization.
Additionally, all SAEs must be reported to Italian Sarcoma Group Pharmacovigilance Office as specified within section 8.6 'SAE Reporting'.
All adverse events must be recorded using medical terminology. Whenever possible the Investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnoses.
All the information related to AEs and/or SAEs must be reported into the AE section of the CRF.

Causality assessment

The investigator is obligated to assess the relationship between study treatments and the occurrence of each AE/SAE.
The investigator will assess whether the AE/SAE reported are drug:

- Related (Y): reasonable possibility
- Not related (No): no reasonable possibility

This assessment will be recorded into CRF for all AE/SAE.

Severity assessment

The severity of each AE/SAE will be assessed by the investigator according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guide:

- **Grade 1** = mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2** = moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
- **Grade 3** = severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care.
- **Grade 4** = life-threatening consequences; urgent intervention indicated.
- **Grade 5** = death related to AE.

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with patient´s usual function) but would not be classified as serious unless it met one of the criteria for serious adverse events listed in section 8.2, within the SAE definition.

8.2 Adverse Event, Serious Adverse Event, Adverse Drug Reaction, Serious Adverse Drug Reaction Definitions

An **Adverse Event (AE)** is defined as any untoward medical occurrence or experience in a patient or clinical investigation subject temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This can include any unfavourable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests, x-rays or scans) or a disease (new or exacerbated) temporarily associated with the use of a medicinal product (ICH GCP).
The following Special Situations also should be considered AE, but only when they lead to an Adverse Drug Reaction (ADR):

- Drug overdose.
- Drug abuse.
- Drug misuse.
- Drug interactions.
- Drug dependency.
- Exposure in uterus.

Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study. See Section “Lack of Efficacy”, for additional information.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

Examples of an AE do not include a/an:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject’s condition.
- An **Adverse Drug Reaction (ADR)** (marketed products) are responses to a drug which are noxious and unintended and which occur at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function (ICH GCP). An Adverse Drug Reaction (ADR) (non-marketed products) is defined as any response to a medicinal product, that is noxious and/or unexpected, related to any dose (ICH GCP).
- Response to a medicinal product (used in the above definition) means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. An Unexpected Adverse Drug Reaction is any adverse reaction for which the nature or severity is not consistent with the applicable product information (e.g., Investigators' Brochure) (ICH-GCP).
- A **Serious Adverse Event (SAE)** is defined as any untoward medical occurrence occurring to a patient, whether or not considered related to a medicinal product and that is considered

serious. A Serious Adverse Event (SAE), which is considered related to the protocol treatment, is defined as a **Serious Adverse Drug Reaction (SADR).**

Adverse events and adverse drug reactions that are considered as serious are those that:

a) Results in Death;

b) Are life threatening events
NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Require in-patient hospitalization or prolongation of existing hospitalization;
NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the AE, and the resulting appendectomy should be recorded as treatment for the AE.
Hospitalizations that do not meet criteria for SAE reporting are:
- Reasons described in protocol [e.g., investigational medicinal product (IMP) administration, protocol-required interventions/investigations, etc]. However, events requiring hospitalizations or prolongation of hospitalization as a result of a complication of therapy administration or clinical trial procedures will be reported as SAEs.
- Hospitalization or prolonged hospitalization for technical, administrative, practical or social reasons, in absence of an AE.
Pre-planned hospitalizations: Any pre-planned surgery or procedure must be documented in the source documentation. Only if pre-planned surgery needs to be performed earlier due to a worsening of the condition, should this event (worsened condition) be reported as SAE.
- An emergency visits due to an accident where the patient is treated and discharged.
- When the patient is held 24 hours for observation and finally is not admitted.
- Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e., laser eye surgery, arthroscopy, etc).

d) Result in Persistent or significant disability/incapacity;
NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e) A congenital anomaly/birth defect;

f) Are Medically Significant events: Any adverse event may be considered serious because it may jeopardize the subject and may require intervention to prevent another serious condition. As guidance for determination of important medical events refer to the "WHO Adverse Reaction Terminology – Critical Terms List". These terms either refer to or might be indicative of a serious disease state.
Such reported events warrant special attention because of their possible association with a serious disease state and may lead to more decisive action than reports on other terms.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

g) Any suspected transmission of an infectious agent via medicinal product.

REMARK: Death, as such, is the outcome of a SAE and should not be reported as the SAE term itself. Instead the cause of death should be recorded as the SAE term. When available, the autopsy conclusions will be provided to the Sponsor in English.

In this study death due to progression of disease will NOT be considered as a SAE and must therefore NOT be reported as a SAE.

8.3 Disease-Related Events or Outcomes Not Qualifying as SAEs

An event which is part of the natural course of the disease under study (e.g., disease progression) does not need to be reported as a SAE. However, if the progression of the underlying disease is greater than that which would normally be expected for the subject, or if the investigator considers that there was a causal relationship between treatment with study medication(s) or protocol design/procedures and the disease progression, then this must be reported as a SAE. Any new primary cancer must be reported as a SAE.

8.4 Lack of Efficacy

'Lack of efficacy' per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the AE or SAE definition (including clarifications).

8.5 Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g., ECG, X-ray, etc.) that are judged by the investigator as clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE, or SAE, as defined in the relevant sections. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.
In addition, any event involving adverse drug reactions, illnesses with onset during the study or exacerbations of pre-existing illnesses should be recorded including but not limited to clinically significant changes in physical examination findings and abnormal objective test findings (e.g., X-Ray, ECG). The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- The test result is associated with clinically significant symptoms, and/or

- The test result leads to a change in the study dosing or discontinuation from the clinical trial, significant additional concomitant drug treatment or other therapy, and /or
- The test result leads to any of the outcomes included in the definition of a SAE, and/or
- The test result is considered to be an AE by the investigator.

8.6 SAE Reporting

Serious Adverse Event The investigator will collect all SAEs from the time of signing of the Informed Consent Form until 30 days after administration of the last dose of the study drug/IMP or until the start of a new antitumor therapy, whichever occurs first.
Whenever possible the investigator will record the main diagnosis instead of the signs and symptoms. Beyond this period of time, only those SAEs suspected to be related to the IMP will be collected. Nonetheless, the Sponsor will evaluate any safety information related to the clinical trial that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

All SAEs suspected to be related to the IMP must be followed until the event or its sequelae resolve or until symptoms stabilization.

All SAEs (initial and follow ups), regardless of relationship to the study drugs, must be reported immediately and no later than 24h after the Investigator's first knowledge of the event. Communication should be made filling in the SAEs Form that is included in the ISF (Investigator Site File). This form will be sent by email or fax to the next contact information:

I.S.G. Italian Sarcoma Group ETS
Clinical Trial Pharmacovigilance Office:
email: clinicaltrials@italiansarcomagroup.org

All SAE-related information needs to be provided in English.

All additional documents in local language must be accompanied by a translation in English, or the relevant information must be summarized in a follow-up SAE report form.

The initial SAE should be as complete as possible; however, information not available at the time of initial report can be send as follow ups using a new SAE Form.
All SAE will be evaluated and delivered to the Principal Investigators participating in the study for their knowledge and awareness.

Tumour progression or appearance of new tumour lesions MUST NOT be reported as a SAE.
Events of "disease progression" (including signs and symptoms of progression) even if they fulfil any seriousness criterion (i.e., fatal, requiring hospitalization, etc.) are exempted from reporting and will only be reported in the applicable section of the CRF.

8.7 SAE and SUSAR delivery to ECs and Regulatory Agencies

The Sponsor is responsible for the appropriate expedited reporting of serious unlisted/ unexpected and related adverse events (SUSAR) following the local and international regulatory requirements. The Sponsor will report all SAEs that are unlisted/unexpected and related to the study drug (IMP), to the Competent Authorities, ECs and Investigators, according to the current legislations unless otherwise required and documented by the ECs.
To assess the listedness/expectedness for each SAE, the Sponsor will use the Reference Safety Information reported in the Trabectedin Investigator Brochure.

8.8 Report of Pregnancy Events

Any pregnancy, suspected pregnancy, or positive pregnancy test, in a female patient or the female partner of a male patient occurring while the patient is on study drug, or within three months from the patient´s discontinuation visit, must be reported to the Sponsor's Pharmacovigilance Department immediately and always within 24 hours from first knowledge, using the Pregnancy Report Form (see the contact details included under section 8.6 'SAE Reporting'). In the case of pregnancy of the female partner of a trial patient, the Investigator will obtain her informed consent to provide the information by using the applicable form provided by the Sponsor who will also advise the Investigator in these situations.
The investigator will report the following events immediately and always within 24 hours from first knowledge:

- Any occurrence of a pregnancy where any kind of exposure to the IMP is suspected.
- Possible exposure of a pregnant woman (this could involve a partner of a male patient of a pregnant female who came in contact with the study drugs).
- All reports of elevated/questionable or indeterminate beta human chorionic gonadotropins (β-hCGs).

8.9 Patient pregnancy

In case the patient and/or his female partner are fertile it is important to advise them in the use of different contraceptive methods during the study. Highly effective contraceptive methods include the use of oral, injected or implanted hormonal methods of contraception and placing intrauterine device (IUD) or system (IUS). Other contraceptive methods such as barrier methods (condoms, diaphragm or cervical caps) with spermicide can be used. Moreover, abstinence from 2 weeks prior to treatment initiation to 6 months after finishing last treatment dose is another option.
It will be indicated to any female patient of childbearing potential to inform immediately to the Investigator if she becomes pregnant or suspects of being pregnant during clinical trial or up to 3 months of last study drug administration. The Investigator will fill up a Pregnancy Report Form and will send it to the Sponsor within 24hours of being aware of this pregnancy. The pregnancy MUST NOT be reported on the CRF. The IMP will be interrupted from the moment investigator is aware of pregnancy. The investigator must facilitate information about the risks for pregnancy and the possible effects on the foetus to support the decision in collaboration with the responsible physician. Patient´s will be followed up until childbirth and the Investigator must notify the Sponsor Pharmacovigilance Department the outcome of the pregnancy and the newborn within 24 hours of first knowledge as a follow-up to the initial report.
For any event during pregnancy which meets a seriousness criterion (including fetal or neonatal death or congenital anomaly) the Investigator will also follow the procedures for reporting SAEs.

8.10 Pregnancy of female partners of male participants

It will be indicated to male participants in the Informed Consent to report immediately if his partner becomes pregnant or suspects of being pregnant during the study and up to 3 months from last study drug administration. The Investigator will fill up a Pregnancy Report Form and will send it to the Sponsor within 24 hours of being aware of this pregnancy.
The Investigator will ask the couple to sign an authorization for disclosure of information from pregnancy records to allow follow up on pregnancy. Once this authorization has been signed the Investigator can update the Pregnancy Report Form with any additional information obtained during pregnancy. All efforts will be made by the Investigator to collect and notify all details about evolution of pregnancy and outcome.
The investigator must facilitate information about the risks for pregnancy and the possible effects on the foetus, to support the decision in collaboration with the physician. The female partner of the male patient will be followed up until childbirth and the Investigator must notify the Sponsor

Pharmacovigilance Department the outcome of the pregnancy and the newborn within 24 hours of first knowledge as a follow-up to the initial report.

For any event during pregnancy which meets a seriousness criterion (including fetal or neonatal death or congenital anomaly) the Investigator will also follow the procedures for reporting SAEs.

8.11 Anomalies and congenital malformations

Any anomaly or congenital malformation in a patient´s son/daughter or his partner´s will be classified as Serious Adverse Event, and will be registered on Adverse Events CRF page and will be notified to the Sponsor immediately, within 24 hours of Investigator being aware, within a SAE form.

If an SAE (including fetal or neonatal death) occurs in conjunction with the pregnancy, follows the procedure for reporting SAEs (complete an SAE form within 24 hours as explained in the SAE Reporting chapter).

8.12 Quality of AEs and SAEs

A double check of AEs will be performed between the CRF data and the received paper SAEs. The SAEs status will be followed daily during an open SAE until the event is closed.

A safety report will be developed by the CRC and the statistician for the SC and the Sponsor covering:

- The status of the study, progress or findings to-date.
- Total number of subject and status (screened, enrolled, completed, lost-to-follow-up)
- CRF AEs tracking, quality of the data.
- Tables of adverse events and serious adverse events
- Tables of clinical laboratory values when necessary
- Listing of SUSAR

Important information of these safety reports may be delivered to the sites in order to be aware of the study status and provide reasons for continuous improvement.

9. Subject registration, quality assurance of the study

9.1 Patient's registration

Patients considered eligible and who have provided a written informed consent will be enrolled in the study. As soon as the patient has signed the Informed Consent, the investigator must notify the sponsor by sending the **Screening Form** to the sponsor which will provide the patient screening ID. In order to start the treatment, the sponsor must receive the **Enrolment Form** and must approve the treatment start.

9.2 Quality assurance

The responsible investigator will ensure that this study is conducted in agreement with both the most updated Declaration of Helsinki and all the international and local laws that apply to clinical trials and to patient protection. The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).
All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. The competent ethics committee for each Institution participating to the study must validate local informed consent documents before the study can be opened. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are included. This must be done in accordance with the national regulatory requirements. The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

9.3 Record Retention and Data collection

To enable evaluations and/or audits from regulatory authorities or Sponsor, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, e.g., CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, serious adverse event forms, source documents, and detailed records of treatment disposition.
The records should be retained by the investigator according to ICH, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. If the investigator relocates, retires, or for any reason withdraws from the trial, Sponsor should be prospectively notified.

The Investigators must enter the information required by the protocol into the CRF provided. Details on tumour response need to be accurately documented in the patient's hospital records.

The trial records must be transferred to an acceptable designee, such as another investigator, another institution, or to Sponsor.
The investigator must obtain Sponsor written permission before disposing of any records, even if retention requirements have been met.

9.4 Case Report Form

As used in this protocol, the term Case Report Form (CRF) should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this trial.

A CRF is required and should be completed for each included subject.
Subject source documents are the physician's subject records maintained at the trial site. In most cases, the source documents will be the hospital's or the physician's chart. In cases where the source documents are the hospital or the physician's chart, the information collected on the CRFs must match those charts. In some cases, the CRF may also serve as the source document. In these cases, the investigator must prospectively document which items will be recorded in the source documents and for which items the CRF will stand as the source document.
Subsequently, the information entered into the database is systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors will be corrected by data management personnel. Other errors or omissions will be entered on Data Query Forms, which will be returned to the investigational site for resolution. A copy of the signed Data Query Form is to be kept with the CRFs, and once the original is received at the data management centre the resolutions will be entered into the database. Quality control audits of all key safety and efficacy data in the database will be made after entering data from each visit.
When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the Clinical Trial Leader, the Trial Statistician and the Data Manager.

9.5 Data monitoring

During trial conduct, Sponsor or its delegates will conduct periodic monitoring visits to ensure that the trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirements so to guarantee the protection, the safety, the well-being of the enrolled patients, as well as the quality of collected data
The study monitor will monitor all trial aspects, with a specific effort on the following points:

- Surveillance for serious adverse events (SAEs) according to regulatory guidelines (pharmacovigilance).
- Routine monitoring of non-serious adverse events as they are recorded in the CRFs or appear in the source documents at the investigational sites.
- Periodic meetings with the Principal Investigators on individual studies to share experiences and ensure communication.
- Site visit audits will be made periodically by the Sponsor's or CRO's qualified compliance auditing team, which is an independent function from the study conduct team.

The monitor will review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow Sponsor monitors or its agents and appropriate regulatory authorities direct access to source documents to perform this verification.
The trial site may be subject to review by the Ethics Committee (EC), and/or to quality assurance audits performed by Sponsor, and/or to inspection by appropriate regulatory authorities to verify consistency and compliance to protocol requirements.
It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

9.6 Protocol Deviations, Violations and exceptions

In general, protocol violations include deviations from inclusion/exclusion criteria, from concomitant medication restrictions, and from any other protocol requirement that could, at least hypothetically, result in significant risk to the subject and/or affect the outcome of the clinical trial. Protocol violations will be noted in the final Clinical Study Report.
The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with and prepared by the Sponsor. The investigator should not implement any

deviation or change to the protocol without prior review and documented approval from the EC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

a. Protocol Deviations

A protocol deviation is non-adherence to protocol-specific study procedures or schedules that does not involve inclusion/exclusion criteria, primary objective variable criteria, and/or GCP guidelines. Deviations are considered minor and do not impact the study.
The investigator is responsible for communicating to the sponsor any deviation occurred within 5 days.

b. Protocol Violations

A protocol violation is any significant divergence from the protocol, i.e., non-adherence on the part of the patient or the investigator to protocol-specific inclusion/exclusion criteria, primary objective variable criteria, and/or GCP guidelines.
The investigator is responsible for communicating within 24 hours to the Sponsor any protocol violation observed. The Steering Committee will decide whether the patient can continue in the study or must be withdrawn from it.

c. Protocol Exceptions

As a matter of policy, Sponsor will not grant exceptions to protocol-specific entry criteria to allow patients to enter a study. If under extraordinary circumstances such action is considered ethically, medically, or scientifically justified for a particular patient, prior approval from the Steering Committee is required before the patient will be allowed to enter the study.

9.7 Access to source documentation

The investigator must permit authorized agents of the Sponsor, IRB/IEC representatives and regulatory agency representatives to enter and inspect any investigational site where the test article or records pertaining to the test article are held and to inspect and copy all records relating to an investigation, including subject records. To ensure the accuracy of data submitted, it is mandatory that representatives of the Sponsor and of the regulatory agencies have direct access to source documents (e.g., subject medical records, charts, laboratory reports). Subject confidentiality will be protected at all times.
In accordance with ICH E6 source documents include, but are not limited to, the following:

- Clinic, office, or hospital charts Copies or transcribed health care provider notes which have been certified for accuracy after production
- Recorded data from automated instruments, x-rays, and other imaging reports, (e.g., so, CT scans, MRIs, radioactive images, ECGs, pulmonary function tests,…) regardless of how these images are stored, including microfiche and photographic negatives
- Medical history questionnaires completed by subjects.
- Records of telephone contacts
- Diaries or evaluation checklists
- Drug distribution and accountability logs maintained in pharmacies or by research personnel
- Laboratory results and other laboratory test outputs
- Correspondence regarding a study subject's treatment between physicians or memoranda sent to or from the ECs that are completed directly by subjects and serve as their own source.

9.8. Amendments to the protocol

There are to be no changes to the protocol without written approval from the Sponsor. Protocols will be followed as written. Any change to the protocol requires a written protocol amendment or administrative change that must be approved by the Sponsor before implementation. Amendments specifically affecting the safety of subjects, the scope of the investigation, or the scientific quality of

the study require submission to health or regulatory authorities, as well as additional approval by the applicable IRBs/IECs of all investigational sites and, in some countries, by the regulatory authority. These requirements should in no way prevent any immediate action from being taken by the investigator or the Sponsor in the interest of preserving the safety of all subjects included in the study. If an immediate change to the protocol is felt by the investigator to be necessary for safety reasons, the Sponsor's medical monitor must be notified promptly, and the IRB/IEC for the site must be informed immediately. Changes affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC approval, but the IRB/IEC must be kept informed of such changes. In these cases, the Sponsor will send a letter to the IRB/IEC (and as regionally required, the heads of the medical institutions) detailing such changes.

9.9 Sponsor discontinuation criteria

Premature termination of this clinical trial may occur because of a regulatory authority decision, change in opinion of the IRB/IEC, drug safety problems, or at the discretion of Sponsor.
If a trial is prematurely terminated or discontinued, Sponsor will promptly notify the investigators. After notification, the investigator must contact all participating patients within 2 weeks. As directed by Sponsor, all trial materials must be collected and all CRFs completed to the greatest extent possible.

10. Translational Study

To gain insights on MCS biology and to investigate molecular basis underlining MCS response to trabectedin, pre-treatment samples (FFPE and/or frozen samples with tumor cellularity greater than 70%) will be molecularly profiled. Moreover, in vitro models will be generated to identify *HEY1-NCOA2*-specific transcriptional targets involved in the response to trabectedin.

In detail, the transcriptome (RNA-seq) and mirnome (miRNA-seq/Nanostring) profile of a sizable and representative subset of responsive and unresponsive MCS will be analyzed as previously described [26]. Functional annotations and RNA/miRNA data integration will be performed by using appropriate bioinformatic tools.

The MCS mutation landscape of patient-matched tumor and normal DNA will be investigated by targeted NGS-based assays.
Wherever possible, *HEY1-NCOA2* from circulating cell-free DNA/RNA will be measured by PCR-based approaches to assess the feasibility of liquid biopsy strategies to monitor MCS respon se and anticipate disease progression. Circulating cell-free DNA/RNA will be extracted from freshly collected and separated plasma EDTA using the QIAamp ccfDNA/RNA Kit (Qiagen).

11. Ethics

11.1 Ethical Conduct of the Trial

This study will be performed according to the Ethic Principles originated in The Declaration of Helsinki adopted by the 18th WMA General Assembly, Helsinki, Finland, june 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, China, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000, clarification note of paragraph 29, added by WMA, Washington 2002, Clarification note of paragraph 30, added by WMA General Assembly, Tokyo 2004, 59th General Assembly, Seúl, Corea, October 2008 (Annex XII), 64th General Assembly, Fortaleza, Brazil, (October 2013) and the Good Clinical Practice issued by the work group of Efficacy of Medicinal Substances of the European Community (1990) (CPMP/ICH/135/95) and all legal requirements and laws of each country participating in the Clinical Trial.
According to EU Regulation 679/2016 on the protection of individuals with regard to the processing of personal data and on the free movement of such data and the Directive 2001/20/EC of the European Parliament and of the council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use 2001/20/EC, all information obtained during the clinical trial can only be used by the Sponsor of the trial to evaluate the results in conformity with the mentioned directives.
The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an inspection by the sponsor representatives and/or Regulatory Authority representatives at any time. The investigator must agree to the inspection of study-related records by the Regulatory Authority/sponsor representatives, and must allow direct access to source documents to the Regulatory Authority/sponsor representatives.
Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation form, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior EC/IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the EC/IRB/Sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

11.2 Competent Authorities and Ethics Committees

The clinical trial protocol and its documents will be sent before initiating the study to the competent Authorities and Ethics Committees of each country participating in the study for approval.
The sponsor is responsible of sending all documentation and protocol to the Competent Authorities and Ethics Committees for approval of the clinical trial in that specific country.

11.3 Informed Consent (IC)

The patient must sign an Informed Consent in order to enter the study, which includes the consent for donating archive tumour for translational studies and diagnosis confirmation.
An optional informed consent is needed to collect the blood samples required for the translational analyses, according section 7.5
The patient must consent before being admitted to enter the clinical trial and before any procedure related to the clinical trial is performed.
The Investigator must explain the nature, purpose and possible consequences of the clinical trial in a comprehensive way for the patient, as well as clearly inform that the patient is free to refuse participation in the study and that can withdraw consent at any time and for any reason. The Patient Information Sheet (PIS) should be provided with detailed information of the study objectives and

procedures but in no case this PIS should be place instead of a meeting with the investigator or delegated research staff.
The subject must consent by signing the Informed Consent form, if patient is not able to perform a written consent then an oral consent in the presence of witnesses independent from the Investigator team must be performed. The Investigator must also sign the Informed Consent form, and will keep the original in the Investigator Trial Master File and a copy of the original must be handed to the patient. If local administration requires a third copy for the patient anamnesis it will also be performed.
The Investigator must not initiate any procedures related to the study, out of the local standard medical care, until the patient´s informed consent has been obtained.
The Informed Consent form used for this study, and all the changes made during the course of the study, must be prospectively approved by the corresponding Ethics Committee of each country participating in the study.
The patient must be informed of any new versions of Informed Consents and perform the signing process of the new Informed Consent.

11.4 Insurance

All subjects participating in the study will have insurance coverage by the sponsor, which is in line with applicable laws D.M. of 14 July 2009.

11.5 Confidentiality

In order to ensure confidentiality of clinical trial data as disposed in the European Parliament Directive 2001/20/EC, data will be only accessible for the trial Sponsor and its designees, for monitoring/auditing procedures, the Investigator and collaborators, the Ethics Committee of each corresponding site and the Health Authority.
All information, oral or written, even unpublished information that is handed to the Investigators, including protocol and CRFs, must be considered as Promoter property. All data or any material from the study cannot be disclosed by the Investigator and/or collaborator to any unauthorized third party, without written consent of the Promoter.
Investigator and the Institution will allow access to data and source documentation for monitoring, auditing, Ethic Committee revision and inspections of Health Authority, but maintaining at all times subject personal data confidentiality according the General Data Protection European Regulation 679/2016.
The Investigator must guarantee that patient anonymity is kept at all times and their identity must be protected from unauthorized persons and institutions. All patients included in the study will be identified with a numeric code, so that no identifiable personal data will be collected (pseudo anonymization).
The Investigator must have and conserve a patients' inclusion registry where it figures the personal data of the patient: name, surname, address and corresponding identification code into the study, this register will be kept on the Investigator File.

11.6 General principles for Human Biological Material (HBM) collection

Human biological material (HBM) collection involves the collection and storage of biological material, residual biological material or derivatives in compliance with ethical and technical requirements.
Biological material (FFPE blocks of tumour sample for diagnosis confirmation used also for gene expression and immunological profile evaluation) stored in the sites' local archives will be sent by the sites to:

Anatomia Patologica
via A Gabelli, 61
35121 Padova
ITALY

or

Anatomia Patologica
Fondazione IRCCS, Istituto Nazionale dei Tumori
Via Giacomo Venezian 1,
20133 Milano
ITALY

The biological material will be then transferred to:

Dr Roberta Maestro
SOC Oncogenetica ed Oncogenomica Funzionale,
Centro di Riferimento Oncologico di Aviano (CRO) IRCCS,
Via Gallini 2
33081 Aviano (PN)
ITALY

for the translational studies.

The material will be handled, transferred, used and stored according with the sample characteristic and applicable regulation.

- Both laboratories Coordinating Center will have a designated person responsible for collection and will act as a communication point
- The collected HBM should be documented, i.e. the amount remaining and its location

12. Publications of results

The results from this clinical trial can be published or shown at scientific conferences. According to usual practice, this multicentric study will be published as a whole, and not with the data obtained separately from each of the participant site. It is expected that other articles are published about the exploratory aspects of this trial once the main data have been published. The final publication of the trial results will be written by the Study Chairmen on the basis of the final analysis performed at Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy. The first author will be one of the Study Chairmen. Investigator who has entered at least 10% of the eligible patients will be included as a co-author. Co-authors will also be a representative of diagnostic review panels (pathology and radiology), the statistician and the data manager. The sequence of the authors in publication will be one of the Study Chairmen, and subsequent authors according to the number of patients entered in the study. The manuscript will also include an appropriate acknowledgment section, mentioning all groups that have contributed to the study, as well as supporting bodies (sponsors…). All publications (papers, abstracts, presentations…) including data from the present trial will be submitted for review to all co-authors prior to submission. Publications on secondary objectives as well as on retrospective unplanned analyses will be allowed, upon approval by the Study Chairmen. This is applicable to any individual patient included in the trial.

13. Sponsor role and responsibility

The sponsor is the sole owner of the data and is responsible of all the clinical trial activities from study design, development, data collection, management, analysis, interpretation of data, writing and the decision to submit the report for publication written by the Global Study Coordinating Investigator according the publication policy reported on section 11.

14. Conflict of interest

Persons participating to the study will have to declare that neither them nor any of their family members have any financial interest (direct or indirect) in relation to the outcome of the study.

15. Funding

This is an academic, investigators driven study.
Trabectedin will be provided free of charge by Pharma Mar (owner of the Marketing Authorization) for each patient treatment aged under 18 years and for all patient with skeletal MCS, since trabectedin is off-label in paediatric patients and in patients with bone sarcomas.
An unconditional financial support has been granted by Pharma Mar to cover the insurance and study management related costs. The support is in compliance with the total independence of the ownership of the study data applicable to the non-commercial studies, driven by investigators.

16. References

1. Huvos AG, Rosen G, Dabska M, Marcove RC. Mesenchymal chondrosarcoma. A clinicopathological analysis of 35 patients with emphasis on treatment. Cancer 1983;51(7):1230-7

2. Nakashima Y, Unni KK, Shives TC et al. Mesenchymal chondrosarcoma of bone and soft tissue. A review of 111 cases. Cancer 1986; 57(12):2444-53

3. Lichtenstein L, Bernstein D. Unusual benign and malignant chondroid tumours of bone: a survey of some mesenchymal cartilage tumours and malignant chondroblastic tumours, including a few multicentric ones, as well as many atypical benign chondroblastomas and chondromyxoid fibromas. Cancer. 1959; 12(6):1142–1157.

4. Fletcher CDM, Bridge JA, Hogendoorn PCW et al. World Health Organization classification of tumours of soft tissue and bone. IARC 2013 4th edition

5. Lee AF, Hayes MM, Lebrun D et al. FLI-1 distinguishes Ewing sarcoma from samll cell osteosarcoma and mesenchymal chondrosarcoma. Appl Immunohistochem Mol Morphol 2011;19:233-238

6. Wang L, Motoi T, Khanin R et al. Identification of a novel, recurrent HEY1-NCOA2 fusion in mesenchymal chondrosarcoma based on a genome-wide screen of exon-level expression data. Genes Chromosomes Cancer 2012. 51(2):127-39

7. Dantonello TM, Int-Veen C, Leuschner I et al. Mesenchymal chondrosarcoma of soft tissue and bone in children, adolescent and young adults: experiences of CWS and COSS study. Cancer 2008;112(11): 2424-31

8. Bielack SS, Schroeders A, Fuchs N et al. Malignant fibrous histiocytoma of bone: a retrospective EMSOS study of 125 cases. European Muscolo-Skeletal Oncology Society. Acta Orthop Scand 1999;70(4):353-60

9. Salvador AH, Beabout JW, Dahlin DC. Mesenchymal chondrosarcoma-observations on 30 new cases. Cancer 1971 Sep;28(3):605-15

10. Cesari M, Bertoni F, Bacchini P et al. Mesenchymal chondrosarcoma. An analysis of patients treated at a single institution. Tumouri 2007;93(5):423-7

11. Frezza AM, Cesari M, Baumhoer D et al. Mesenchymal chondrosarcoma: prognostic factors and outcome in 113 patients. A European Muscolo-Skeletal Oncology Society study. Eur J Cancer 2015;51:374-381

12.Van Maldegem A, Conley AP, Rutkowski P et al. Outcome of First-Line Systemic Treatment for Unresectable Conventional, Dedifferentiated, Mesenchymal, and Clear Cell Chondrosarcoma. The Oncologist 2019;24:110–116

13. Casali PG, Bielack S, Abecassis N et al. Bone sarcomas: ESMO-PaedCan- EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018 Oct 1;29(Supplement_4):iv79-iv95

14. Demetri JD, Chawla SP, von Mehren M et al. Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and

ifosfamide: results of a randomised phase II study of two different schedules. J Clin Oncol 2009 Sep 1;27(25):4188-96

15. Demetri JD, von Mehren M, Jones RL et al. Efficacy and safety of trabectedin and dacarbazine for metastatic liposarcoma or leiomyosarcoma after failure of conventional chemotherapy: results of a phase III randomized multi center clinical trial. J Clin Oncol 2016 Mar 10;34(8): 786-93
16. Le Cesne A, Cresta S, Maki RG et al. A retrospective analysis of antitumour activity with trabectedin in traslocation-related sarcoma. Eur J Cancer 2012;48(16):3036-44

17. Kawai A, Araki N, Sugiura H et al. Trabectedin monotherapy after standard chemotherapy versus best supportive care in patients with advanced traslocation-related sarcoma: a randomized phase 2 study. Lancet Oncol 2015;16(4):406-16

18. Blay JY, Leahy MG, Nguyen BB et al. Randomized phase III trial of trabectedin versus doxorubicin-based chemotherapy as first-line therapy in translocation-related sarcomas. Eur J Cancer. 2014 Apr;50(6):1137-47

19. Forni C, Minuzzo M, Virdis E et al. Trabectedin (ET-743) promotes differentiation in myxoid liposarcoma tumours. Mol Cancer Ther 2009;8(2):449-457

20. Grohar PJ, Griffin LB, Yeung C et al. Ecteinascidin 743 interferes with the activity of EWS-FLI1 in Ewing sarcoma cells. Neoplasia 2011;13(2):145-153

21. Morioka H, Takahashi S, Araki N et al. Results of a sub-analysis of a phase 2 study on trabectedin treatment for extraskeletal myxoid chondrosarcoma and mesenchymal chondrosarcoma. BMC Cancer 2016; 16:479

22. The Criteria Committee of the New York Heart Association, Inc: Diseases of the Heart and Blood Vessels; Nomenclature and Criteria for Diagnosis, 6th ed Boston, Little, Brown 1964

23. Stacchiotti S, Collini P, Messina A, et al. Tumour response assessment in high-grade soft tissue sarcomas (STS): a pilot study to assess the correlation between radiological and pathological response using both RECIST and Choi's criteria. Radiology, 2009; 251:447-56

24. Baruchel S, Pappo A, Krailo M et al. A phase 2 trial of trabectedin in children with recurrent rhabdomyosarcoma, Ewing sarcoma and non-rhabdomyosarcoma soft tissue sarcomas: a report from the Children's Oncology Group. Eur J Cancer. 2012 Mar;48(4):579-85.

25. Choi H, Charnsangavej C, Faria SC, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumour treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol 2007; 25:1753–1759

26. Brenca M, Stacchiotti S, Fassetta K, et al. NR4A3 fusion proteins trigger an axon guidance switch that marks the difference between EWSR1 and TAF15 translocated extraskeletal myxoid chondrosarcomas. J Pathol. 2019 Sep;249(1):90-101

APPENDIX 1: Flow chart

	Screening	Cycle 1 Day1	Cycle 2 Day 1	Cycle 3 Day 1	Cycle 4 Day 1	Cycle 5 Day 1	Cycle 6 Day 1	Cycle n Day n	End of Treatment	FU (every 6 months)
	- 28 days	D1	Week 3 (±3 d)	Week 6 (±3 d)	Week 9 (±3 d)	Week 12 (±3 d)	Week 15 (±3 d)	Week n (±3 d)		
Informed consent signature	X									
Biological optional IC signature	X									
Medical history	X									
Cancer signs and symptoms	X									
Physical examination[a]	X	X	X	X	X	X	X	X	X	
ECOG [a]	X	X	X	X	X	X	X	X	X	
ECG [c]	X				X				X	
ECHOCARDIOGRAM[c]	X				X				X	
Hematology [b]	X	X	X	X	X	X	X	X	X	
Serum Chemistry [b]	X	X	X	X	X	X	X	X	X	
Coagulation [b]	X	X	X	X	X	X	X	X	X	
Tyroid Exam [b]	X	X	X	X	X	X	X	X	X	
Pregnancy test [b]	X	X	X	X	X	X	X	X	X	
Bone scan	X									
CT/MRI and whole body CT [d]	X		X		X		X	X	X	X
Brain and CAP CT [d]	X									
Central diagnosis confirmation	X									
Tumor sample for gene expression and immunological profile [e]	X								X	
Blood sample for biological study [f]		X			X				X	
Study drug administration		X	X	X	X	X	X	X		
AE		X	X	X	X	X	X	X	X	
Survival status										X

a = the visit, including vital signs (pulse, temperature, blood pressure), physical examination (body weight, height, pain assessment, neurological examination), cancer symptoms, AE and ECOG assessments, must be performed at baseline, on D1 every cycle (±3 days)

b = blood samples must be drawn within 24 hours before study drug administration. If necessary, for logistical or scheduling reasons, blood samples may be drawn with a 3-day window. The following tests will be done:

Hematology : WBC, ANC, Haemoglobin, platelet count
Serum Chemistry: sodium, potassium, phosphorus, calcium, creatinine, urea, total bilirubin, alkaline phosphatase, AST, ALT, lipase, creatine phosphokinase, albumin, total protein
Coagulation: PT, PTT
Thyroid exams: TSH, FT3, FT4
Urine exam pregnancy test for female subjects as alternative to plasmatic beta-HCG dosage, if applicable

c = Cardiac function (ECG and Echocardiogram) will be performed at screening then every 3 Cycles and at End of Treatment

d = CT/MRI of tumour site(s) disease and Chest Abdomen Pelvis + Brain CT at screening (if not already done for the tumour lesion(s) evaluation)
CT/MRI evaluation will be performed after 2 cycles of CT (just before cycle 3 week 6, just before the cycle 5 week 12, just before the cycle 7 week 18), then every 12 weeks

e = for all patients who have provided the optional biological consent to the study, the tumor sample used for the centralized revision of the diagnosis will be analyzed for the planned molecular analysis (section 10 of the protocol)
A post-treatment tumour sample will be collected when possible (selected patients for whom post-treatment biopsy could be performed).

f = for all patients who have provided the optional biological consent to the study, 2 tubes of 5-mL blood samples will be taken at the following times:
baseline (within 72 hrs prior starting the treatment),
at documented radiological response (within 72 hrs after the radiological response is documented)
and at the time of documented progression (within 72 hrsafter the progressive disease is documented)

APPENDIX 2: SAFETY ASSESSMENTS

Safety assessments will consist of evaluation of adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies. Toxicity will be evaluated according to the NCI Common Terminology Criteria for Adverse Events, v. 5.0, accessible at the URL address:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

APPENDIX 3. EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS SCALE

Description	Grade
Fully active, able to carry on all pre-disease activities without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light housework, office work work.	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4

APPENDIX 4. RECIST 1.1 and Choi criteria applied even to MR Response

	RECIST	***CHOI***
CR	- Disappearance of all lesions. - No new lesions.	- Disappearance of all lesions. - No new lesions.
PR	A ≥ 30% decrease in the sum of greatest diameters. - No new lesions.	- A > 10% decrease in the greatest maximal diameter or a >15% decrease in tumour density (HU) / contrast enhancement on CT/ MRI. - No new lesions.
SD	- Does not met criteria for CR, PR or PD.	- Does not met criteria for CR, PR or PD.
PD	A ≥ 20% increase in the sum of greatest diameters. - New lesion	- >10% increase in the greatest maximal diameter and does not meet criteria for PR by tumour density (HU) /contrast enhancement on CT / MRI or >15% increase in tumour density (HU) contrast enhancement on CT/MRI and does not meet the criteria for PR by tumour size. - New lesion. - New intra-tumoural nodule or increase in size of existing intra-tumour nodule.

ANNEX 5 : CYP3A4 INHIBITORS AND INDUCERS

INHIBITORS							
1A2	**2B6**	**2C8**	**2C9**	**2C19**	**2D6**	**2E1**	**3A4,5,7**
fluvoxamine ciprofloxacin cimetidine amiodarone fluoroquinolones furafylline interferon methoxsalen mibefradil	thiotepa ticlopidine	gemfibrozil trimethoprim glitazones montelukast quercetin	fluconazole amiodarone fenofibrate fluvastatin fluvoxamine isoniazid lovastatin phenylbutazone probenecid sertraline sulfamethoxazole sulfaphenazole teniposide voriconazole zafirlukast	PPIs: lansoprazole omeprazole pantoprazole rabeprazole chloramphenicol cimetidine felbamate fluoxetine fluvoxamine indomethacin ketoconazole modafinil oxcarbazepine probenicid ticlopidine topiramate	bupropion fluoxetine paroxetine quinidine duloxetine terbinafine amiodarone cimetidine sertraline celecoxib chlorpheniramine chlorpromazine citalopram clemastine clomipramine cocaine diphenhydramine doxepin doxorubicin escitalopram halofantrine histamine H1 receptor antagonists hydroxyzine levomepromazine methadone metoclopramide mibefradil midodrine moclobemide perphenazine ranitidine red-haloperidol ritonavir ticlopidine tripelennamine	Diethyl- dithiocarbamate disulfiram	HIV Antivirals: indinavir nelfinavir ritonavir clarithromycin itraconazole ketoconazole nefazodone saquinavir telithromycin aprepitant erythromycin fluconazole grapefruit juice verapamil diltiazem cimetidine amiodarone NOT azithromycin chloramphenicol ciprofloxacin delaviridine diethyldithiocarbamate fluvoxamine gestodene imatinib mibefradil mifepristone norfloxacin norfluoxetine star fruit voriconazole

INDUCERS							
1A2	**2B6**	**2C8**	**2C9**	**2C19**	**2D6**	**2E1**	**3A4,5,7**
broccoli brussel sprouts char-grilled meat insulin methylcholanthrene modafinil nafcillin beta-naphthoflavone omeprazole tobacco	phenobarbital rifampin	rifampin	rifampin secobarbital	carbamazepine norethindrone NOT pentobarbital prednisone rifampin	dexamethasone rifampin	ethanol isoniazid	HIV Antivirals: efavirenz nevirapine barbiturates carbamazepine efavirenz glucocorticoids modafinil nevirapine oxcarbazepine phenobarbital phenytoin pioglitazone rifabutin rifampin St. John's wort troglitazone