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Istituto di Ricovero e Cura a Carattere Scientifico

An observational study of local relapse after high grade osteosarcoma

Study Code	Osteo LR
Sponsor's Name	IRCCS Istituto Ortopedico Rizzoli Via di Barbiano 1/10 40136 Bologna, Italy
Study Number/Version/Date:	Vers. 1.0 Jan 10th, 2022
Coordinating Center and Principal Investigator:	Osteoncology, Bone and Soft Tissue Sarcomas and Innovative Therapies Unit IRCCS Istituto Ortopedico Rizzoli Via pupilli 1 40136 Bologna, Italy Dott.ssa Emanuela Palmerini Phone: 051-6366.199 Email: emanuela.palmerini@ior.it
Methodology:	Prospective and retrospective study (multi-institutional cases series review of clinical data)
Type:	Academic/No profit
Funding	None
Principal Investigator Signature	I confirm that I've read this protocol and I accept to run the study in compliance with what is stated in the protocol and with the ICh-GCP and all applicable law Emanuela Palmerini M.D., Ph.D. Firma _____

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1. Background

Treatment of osteosarcoma has evolved in the last 40 years: with the introduction of neo-adjuvant chemotherapy, 5-year survival rates for patients affected by high-grade osteosarcoma have improved from 15% to more than 70%[1-6].

To date, many osteosarcomas are cured by multiagent chemotherapy and surgery, but a significant number still relapse: after treatment of the primary osteosarcoma, 40% to 50% of patients will develop local relapse (LR), distant metastases (DM) or both[7-15].

Few analyses in literature report on locally recurrent disease as it remains uncommon, with LR rate ranging from 4% to 10%[10-17]. Prognosis of patients experiencing LR is generally considered poor, however most data available do not provide sufficient details[7,8,15]. DM are such an overriding determinant of survival, and LR is so infrequent, that it is difficult to study local recurrence as an independent variable[15-22].

Also, in case of LR, while the importance of achieving a second surgical complete remission is well known (CR2), the role of second line chemotherapy is under discussion, with a mono-institutional experience showing no advantage in of chemotherapy after local relapse[23]. Several molecules and their receptors are known to be involved in migration and invasiveness of tumour cells including CXCL12 (SDF-1) which is the ligand of Receptor-4 (CXCR4). The binding of CXCL12 to CXCR4 it is known to activate downstream cascades involving many signalling pathways, such as JAK/STAT, PI3K/Akt, MAPK, JNK which are key in cell migration. The expression of CXCR4 is low or absent in normal cells, while it is elevated in tumor cells. There are preclinical evidences that CXCR4 has an important role on tumor progression in sarcoma[24-25.] Also, nuclear expression of CXCR4 and IGF-1R negatively influences survival in patients with localized soft tissue sarcoma[26].

2. Objectives

Aim of this study is to analyse the modalities of diagnosis (symptoms versus planned radiology) of LR for high-grade osteosarcoma patients, pattern of recurrence, treatment of LR and factors influencing post-LR survival (PLRS) on the role of second line chemotherapy, margins, Local relapse-free interval (LRFI) ("early LR" recurrences with an LRFI ranging between 1 and 24 months, and "late LR" as those having an LRFI longer than 24 months.

Primary Objective

- post-LR survival (PLRS)

Secondary Objectives

- analyse the modalities of diagnosis of LR in high-grade osteosarcoma
- pattern of recurrence
- treatment of LR
- Prognostic factors for PLRS (second line chemotherapy, surgical margins, surgical complete remission after LR (CR2), type of surgery)

Exploratory Objectives

Assessment of CXCR4 and CXCL12 (SDF-1) expression classified as nuclear, cytoplasmatic, and both in primary tumor, LR and DM

- Comparison of CXCR4 and CXCL12 (SDF-1) expression in the LR samples between the groups of patients with isolated LR and those presenting with LR and DM
- Comparison of CXCR4 and CXCL12 (SDF-1) expression on primary tumor in 3 group of patients:

- 1- patients with non LR, nor DM (archival series from Rizzoli database)
- 2- patients with isolated LR
- 3- patients with LR + DM

3. STUDY DESIGN

This is a multicenter retrospective and prospective study that will analyze all cases with of

diagnosis of LR in high-grade osteosarcoma from 01 January 1980 and all new cases referring to each participant site.

4. Prospective observational analysis and data collection

Clinical data will be retrieved by patient charts.

A protocol-specific CRF reporting the results of the review will be provided.

Data from all patients diagnosed with OS will be prospectively entered in this platform.

Each patient will be provided with an informational leaflet for his/her general practitioner and asked to sign an informed consent prior to data entry.

The following information will be recorded in the database:

- Demographics – patient name, DOB, gender, date of first consultation, data of diagnosis
- Data on primary tumor – size, site, evidence of multifocality
- Data on histological diagnosis – morphology (classical type, proximal type, mixed), mitotic count, depth
- Data on staging at diagnosis – evidence of lymph-nodal and/or distant metastases
- Data on treatment of primary tumor – surgery, radiotherapy, chemotherapy
- Data on recurrence – type of recurrence, site, date
- Data on treatment of local relapse – surgery, radiotherapy, chemotherapy
- Data on treatment of metastatic disease – pulmonary metastasectomy, radiotherapy
- Data on chemotherapy for metastatic disease – line, regimen, number of cycles, best response, duration of response
- Patient status at last follow-up (dead of disease, DOD, alive with disease, AWD, nonevidence of disease, NED)

Optional sub-study

For both prospective and retrospective analysis, in all cases with formalin - fixed paraffin

embedded (FFPE) tissue available, and if funding might be secured, an immunohistochemistry analysis to assess the expression of CXCR4 and CXCL12 (SDF- in LR, DM and primary tumor) will be assessed both in the nucleus and cytoplasm, as described [10]. The optional exploratory revision will start only if funding might be secured.

4.1 Study population

We plan to include approximately 50 patients in 36 months.

Inclusion criteria

- Patients who experienced LR as first event of recurrence after treatment for localized high-grade osteosarcoma of bone, including cases with synchronous lung metastases
- Adequate patient compliance to follow-up visits
- Written informed consent prior to any study-specific analysis and/or data collection
- No age limits

Exclusion criteria

- Cases of LR demonstrated after > 3 months after metastatic disease
- Impossibility to ensure adequate compliance

5. Retrospective observational analysis and data collection

Together with the prospective observational study, a retrospective analysis including all the cases with a histological diagnosis of localized high-grade osteosarcoma who experienced LR as first event of recurrence after treatment from 01 Jan 1980 to 31 Dec 2021 will be performed.

The data will be extracted retrospectively from prospectively maintained institutional database and will be checked through a revision of patient records.

The following data will be collected:

- demographics (gender, date of birth, age at diagnosis),

- disease characteristics (morphological subtype, dimension, primary site),
- treatment received (date of surgery, surgical margins, radiation therapy, timing of radiation therapy, chemotherapy, timing of chemotherapy, type of regimen used, best response according to RECIST 1.1)
- date of LR, modality of LR diagnosis, clinical suspect or imaging, presence, or absence of DM at LR diagnosis, type of treatment after LR, date of surgical complete remission after LR (CR2), surgical margins
- outcome (recurrence type and timing, patient status at last follow-up)

5.1 Study population

Through the collaboration of the joining institutions, we plan to include approximately 100 patients

Inclusion criteria

- Male and female patients treated for localized high-grade osteosarcoma from 01 Jan 1980 to 31 Dec 2021.
- Clinical Information available
- No age limits

Exclusion criteria

- Cases of LR demonstrated after > 3 months after metastatic disease
- Evidence of metastatic disease at presentation

6. Statistics

To the case series will be applied a descriptive statistic.

For the exploratory objectives, positive and negative expression of CXCR4 and CXCL12 (nuclear and cytoplasmatic) will be correlated with ES and OS.

7. Enrolment procedures

Patients considered eligible and who have provided a written informed consent will be

included into the study and their material will be used for the purpose of this research according to the protocol and to the informed consent provided.

For the retrospective analysis, the investigator will extract from Institute database the list of potential eligible patients for whom the tumor material is available. After the verification of the presence of the study enrollment criteria, the patient will be included in the study.

Due to the high incidence of mortality of the disease under investigation, it would be possible that some eligible subjects will be deceased

8. Data Collection

Clinical data will be retrieved by patient charts.

A protocol-specific CRF reporting the results of the review will be provided.

A CRF is required and should be completed for each included subject.

9. Ethics and quality assurance

The clinical trial protocol and its documents will be sent before initiating the study to the competent Authorities and Ethics Committees of each participating country for its approval.

The responsible investigator will ensure that this study is conducted in agreement with either 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 principles of the ICH Harmonized Tripartite Guideline for Good Clinical Practice

(ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

10. Informed consent

All Patients will be informed, by the investigator, of the aims of the study, the possible risks and benefits that will derive from the study participation.

The Investigator must 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.

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 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".

The Investigator must also sign the Informed Consent form and will keep the original at the site and a copy of the original must be handed to the patient.

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 study whenever he/she wants. This will not prejudice the patient's subsequent care.

11. General principles for human biological material (HBM) collection

In all cases with formalin - fixed paraffin embedded (FFPE) tissue available, and if funding accessible, 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 tumor sample) are stored in the sites' local archives. The biological material will be used and stored according with the sample characteristic and applicable regulation.

- Each institute 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 act as a communication point.

12. Confidentiality

To ensure confidentiality of clinical trial data as disposed the national and European applicable regulation, 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.

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 as specified in the "Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995".

The Investigator must guarantee that patient anonymity is always kept 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.

13. Publication of results

The results from this study can be published or shown at scientific conferences. According to usual practice, this multicentred study will be published as a whole, and not with the data obtained separately from each of the sites participants. It is expected that other articles are published about the exploratory aspects of this trial once the main data has been published. The final publication of the trial results will be written by the Coordinator Investigator.

All publications (papers, abstracts, presentations…) including data from the present trial

will be submitted for review to all co-authors prior to submission.

14. 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 Principal Investigator.

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