SERVIZIO SANITARIO REGIONALE
EMILIA - ROMAGNA
Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

"Analysis of the molecular profile of the mixofibrosarcoma of the extremities: diagnostic and prognostic impact "

Study code	**MFS**
Sponsor's Name and Address:	Istituto Ortopedico Rizzoli Via Pupilli 1 40136 Bologna Italy
Study Number/Version/Date:	Vers 1.0 12 June 2020
Coordinating Center:	IRCCS Istituto Ortopedico Rizzoli Department of Orthopaedic Oncology Via Pupilli 1 40136 Bologna, Italy
Coordinating Investigator and address:	Dr. Giuseppe Bianchi Phone: 0516366523 Email: giuseppe.bianchi@ior.it
Scientific/Medical study responsible and developer	Dr. Giuseppe Bianchi
Methodology:	Retrospective study (Single institution case series review of clinical and histological data)
Type:	Academic
Founding:	
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 Firma

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BACKGROUND

Myxofibrosarcoma (MFS) represents one of the most common sarcomas in the adult / elderly age and its incidence is increasing due to the increase in the average age of the population; mainly affects the limbs and limbs, especially the lower limb. When compared to other histotypes, the typical feature of MFS is the tendency to a greater risk of local recurrence (RL) regardless of the degree of histological malignancy and surgical margins (60% vs. 25-30%)(1). Such local aggression could be caused by the specific histological pattern of infiltrative growth. Local recurrence is also characterized by a variable, but high (15–38%) progression of degree, thus increasing the metastatic potential at the time of any local relapse (2).The use of local (radiotherapy) or systemic (chemotherapy) adjuvant therapies have not shown efficacy in terms of increasing the control of local and / or systemic diffusion. Despite this, the prognosis of MFS is relatively good with a metastasis rate of 20-25% and an estimated survival of approximately 75% at 5 years and 70% at 10 years(3).Morphologically, it appears as a tumor with distinctive characteristics (association of hypocellular mixoid areas and hypercellular fibrous areas); however, it does not have a specific immunohistochemical pattern, so the histological diagnosis is mainly based on the hematoxylin-eosin morphology (4). MFS is characterized by nonspecific cytogenetic aberrations and complex and unbalanced karyotypes, which increase with increasing degree, suggesting a multi-step tumor progression process due to the acquisition of genetic instability (5).Recently, Heitze et al. in a series of 25 cases of MFS (grade 1 and 3) they identified some genes with amplified expression including CCND1, CCNE1, EGFR, PTEN, RET (MET) and some deleted genes including TP53 and RB1. The mutation analysis also highlighted the presence of somatic mutations in TP53, PTEN, FGFR3, CDKN2A, and RB1 (6).

However, for none of these alterations has a correlation been observed with the prognosis (in terms of overall or progression-free survival). On this basis, knowledge of genetic changes appears essential for the identification of potential biomarkers useful both at diagnostic / prognostic and therapeutic level.

OBJECTIVE OF THE STUDY

The aim of the present study, in a serie of patients with a diagnosis of Mixofibrosarcoma treated at the Rizzoli Institute from *1 January 1993 up to 01 December 2017,* is to identify patients with different risk classes in terms of disease-free survival (local and systemic recurrence) and total survival. The study will exam all the clinical, radiological, histological and immunohistochemical features of this tumour in all samples and in a limited serie of cases the presence of mutation of 50 genes cancer related.

STUDY DESIGN

To identify patients with different risk classes in terms of disease-free survival (local and systemic recurrence) we will review about 200 cases of mixofibrosarcoma of extremities treated at our institution. We will perform immunohistochemical and molecular analysis by NGS, Sanger sequencing and Digital PCR considering 50 cancer-related genes. Survival analysis for disease free-survival and overall survival was carried out using Kaplan-Meier curves and log-rank tests.

POPULATION

Inclusion criteria

1) Male and female patients treated at Rizzoli Institute from 01 Jan 1993 to 01 Dec 2017
2) Diagnosis of mixofibrosarcoma of extremities
3) Histological slides/formalin-fixed paraffin-embedded tissue tumor (FFPE) blocks from archive available to perform the histology analysis and molecular analysis
4) Enough tumor biobanked material available for molecular analysis
5) Presence of the biobank signed informed consent
6) Written informed consent prior to any study-specific analysis and/or data collection

Exclusion criteria

1) Patients with histological diagnosis different from mixofibrosarcoma of extremities

MATERIAL AND METHODS

We will retrieve from the database of the Rizzoli institute all the cases with a histological diagnosis of mixofibrosarcoma of the extremities from 01 Jan 1993 to 01 Dec 2017

We aspect to find approximately 200 cases.

We will review all the medical records, radiological imaging, and histological data of these cases.

The immunohistochemical analysis of the principal alterated genes identified in this tumor by the literature will be performed. In a limited serie of cases, we will perform NGS analysis of specific hot spot mutations cancer-related in 50 genes. Results obtained will be validated by Sanger sequencing and Digital-PCR in all the selected cases.

STATISTICS

To the case series will be applied a descriptive statistic.

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.

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).

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.

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

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 and frozen tissue of tumor sample) are already stored in the archive of the Istituto Ortopedico Rizzoli, Pathology Department.

The biological material will be used and stored according with the sample characteristic and applicable regulation.

- The Istituto Ortopedico Rizzoli 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

CONFIDENTIALITY

In order 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 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.

PUBBLICATION OF RESULTS

The results from this study will be published or shown at scientific conferences.

The final publication of the study results will be written by the Principal Investigator.

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.

REFERENCES

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