Novel models of Myxoid Liposarcoma Xenografts mimicking the biological and pharmacological features of human tumors

Roberta Frapolli¹, Elena Tamborini², Emanuela Virdis², Ezia Bello¹, Eva Tarantino², Sergio Marchini¹, Federica Grosso³, Roberta Sanfilippo³, Alessandro Gronchi⁴, Juan Carlos Tercero⁵, Gabriella Peloso⁶, Paolo Casali³, Silvana Pilotti²* and Maurizio D’Incalci¹*.

¹Department of Oncology, Mario Negri Institute for Pharmacological Research, Milan, Italy; ²Department of Pathology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; ³Adult Sarcoma Medical Treatment Unit, Cancer Medicine Department, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; ⁴Department of Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; ⁵PharmaMar S.A., Madrid, Spain. ⁶Department of Forensic Medicine, University of Pavia, Italy.

**Running title:** New models of Myxoid Liposarcoma Xenografts

**Key words:** Myxoid liposarcoma, FUS-CHOP fusion gene, xenografts, trabectedin

*Corresponding authors: Dr Maurizio D’Incalci, Oncology Department, Istituto di Ricerche Farmacologiche Mario Negri, via La Masa 19, 20156 Milan, Italy. Phone: +39-0239014571. Fax: +39-0239014734. E-mail: maurizio.dincalci@marionegri.it

Dr Silvana Pilotti, Department of Pathology, Fondazione IRCCS Istituto Nazionale Tumori, via Venezian 1, 20133 Milan, Italy. Phone: +39-0223902293 Fax: +39-02-23902877 E-mail: silvana.pilotti@istitutotumori.mi.it
Statement of clinical relevance

This study describes the development of human myxoid liposarcoma (ML) xenografts that are morphologically and biologically very similar to the human tumor they derive from. All the xenografts that grew successfully in different passages belonged to the round cell subtype and maintained the chromosomal translocation t(12;16)(q13;p11) with the expression of the FUS-CHOP transcript that is responsible for the oncogenic transformation. The breakpoint characteristic of type I, II or III FUS-CHOP was identical to that in patients.

Since trabectedin has been recently reported to be very effective in a high percentage of ML patients we investigated the effects of this drug in ML xenografts. The response was histologically comparable to that in patients, with much greater activity in type II than type III ML. These new models offer a useful tool for clarifying the mechanism of selectivity of trabectedin and identifying novel drugs and combinations to be tested rationally in clinical practice.
ABSTRACT

Purpose: Myxoid liposarcoma (ML) is a common subtype of liposarcoma. It is associated in more than 90% of cases with the chromosomal translocation t(12;16)(q13;p11) leading to the fusion FUS-CHOP gene that is responsible for the oncogenic transformation of pre-adipocytes. Recently the marine natural product trabectedin has shown highly selective activity for ML, even in the most aggressive round cell (RC) subtype.

Experimental design: Fragments of 17 sarcomas were transplanted s.c. in female athymic NCr-nu/nu mice. Xenografts were established and characterized by morphology, FISH analysis for the translocation and RT-PCR analysis for fusion transcripts. Trabectedin was injected i.v..

Results: Seven out of 17 tumors grew as continuous xenografts, five of them were ML of the RC subtype. The chromosomal rearrangement and fusion transcripts in different passages were the same as in the human tumors from which they were derived. The responsiveness to trabectedin in type II ML xenografts was as high as in patients. The pathological response was associated with the presence of FUS-CHOP fusion gene, indicating that the drug does not totally eradicate the disease. Type III ML xenografts appeared much less sensitive to trabectedin, confirming previous clinical observations.

Conclusions: This study reports for the first time the characterization of human ML xenografts that adequately mimic the biological and pharmacological features of the human tumor. These models offer a useful tool for investigating the mechanism of selectivity of trabectedin, testing new combinations with this drug and evaluating novel therapies for ML.
Introduction

Myxoid liposarcoma (ML) is the second most common subtype of liposarcoma. It accounts for one third of liposarcomas which, in turn, amount to approximately 10% of all mesenchymal malignancies.

Microscopically ML is made up of uniform round-oval primitive non-lipogenic cells and variable numbers of uni-multivacuolated lipoblasts intermixed with a well-developed plexiform capillary network and embedded in a myxoid matrix composed of hyaluronic acid. Depending on the proportion of the cellular component to the stroma and the prevalence of immature and mature cellular features ML is divided into a usual or pure subtype, and a round cell or cellular (RC) subtype. Usual ML is the most differentiated form and shows low cellularity, evidence of lipoblast differentiation and a conspicuous vascular network while the RC subtype presents the opposite extreme of the differentiation gamut, and shows high cellularity made up of primitive non-lipogenic cells, little or no intervening myxoid stroma and a capillary vascular pattern that is not easy to visualize. Diagnosis of the RC subtype requires >5% hypercellular area (1). Irrespective of the grade of differentiation and consistent with the notion that these histologic variants belong to the same tumor category, the subtypes share the same cytogenetic abnormalities, represented by t(12;16)(p13;p11). This translocation is present in more than 90% of ML and results in fusion of the FUS gene, on chromosome 16, with the CHOP gene on chromosome 12. Rarely, a translocation occurs between chromosome 12 and 22 (EWS gene). The translocation is specific and useful in diagnosis because of the morphologic overlap of ML with other myxoid malignancies, particularly myxofibrosarcoma and well differentiated/dedifferentiated (WD/DD) liposarcoma with exclusive myxoid-like changes (retroperitoneum).

Several subtypes of chimeric transcripts with FUS/CHOP have been described, mainly involving different breakpoints in the FUS gene. Interestingly, unlike alveolar rhabdomyosarcoma and synovial sarcoma (2, 3) the prognostic significance of these variant transcripts remains to be clarified (4, 5) although the type III transcript seems correlated with a more severe outcome (6). Surgery, alone or with radiotherapy is the treatment of choice for localized ML. However, approximately half the patients die from metastatic disease. Chemosensitivity is limited and after failure of conventional chemotherapy (anthracyclines and ifosfamide) there is no standard of care. Interestingly, in a recent series of advanced pretreated patients with ML (7), subsequently updated (8), trabectedin showed both a high response rate and prolonged progression free survival (PFS).
Trabectedin is a marine natural product isolated from the tunicate Ecteinascidia turbinate. It binds DNA in the minor groove at the N2 position of guanine and appears to have unique properties as far as DNA repair mechanism (9-12) and transcription regulation (13, 14). In addition this drug seem to be able to modify the tumor microenvironment (15, 16). See as a review (17).

Surgical tumor specimens of ML analyzed after trabectedin showed a definite pathologic response mainly involving loss of cellular and vascular tumor components and evidence of an increase in mature lipoblasts. These findings are in keeping with the results of a recent study aimed at clarifying the mechanism of action of trabectedin on cell lines which showed a prodifferentiation effect of the drug (18), and with one of the model proposed for sarcoma development which suggests that the fusion oncoprotein might lead to tumor initiation through a block of differentiation (19). However, even with the highest regression score translocation gene footprints (transcript or fusion gene) persisted, indicating that the treatment was unable to eradicate the tumor completely (7).

In order to elucidate the mechanisms of selectivity of trabectedin and to optimize its use, alone or with other compounds, we set up preclinical experimental models of this human disease and conducted experiments to obtain and characterize ML xenografts from surgical tumor biopsies. We report the successful development of MLs reproducibly growing in immunodeficient mice, and mimicking the pathological and biological features of this disease, with very high sensitivity to trabectedin.
Materials and methods

Animals

Female athymic NCr-nu/nu mice, seven weeks old, were obtained from Harlan Laboratories (Bresso, Italy). They were maintained under specific pathogen-free conditions, housed in individually ventilated cages and handled using aseptic procedures. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (Legislative Decree 116 of Jan. 27, 1992 Authorisation n.169/94-A issued Dec. 19, 1994 by Ministry of Health) and international laws and policies (EEC Council Directive 86/609, OJ L 358. 1, December 12, 1987; Standards for the Care and Use of Laboratory Animals, United States National Research Council, Statement of Compliance A5023-01, November 6, 1998).

Diagnostic assessment of human and murine xenografts

- Histological characterization

The histological criteria applied to define myxoid liposarcoma histotype and its usual and round cell (RC) subtypes were as described in the WHO classification (1). To obtain a diagnostic confirmation, we did cytogenetic analysis (FISH) and/or molecular analysis (RT-PCR) on all the tumor specimens, both human and murine xenografts.

- Cytogenetic analysis (FISH)

The characteristic translocation t(12:16) involving FUS and CHOP genes was evaluated on a selected area of sections from formalin-fixed paraffin-embedded samples, as previously reported (7). The cases resulted negative for this chromosomal rearrangement were also tested for the alternative translocation t(12:22) involving EWS and CHOP genes (7) before ruling out the diagnosis of ML. Morphologic overlap between myxofibrosarcoma and dedifferentiated liposarcoma may in fact result in misdiagnosis.

- Molecular analysis of fusion transcripts

When frozen material was available a RT-PCR was done in order to characterize fusion transcript types. Total RNAs was extracted by the Trizol method (Life Technologies, Fredrick, MA, USA), reverse transcribed into cDNA and amplified using specific primers detecting the FUS-CHOP fusion transcript, as already described (7). All the PCR products were sequenced by an automated sequencer (3100 Genetic Analyzer, Applied Biosystem) following standard protocols and aligned with the GeneBank sequences NT_010393 (FUS gene) and NT_029419 (CHOP gene).
- **PTEN sequencing**

cDNA was specifically amplified for exons 2-9 of PTEN using previously published primers and conditions (20). Exon 2 mutations were confirmed on genomic DNA using the following primers:

Fw 5'-GTTTGATTTGCTGATATTTCAG-3'
Rev 5'-CTGTGGCTTAGAAATTTTC-3'

in the following conditions: 40 cycles at 96°C for 8 min; 95°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and an extension of 72°C for 5 min.

**Patients and tumors**

Samples from 17 patients treated at the Fondazione IRCCS Istituto dei Tumori, Milan, whose fragments were grafted into nude mice, were revised by a pathologist. At the same time, FISH and RT-PCR analyses were done. On the basis of this combined diagnostic approach, three tumors were classified as usual myxoid liposarcoma (cases 007, 010 and 011), nine as RC myxoid liposarcoma (cases 002, 004, 006, 009, 012, 014, 015, 016 and 017), three as myxofibrosarcoma (cases 001, 003 and 008), one as leiomyosarcoma (case 005) and one as a dedifferentiated liposarcoma (case 013) (Table 1 A).

**Establishment of tumor xenograft**

Nude mice under isoflurane anesthesia were grafted bilaterally s.c. with representative human tumor fragments of about 4x4 mm derived from the samples described above. Fresh material was obtained from a surgical specimen (n=13) or from a dedicated Tru-cut (n=4) and cytogenetically/molecularly characterized (Table 1 B). Tumors were implanted in mice at different times of disease progression and during the period some patients were treated as detailed in the column “previous treatment”. Two to five animals were used for each specimen depending on tissue availability. The xenografts were further transplanted from mouse to mouse and a portion of each neoplastic tissue was used for histologic and cytogenetic/molecular studies. A xenograft model was considered established after the tumor tissue had been passaged three or more times in mice.

**Genetic profiles**

PCRs were performed using the STR multiplex kits AmpFISTR IdentifilerTM PCR amplification kit (Applied Biosystem), which co-amplifies 15 STRs loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPO, D18S51, D5S818 and FGA) and the Amelogenin, the gender marker, with 1.5-2 ng of DNA.
samples. The amplification protocols and the thermal cycling conditions were according to the manufacturer's instructions. A positive and a negative control were co-analysed in each amplification reaction. Electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using the GeneMapper software following the manufacturer’s recommendations. Positive control and allelic ladder were also analysed. Alleles were designated according to the guidelines of the International Society of Forensic Genetics (ISFG), (21).

The obtained profiles have a frequency $\leq 1\times10^{-17}$ using Italian gene frequencies, then each cell line belongs to just one person, so it’s unique. The profiles aren’t reported for privacy reasons, but the electropherograms are available as requested (gabriella.peloso@unipv.it).

**Drug treatment**

Female athymic nude mice were engrafted s.c. with tumor fragments. The growing tumor masses were measured with a Vernier caliper, and the tumor weights (1mm$^3 = 1$mg) were calculated with the formula: length x (width)$^2$/2. When the tumor weight reached about 130-140 mg (early-stage) treatment started. Trabectedin (generously provided by PharmaMar, Madrid, Spain) was given i.v. at the dose of 0.1 mg/kg, every four days for three times (q4dx3) as previously reported (22). Each group comprised seven mice.

Drug efficacy was calculated as T/C%, where T and C are the mean tumor weights of treated and control groups, respectively.

In order to simulate the clinical situation and to evaluate the histopathological response better, ML015 xenografts were treated with trabectedin 0.1 mg/kg every seven days for six times (q7dx6). Treatment started when tumor weight was about 900 mg (late-stage).
Results

We implanted a total of 17 sarcomas s.c. into nude mice (Table 1 A and B). In ten cases tumors grew over two passages but only in seven were continuous xenografts established. Table 2 reports the xenograft identification and their histological and molecular features (column A). The success of tumor take was not related to chemotherapy or radiotherapy before biopsy. Tumors appeared between two and nine months after transplantation. The latency decreased slightly after the first passage but still remained variable. Tumors grew very slowly with doubling time variable from 10 to more than 70 days (column B, left side). After each passage, we compared the histology and the molecular features of the growing tumors with the original sarcoma.

Tumor fragments from established xenografts were frozen and subsequently retransplanted in mice, making these models potentially available for the use in other laboratories.

Histology and molecular characterization of the established xenograft models: comparison with the corresponding human tumors

The morphological features of the tumors grown in mice were very similar to those observed in the Tru-cut biopsies or surgical specimens from patients and were consistent with those recorded at the initial diagnosis on surgical specimens including the ML006 xenograft classified as RC>20% but for which the material transplanted (Tru-cut) corresponded to the usual subtype component. In all the ML xenografts (ML004, ML006, ML014, ML015 and ML017) the RC component was retained in all passages. Figure 1, panel A, shows one representative case (ML014), illustrating the morphology of the human tumor and the xenografts at different passages. Molecular analysis (panel B) and FISH (panel C) at all the passages always confirmed the chromosomal rearrangement and fusion transcript type detected in the human tumors.

Interestingly, xenograft ML017, from a patient whose bioptic tissue had been found to have a PTEN mutation (Negri et al, personal communication), retained the same alteration throughout all the passages analyzed (Figure 2).

From the ML xenograft we obtained primary culture that grew very slowly and after few passages they lost the FUS-CHOP transcript and started to grow faster.

Drug sensitivity

Considering the recent evidence that ML are highly sensitive to the marine natural product trabectedin (7, 8), we investigated the response of ML xenografts to this drug. The first experiments
were done in two different models, ML006 and ML014 selected because they are characterized by type III and type II FUS-CHOP transcripts, respectively. As it can be seen in figure 3 panel A, control mice with ML006 myxoid liposarcoma reached a tumor weight of about 1.2 g on day 109 after tumor inoculum. Trabectedin slowed the tumor growth, with a best T/C of 41% on day 70. Histological examination before and after treatment showed no response (panel B, left column).

Figure 3 panel C shows the effect of trabectedin in ML014 bearing mice. The drug completely stopped tumor growth and this effect was long-lasting: about seven months after the end of treatment, when control mice had a tumor weighting 1.8 g, treated mice were still in response with the mean tumor weight about 150 mg, not different from that at the moment of randomization (130 mg). The best T/C was 8% on day 265. H/E sections showed a partial response in post-treatment samples (panel D, left column). In both tumor models, FISH and molecular analysis confirmed the presence and the subtype of the FUS-CHOP transcripts in pre- and post- trabectedin tumor (Figure 3 panel B and D, right columns).

Table 2 (column B, right side) reports the best T/C registered for all models tested. As it can be seen the sensitivity of both type II MLs was higher compared to the type III tumors. The myxofibrosarcoma MF003 did not respond to trabectedin, whereas the dedifferentiated liposarcoma DD013 was very sensitive to the drug.

Since the xenograft ML015 was obtained from a patient who had been treated with trabectedin for six cycles, achieving a significant response, we compared the pathological and molecular response in the patient and in the correspondent xenograft after six doses. Figure 4 Panel A shows H/E sections of the pre- and post-treatment mouse tumors and the cytogenetic/molecular analyses indicating the chromosomal rearrangement leading to the type II FUS-CHOP transcript. Figure 4 panel B shows the corresponding pre- and post-treatment human tumors. Morphological and cytogenetic/molecular features are superimposable both before (mouse and human) (left column) and after trabectedin treatment (mouse and human) (right column). The pathological evaluation of tumor regression (human and mouse) was scored as grade 2, according to the published criteria (7, 8).
Discussion

The present study describes, for the first time, the characterization of a series of human myxoid liposarcoma (ML) xenografts. These models closely mimic the biological and pharmacological features of the human ML tumors from which they derive. Morphologically, the tumor grown in each mouse, for each passage and for each xenograft, closely mirrored the subtype of the corresponding human tumor. The morphological diagnosis was confirmed in all cases by FISH and RT-PCR and, interestingly, xenografts harbored the same translocation as the patient’s tumor which, in turn, expressed the same fusion gene (FUS-CHOP), and involved the identical breakpoint. Excluding the xenografts not confirmed as ML (cases. 001, 003, 005, 008, and 013), the establishment of ML xenografts turned out to be closely related to the RC subtype. Five of the nine RC (55.5%) but none of the three usual subtypes grafted into the animals gave rise to the xenograft. Previous chemotherapy or radiotherapy did not seem to influence the success of the implant. This may be ascribable to the retention of molecular/cytogenetic hallmarks in post-treatment human specimens even in those cases with the highest response rate (8). Furthermore, two of the five ML xenografts harbored the type III transcript (ML004 and ML006, Table 2) which is less frequent than types II and I (6, 7), contains a large portion of FUS transcription factor (6) and also seems to be correlated with worse overall survival (6) even if a demonstrable prognostic effect is still lacking (5, 7).

Finally, the case with a type I transcript (ML017, Table 2) presented, in the human specimen and the xenograft, a mutated PTEN oncosuppressor gene, known to correlate with activation of the PI3K/AKT pathway. In addition to the more aggressive subtype, i.e. the RC one, three out of five of these cases (60%) had hallmarks related to aggressive clinical behavior, suggesting that establishment of the xenograft is likely to be related to the more biologically active tumor subtype. Recent clinical evidence has been published that the marine natural product trabectedin is particularly effective in ML (7, 8) and studies in a immortalized ML cell line indicate that the high sensitivity of this tumor might be related to the drug’s ability to act as a differentiating agent by blocking the transactivating ability of the fusion gene product (18).

The present study indicates that ML xenografts are sensitive to trabectedin. In fact, in the ML015 case treated with the drug, the response was similar to that in previously reported series of treated patients (7), where there was a variable depletion of the tumor cells and the vascular component.
Notably, ML015 showed the same histological response as in the patient from which the tumor was originally obtained (Figure 4). This strongly suggests that this preclinical model could serve as a good starting point for future pharmacological studies (12).

Investigations are now in progress to explore whether longer treatment with trabectedin alone or combined with other compounds leads to complete eradication of the disease or, at least, reaches the high regression score seen in patient’s specimens, corresponding to more than 90% loss of the tumor component. The fact that trabectedin seems very effective in ML xenografts as in clinical settings will provide suitable experimental models to explore the drug’s mode of action, to investigate its potential activity alone or in combinations with other anticancer agents, and possibly to clarify differences related to the different FUS/CHOP transcript.

The main drawback of these models is the very low growth rate with an estimated doubling time of 20->70 days implying long observation times for any pharmacological experiment. This is unexpected in view of the selection in xenografts of the more aggressive subtype, which in the mouse seems to reproduce outcome characteristics more similar to the usual subtype than the RC one.

In summary the new xenografts reported offer a powerful new tool to investigate the biology of ML and to develop more selective and effective therapies, particularly for the advanced RC subtype requiring pharmacological treatment, since the localized usual myxoid subtype is mostly successfully managed by surgery with or without radiotherapy.
References


Acknowledgments

The study was supported by two AIRC grants to MD'I and SP
Tables

Table 1. Clinical characteristics of sarcoma patients

Table 2. Xenograft identification and main characteristics
Legend to figures

Figure 1. Histological, molecular and cytogenetic comparison of human surgical specimens and mouse xenografts

One representative case (ML014) is reported. **A.** Hematoxylin/eosin sections of a human surgical specimen corresponding to a RC subtype of ML and its derived xenografts at the second (a), fourth (b) and fifth passages (c, d and e: three different mice) all with very similar morphology. **B.** A specific RT-PCR for FUS-CHOP fusion transcript revealed the type II transcript in all samples, human and murine. **C.** FISH analysis showed the fusion between FUS and CHOP genes corresponding to the orange spots present in each cell. The green and red spots identified respectively FUS and CHOP genes localized on the non-rearranged chromosomes. As an example of the cytogenetic analysis, at the fifth passage the mouse identified as d is reported. C+: positive control, C-: negative control. PR: Proband sample, in this case patient 014.

Figure 2. PTEN molecular analysis

cDNA sequencing showed a GC insertion between bp 1164 and 1165 in exon 2 of PTEN. This mutation was confirmed at genomic level in the human surgical specimen before and after trabectedin treatment (panel A shows only the pre-treatment tumor) and in the corresponding xenograft (mouse at the second passage) (panel B).

Figure 3. Trabectedin treatment in xenografts

**A.** Antitumor activity of trabectedin in ML006 xenografts. Mice were transplanted s.c. with ML006 fragments and treatment started after 36 days (mean tumor weight 140 mg). Trabectedin was injected i.v. q4dx3 at 0.1 mg/kg. Bars, ± SEM. **B.** Hematoxylin/eosin sections of the mouse tumor (ML006) before and after treatment did not show any morphological change. The cytogenetic/molecular analysis in this case showed a type III FUS-CHOP transcript. **C.** Antitumor activity of trabectedin in ML014 xenografts. Mice were transplanted s.c. with ML014 fragments and treatment started after 109 days (mean tumor weight 130 mg). Trabectedin was injected i.v. q4dx3 at 0.1 mg/kg. Bars, ± SEM. **D.** Pre-treatment hematoxylin/eosin sections of the mouse tumor.
(ML014) featuring a RC subtype of ML and post-treatment tumor, with consistent cellular depletion and, in this case, the ML cytogenetic/molecular hallmarks type II FUS-CHOP fusion transcript. C+: positive control, C-: negative control. PR: Proband samples.

Figure 4. Comparison pathological response after trabectedin treatment in the human and the derived xenograft

Hematoxylin/eosin sections of tumors before (left column) and after (right column) trabectedin treatment in a RC subtype xenograft (ML015, panel A) and in the corresponding human samples (panel B). Very similar, marked cellular depletion patterns were observed, both corresponding to score 2 of regression in comparison with the pre-treatment samples. In all the samples FISH and RT-PCR revealed the FUS-CHOP rearrangement. C+: positive control, C-: negative control. PR: Proband samples.
### Table 1. Clinical characteristics of sarcoma patients

<table>
<thead>
<tr>
<th>PT no.</th>
<th>Age/Sex</th>
<th>Primary tumor site (year)</th>
<th>Histological subtype at onset (Year)</th>
<th>FISH analysis</th>
<th>FUS/CHOP f.t.</th>
<th>Specimen type</th>
<th>Previous treatment</th>
<th>Time</th>
<th>Histology</th>
<th>FISH RTPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>51/F</td>
<td>Right arm (2005)</td>
<td>MFS</td>
<td>Neg</td>
<td>Not present</td>
<td>Surgical</td>
<td>No</td>
<td>2005</td>
<td>MFS</td>
<td>Neg</td>
</tr>
<tr>
<td>002</td>
<td>48/M</td>
<td>Left thigh (2001)</td>
<td>RC &gt;20%</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>RT and CT</td>
<td>2005</td>
<td>RC &gt;20%</td>
<td>Pos</td>
</tr>
<tr>
<td>003</td>
<td>80/M</td>
<td>Right thigh (1992)</td>
<td>MFS</td>
<td>Neg</td>
<td>Not present</td>
<td>Surgical</td>
<td>RT and CT</td>
<td>2005</td>
<td>MFS</td>
<td>Neg</td>
</tr>
<tr>
<td>004</td>
<td>58/M</td>
<td>Retroperitoneum (2003)</td>
<td>RC =30%</td>
<td>Pos</td>
<td>Type III</td>
<td>Tru cut</td>
<td>RT and CT</td>
<td>2005</td>
<td>RC =30%</td>
<td>Pos</td>
</tr>
<tr>
<td>005</td>
<td>44/F</td>
<td>Retroperitoneum (2002)</td>
<td>LMS</td>
<td>Not done</td>
<td>Not done</td>
<td>Surgical</td>
<td>RT and CT</td>
<td>2005</td>
<td>LMS</td>
<td>Not done</td>
</tr>
<tr>
<td>006</td>
<td>53/M</td>
<td>Left leg (2004)</td>
<td>RC &gt;20%</td>
<td>Pos</td>
<td>Type III</td>
<td>Tru cut</td>
<td>CT</td>
<td>2006</td>
<td>USUAL</td>
<td>Pos</td>
</tr>
<tr>
<td>007</td>
<td>47/M</td>
<td>Left thigh (2004)</td>
<td>USUAL</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>CT</td>
<td>2006</td>
<td>USUAL</td>
<td>Pos</td>
</tr>
<tr>
<td>008</td>
<td>24/F</td>
<td>Right thigh (2002)</td>
<td>MFS</td>
<td>Neg</td>
<td>Neg</td>
<td>Surgical</td>
<td>No</td>
<td>2006</td>
<td>MFS</td>
<td>Neg</td>
</tr>
<tr>
<td>009</td>
<td>64/M</td>
<td>Left thigh (2006)</td>
<td>RC &gt;90%</td>
<td>Pos</td>
<td>Type III</td>
<td>Surgical</td>
<td>No</td>
<td>2006</td>
<td>RC &gt;90%</td>
<td>Pos</td>
</tr>
<tr>
<td>010</td>
<td>34/F</td>
<td>Left thigh (2006)</td>
<td>USUAL</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>No</td>
<td>2006</td>
<td>USUAL</td>
<td>Pos</td>
</tr>
<tr>
<td>011</td>
<td>57/M</td>
<td>Right thigh (2006)</td>
<td>USUAL</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>No</td>
<td>2006</td>
<td>USUAL</td>
<td>Pos</td>
</tr>
<tr>
<td>012</td>
<td>45/M</td>
<td>Right thigh (2002)</td>
<td>RC &gt;90%</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>RT and CT</td>
<td>2006</td>
<td>RC &gt;90%</td>
<td>Pos</td>
</tr>
<tr>
<td>013</td>
<td>65/M</td>
<td>Right axilla (2004)</td>
<td>DD</td>
<td>Neg</td>
<td>Neg</td>
<td>Tru cut</td>
<td>CT</td>
<td>2006</td>
<td>DD</td>
<td>Neg</td>
</tr>
<tr>
<td>014</td>
<td>33/F</td>
<td>Left thigh (2006)</td>
<td>RC about 70%</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>No</td>
<td>2006</td>
<td>RC about 70%</td>
<td>Pos</td>
</tr>
<tr>
<td>015</td>
<td>56/M</td>
<td>Left thigh (1992)</td>
<td>RC about 20%</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>CT</td>
<td>2007</td>
<td>RC about 20%</td>
<td>Pos</td>
</tr>
<tr>
<td>016</td>
<td>61/F</td>
<td>Right thigh (2007)</td>
<td>RC = 80%</td>
<td>Pos</td>
<td>Type II</td>
<td>Surgical</td>
<td>No</td>
<td>2007</td>
<td>RC = 80%</td>
<td>Pos</td>
</tr>
<tr>
<td>017</td>
<td>31/M</td>
<td>Left leg (2007)</td>
<td>RC &gt;5% **</td>
<td>Pos</td>
<td>Type I</td>
<td>Tru cut</td>
<td>No</td>
<td>2007</td>
<td>RC &gt;5% **</td>
<td>Pos</td>
</tr>
</tbody>
</table>

**Abbreviations:** MFS, myxofibrosarcoma; LMS leiomyosarcoma; DD, dedifferentiated liposarcoma; CT, chemotherapy; RT, radiotherapy; **In this case only a biopsy was available and the percentage of the round cell component was not assessed.**
Table 2. Xenograft identification and main characteristics.

<table>
<thead>
<tr>
<th>Xenograft identification</th>
<th>Source (patient no.)</th>
<th>No. of passages in mice</th>
<th>Histological type</th>
<th>FISH</th>
<th>FUS/CHOP transcript</th>
<th>Doubling time (Days)</th>
<th>Best T/C% (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF003</td>
<td>003</td>
<td>9</td>
<td>MFS</td>
<td>Neg</td>
<td>Neg</td>
<td>10</td>
<td>99 (39)</td>
</tr>
<tr>
<td>ML004</td>
<td>004</td>
<td>8</td>
<td>RC</td>
<td>Pos</td>
<td>Type III</td>
<td>35</td>
<td>38 (120)</td>
</tr>
<tr>
<td>ML006</td>
<td>006</td>
<td>12</td>
<td>RC</td>
<td>Pos</td>
<td>Type III</td>
<td>23</td>
<td>41 (70)</td>
</tr>
<tr>
<td>DD013</td>
<td>013</td>
<td>8</td>
<td>DD</td>
<td>Neg</td>
<td>Neg</td>
<td>12</td>
<td>15 (68)</td>
</tr>
<tr>
<td>ML014</td>
<td>014</td>
<td>5</td>
<td>RC</td>
<td>Pos</td>
<td>Type II</td>
<td>60</td>
<td>8 (265)</td>
</tr>
<tr>
<td>ML015</td>
<td>015</td>
<td>6</td>
<td>RC</td>
<td>Pos</td>
<td>Type II</td>
<td>30</td>
<td>12 (134)</td>
</tr>
<tr>
<td>ML017</td>
<td>017</td>
<td>4</td>
<td>RC</td>
<td>Pos</td>
<td>Type I</td>
<td>&gt;70</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: MFS, myxofibrosarcoma; DD, dedifferentiated liposarcoma; RC, round cell.
Figure 1

Human Surgical specimen
Formalin fixed material

Xenograft ML014
Cryopreserved material

A

Type II
B

C
Figure 2

PTEN molecular analysis

A. Human sample

B. ML017

GC insertion

GC insertion
**Figure 3**

**ML006**

**A.**

![Graph showing relative tumor weight over days from inoculum for ML006 with treatment groups labeled as Controls, Trabectedin 0.1mg/kg q4x3.](image)

**B. Pre-treatment**

- **c+** and **c-** PR

**Post-treatment**

- **c+** and **c-** PR

**ML014**

**C.**

![Graph showing relative tumor weight over days from inoculum for ML014 with treatment groups labeled as Controls, Trabectedin 0.1mg/kg q4x3.](image)

**D. Pre-treatment**

- **c+** and **c-** PR

**Post-treatment**

- **c+** and **c-** PR
**Figure 4**

<table>
<thead>
<tr>
<th>A. ML015</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="ML015 Pre-treatment" /></td>
<td><img src="image2" alt="ML015 Post-treatment" /></td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="ML015 c+ c- PR Type II" /></td>
<td></td>
<td><img src="image4" alt="ML015 c+ c- PR Type II" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Human sample</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5" alt="Human sample Pre-treatment" /></td>
<td><img src="image6" alt="Human sample Post-treatment" /></td>
<td></td>
</tr>
<tr>
<td><img src="image7" alt="Human sample c+ c- PR Type II" /></td>
<td></td>
<td><img src="image8" alt="Human sample c+ c- PR Type II" /></td>
</tr>
</tbody>
</table>
Clinical Cancer Research

Novel models of Myxoid Liposarcoma Xenografts mimicking the biological and pharmacological features of human tumors

Roberta Frapolli, Elena Tamborini, Emanula Virdis, et al.

Published OnlineFirst August 20, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-0317

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.