

Xenograft models for undifferentiated pleomorphic sarcoma not otherwise specified are essential for preclinical testing of therapeutic agents

MARC BECKER^{1,2*}, CLAUDINE GRAF^{3*}, MARCUS TONAK^{2,4}, MARKUS P. RADSACK⁵,
TOBIAS BOPP⁵, ROBERT BALS⁶, RAINER M. BOHLE¹, MATTHIAS THEOBALD³,
POL-MARIA ROMMENS², DIRK PROSCHEK^{2**} and THOMAS C. WEHLER^{3**}

¹Institute of Pathology, Saarland University Medical Center, Homburg, Saarland D-66421;

²Interdisciplinary Sarcoma Working Group, Center of Orthopaedic and Trauma Surgery;

³III Department of Internal Medicine, University Medical Center, Johannes Gutenberg University of Mainz, Mainz, Rhineland-Palatinate D-55131; ⁴Department for Orthopaedic and Trauma Surgery, University Hospital Mannheim, Mannheim, Baden-Württemberg D-68167; ⁵Institute for Immunology, University Medical Center, Johannes Gutenberg University of Mainz, Mainz, Rhineland-Palatinate D-55131;

⁶Department of Internal Medicine V - Pulmonology, Allergology and Respiratory Critical Care Medicine, Saarland University Medical Center, Homburg, Saarland D-66421, Germany

Received April 27, 2015; Accepted December 11, 2015

DOI: 10.3892/ol.2016.4784

Abstract. Undifferentiated pleomorphic sarcoma not otherwise specified belongs to the heterogeneous group of soft tissue tumors. It is preferentially located in the upper and lower extremities of the body, and surgical resection remains the only curative treatment. Preclinical animal models are crucial to improve the development of novel chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma. However, this approach has been hampered by the lack of reproducible animal models. The present study established two xenograft animal models generated from stable non-clonal cell cultures, and investigated the difference in chemotherapeutic effects on tumor growth between undifferentiated pleomorphic sarcoma *in vivo* and *in vitro*. The cell cultures were generated from freshly isolated tumor tissues of two patients with undifferentiated pleomorphic sarcoma. For the *in vivo* analysis, these cells were injected subcutaneously into immunodeficient mice. The mice were monitored for tumor appearance and treated with the most common or innovative chemotherapeutic agents available to date. Furthermore, the same drugs were administered to *in vitro* cell

cultures. The most effective tumor growth inhibition *in vitro* was observed with doxorubicin and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA), also known as vorinostat. In the *in vivo* xenograft mouse model, the combination of doxorubicin and the tyrosine kinase inhibitor pazopanib induced a significant tumor reduction. By contrast, treatment with vorinostat did not reduce the tumor growth. Taken together, the results obtained from drug testing *in vitro* differed significantly from the *in vivo* results. Therefore, the novel and reproducible xenograft animal model established in the present study demonstrated that *in vivo* models are required to test potential chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma prior to clinical use, since animal models are more similar to humans, compared with *in vitro* cell cultures.

Introduction

Soft tissue sarcoma (STS) is a heterogeneous group of neoplasms arising from degenerated cells of mesenchymal origin (1). Currently, STS is differentiated into >20 distinct subtypes, which are classified according to their tissue of origin (2). The most common type of STS observed in adults is undifferentiated pleomorphic sarcoma not otherwise specified (NOS), which was previously known as malignant fibrous histiocytoma, and presents five histological subtypes (3-5). Currently, surgical resection remains the only method of curative treatment for undifferentiated pleomorphic sarcoma NOS, which often occurs at a high malignancy grade, possesses a high risk of metastasis, and exhibits resistance to radiotherapy and chemotherapy (6-8). Previous studies have demonstrated that additional radiation therapy improves local tumor control; however, chemotherapy remains palliative, since there are

Correspondence to: Dr Thomas C. Wehler, III Department of Internal Medicine, University Medical Center, Johannes Gutenberg University of Mainz, Mainz, Rhineland-Palatinate D-55131, Germany
E-mail: thomas.wehler@uks.eu

*Contributed equally; **Joint senior authorship

Key words: pleomorphic sarcoma, doxorubicin, pazopanib, HDAC inhibitor, xenograft, animal model

no effective chemotherapy drugs (9,10). Due to the limited number of patients with specific subtypes of undifferentiated pleomorphic sarcoma NOS, clinical studies often present limitations, and the majority of the data available may not apply to certain subtypes. The application of an anthracycline-based chemotherapy, including doxorubicin alone or in combination with ifosfamide, is often the first-line treatment of undifferentiated pleomorphic sarcoma NOS, and there are no widely recognized second-line therapies available (11-13). Recently, improved second-line drugs have been developed, including histone deacetylase (HDAC) inhibitors, trabectedin and tyrosine kinase inhibitors such as pazopanib, which are more effective than those currently available (14-19). With these drugs, a progression-free survival time of 3-5 months may be achieved (14-19). However, the overall survival time is not increased. Therefore, to prolong the survival time of patients with undifferentiated pleomorphic sarcoma NOS, additional improvements are required.

Cell lines and animal models are powerful tools for the development of innovative therapeutics. In the past recent years, numerous cell lines derived from undifferentiated pleomorphic sarcoma have been generated and characterized (20-24). A number of these cell lines were injected subcutaneously into immunodeficient mice, and 4 weeks subsequent to injection measurable tumor tissue was formed (20,21,24). However, investigations comparing the effect of therapeutical approaches *in vivo* and *in vitro* have not been performed thus far. In other studies using xenotransplantation models, original human tumor tissue was transplanted into immunodeficient mice (25-27). These animal models appear to be more similar to the tumors observed in humans, but hypoxia following tissue transplantation remains a problem (25-27).

In the present study, two stable cell cultures were generated from two patients with undifferentiated pleomorphic sarcoma. These cells were subcutaneously injected into immunodeficient mice, whereby remained tumorigenic. Following chemotherapeutic treatment of the tumor, clear differences were observed between the *in vitro* and *in vivo* models, which confirms that it is imperative to test innovative chemotherapeutics in appropriate animal models prior to clinical use.

Materials and methods

Animals. In total, 4 immunodeficient non-obese diabetic (NOD) severe combined immunodeficiency (SCID) gamma (NSG) mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) (2 male and 2 female; 8 weeks-old) were purchased from the Jackson Laboratory (Sacramento, ME, USA), and bred in the animal facility at the Johannes Gutenberg University of Mainz (Mainz, Germany). The mice were bred, maintained and manipulated under specific pathogen-free conditions. All the food, water and litter were sterilized prior to use. The temperature and humidity were controlled at 20-24°C and 45-65%, respectively. Daily light cycles consisted of 12 h light and dark cycles. The cages were fully cleaned once or twice per week. Mice that were 6-8 weeks-old were used for subcutaneous injections. All animal procedures were conducted in accordance with the Institutional Guidelines of the Johannes Gutenberg University of Mainz, and approved by the responsible national authority (National Investigation

Office Rheinland-Pfalz; Koblenz, Germany; approval no. 23 177-07/G 13-1-027).

Isolation of sarcoma samples and cell culture. Stable oligoclonal cell cultures, termed MZ-UPS-1 and MZ-UPS-2, were generated from freshly isolated tumor tissue from two patients diagnosed with undifferentiated pleomorphic sarcoma NOS. These two patients were treated in 2011 and 2012, respectively, at the University Medical Center in Mainz and had undergone surgeries where the local tumor tissue was removed. Immediately following resection, the tumor tissue was minced, placed onto 6-well plates (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and cultivated at 37°C in a humidified atmosphere with 5% CO₂ with Gibco® Dulbecco's modified Eagle's medium [Nutrient Mixture F-12 containing GlutaMAX™ Supplement (DMEM/F12; Thermo Fisher Scientific, Inc., Waltham, MA, USA)], 1% sodium pyruvate (100 mM; Thermo Fisher Scientific, Inc.), 10% fetal calf serum (FCS; GE Healthcare Life Sciences, Chalfont, UK) and 1% penicillin-streptomycin (Invitrogen™; Thermo Fisher Scientific, Inc.). The cells were expanded to form a sub-confluent layer of adherent cells 2-4 weeks subsequent to initial seeding. Subsequently, the adherent tumor cells were digested using Accutase (GE Healthcare Life Sciences) and transferred into 175 cm² cell culture flasks (Sigma-Aldrich Chemie GmbH). Since the MZ-UPS-1 cells were a fast-growing culture, they were passaged and serially subcultured at a dilution of 1:3-1:5 every week. By contrast, the MZ-UPS-2 cells were passaged every two weeks and serially subcultured at a dilution of 1:2. The two undifferentiated pleomorphic cell cultures MZ-UPS-1 and MZ-UPS-2 were maintained *in vitro* for ~30 passages for >1 year.

The two patients provided written informed consent for biobanking. The present study was approved by the Ethics Committee of the University Medical Center of Johannes Gutenberg University of Mainz [Mainz, Germany; approval no. 837.250.13 (8935)].

Chemotherapeutic treatment in vitro and cell viability assay. MZ-UPS-1 and MZ-UPS-2 cells from the fourth passage were harvested, washed in phosphate-buffered saline (PBS; Thermo Fisher Scientific, Inc.) and resuspended in DMEM/F12-GlutaMAX™ Supplement with 10% FCS. One day prior to chemotherapeutic treatment, the cells were seeded onto 96-well plates (1x10⁴ cells/100 µl medium/well; Greiner Bio-One GmbH, Frickenhausen, Germany) to ensure adherence. On day 1, the supernatants were discarded, and a cell viability assay was conducted with alamarBlue™ Cell Viability Assay Reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Briefly, alamarBlue™ solution was diluted 1:10 in DMEM/F12-GlutaMAX™ Supplement, and 100 µl/well was added to the cells, which were incubated for 1-2 h. Subsequently, the supernatants were removed, and the absorbance was measured at 570 nm (reference wavelength, 600 nm) in a multiplate spectrophotometer (Sunrise™; Tecan Group Ltd., Männedorf, Switzerland). In parallel assays, the plated tumor cells were cultured in 200 µl DMEM/F12-GlutaMAX™ Supplement in the presence of the appropriate concentrations of the chemotherapeutic agents for 1, 2, 4, 7, 10 and 14 days. alamarBlue™ assays were performed

Table I. Chemotherapeutic treatment regimen of xenograft mouse models of undifferentiated pleomorphic sarcoma.

Chemotherapy	Body concentration, mg/kg	Type of application	Application mode
Doxorubicin	6	Intravenous	Weekly
Suberoylanilide hydroxamic acid	50	Intraperitoneal	Daily
Pazopanib	100	Oral	Daily

at each of the above time points to determine cell viability. At least three experiments were performed.

Xenotransplantation. Cultured MZ-UPS-1 and MZ-UPS-2 cells from the fourth passage were harvested by detachment with Accutase and washed twice in PBS. Live cells were counted using trypan blue staining (Sigma-Aldrich Chemie GmbH) and a Neubauer counting chamber (Sigma-Aldrich Chemie GmbH). In total, 1×10^6 cells were injected subcutaneously into the right flank of NSG mice. Tumor growth was verified 4 weeks later. The effectiveness of the xenograft transplant was 100% for the two undifferentiated pleomorphic sarcoma cell cultures MZ-UPS-1 and MZ-UPS-2.

Histology. Samples from the original tumors of the two patients and isolated xenografts from the mice were fixed in 4% phosphate-buffered formaldehyde solution (Roti[®]-Histofix; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and embedded in paraffin (Sigma-Aldrich Chemie GmbH). The tissue sections (5 μ m) were subsequently deparaffinized using xylol (Sigma-Aldrich Chemie GmbH) and a descending sequence of ethanol (100, 90 and 70% and distilled water), stained with hematoxylin and eosin (Sigma-Aldrich Chemie GmbH) and viewed under a microscope (Axioskop 40; Zeiss GmbH, Jena, Germany).

Immunohistochemical staining were performed according to manufacturers's protocol on a Ventana BenchMark XT platform (Ventana Medical Systems, Inc., Tucson, AZ, USA). Following deparaffinization, antigen retrieval was performed using peroxidase and alkaline phosphatase blocking reagent (Dako, Glostrup, Denmark) for 10 min at 95-99°C. The tissue sections were incubated with mouse anti-human monoclonal vimentin (for 32 min; catalog no., M0725), mouse anti-human monoclonal actin (for 32 min; catalog no., M0851) and rabbit monoclonal anti-human Ki-67 antibodies (for 36 min; catalog no., M7240) at room temperature. All primary antibodies were purchased from Dako. Following washing two times with reaction buffer (Ventana Medical Systems, Inc.), the slides were then incubated with anti-mouse secondary antibody (ultraView Universal Alkaline Phosphatase Red Detection Kit; Ventana Medical Systems, Inc.) for 30 min at room temperature and visualized according to manufacturer's instructions.

Animal treatment and tumor measurement. The size of the tumors in the mice were measured using a digital caliper 6-8 weeks subsequent to subcutaneous injection of 1×10^6 MZ-UPS-1 or MZ-UPS-2 cells. Tumor size was measured from caudal to cranial and dorsal to ventral, and the values were multiplied to calculate the tumor area. Chemotherapeutic agents were prepared prior to application.

Ready-to-use doxorubicin was purchased at a concentration of 2 mg/ml (Hexal AG, Holzkirchen, Germany), and suberoylanilide hydroxamic acid (SAHA; Sigma-Aldrich Chemie GmbH) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich Chemie GmbH) at a concentration of 25 mg/ml. Pazopanib was purchased as 400 mg capsules Votrient[®] (GlaxoSmithKline, Brentford, UK). One Votrient[®] capsule was ground using a pestel and mortar, and dissolved in DMSO at a concentration of 30 mg/ml upon mixing overnight. The mice were weighed and treated as described in Table I. Tumor size was measured every 2 days.

Statistical analysis. Student's *t* test was used to compare the mean values between two experimental groups where appropriate using GraphPad Prism version 6.0f (GraphPad Software, Inc., La Jolla, CA, USA). For non-Gaussian distributions, the Mann Whitney U test was used for the calculation of statistical significance. Gaussian distribution was analyzed with the Kolmogorov Smirnov test. Data are expressed as the mean \pm standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Two undifferentiated pleomorphic sarcoma cell lines were established. Histology of the biopsies and resected tumors of the two patients provided a diagnosis of undifferentiated pleomorphic sarcoma NOS grade 3, according to the FNCLCC classification (28). The tumors were located in the left axillary region or the gluteus maximus. The two primary tumor tissues histologically exhibited a storiform and pleomorphic growth pattern with specific myxoid regions (Fig. 1).

Immunohistochemical staining of the MZ-UPS-1 cells and the original tumor from which they were derived, revealed that the cells expressed vimentin and actin and possessed a Ki-67 index of $>20\%$. The MZ-UPS-2 cells and the cells from their original tumor expressed vimentin and possessed a Ki-67 index of $>20\%$ (data not shown). The features of the two undifferentiated pleomorphic sarcoma cell lines are revealed in Table II.

Tumor formation following subcutaneous xenotransplantation. Early passages of the two undifferentiated pleomorphic sarcoma cells were used for subcutaneous xenotransplantation into NSG mice. Tumors from the MZ-UPS-1 cells grew ≤ 1.5 cm³ in diameter 4-6 weeks subsequent to a subcutaneous injection of 1×10^6 cells. The histology and type of growth of the tumor was similar to the original human resected tumor (Fig. 1). In the boundary region, the tumor tissue exhibited a solid and predominantly storiform growth pattern with

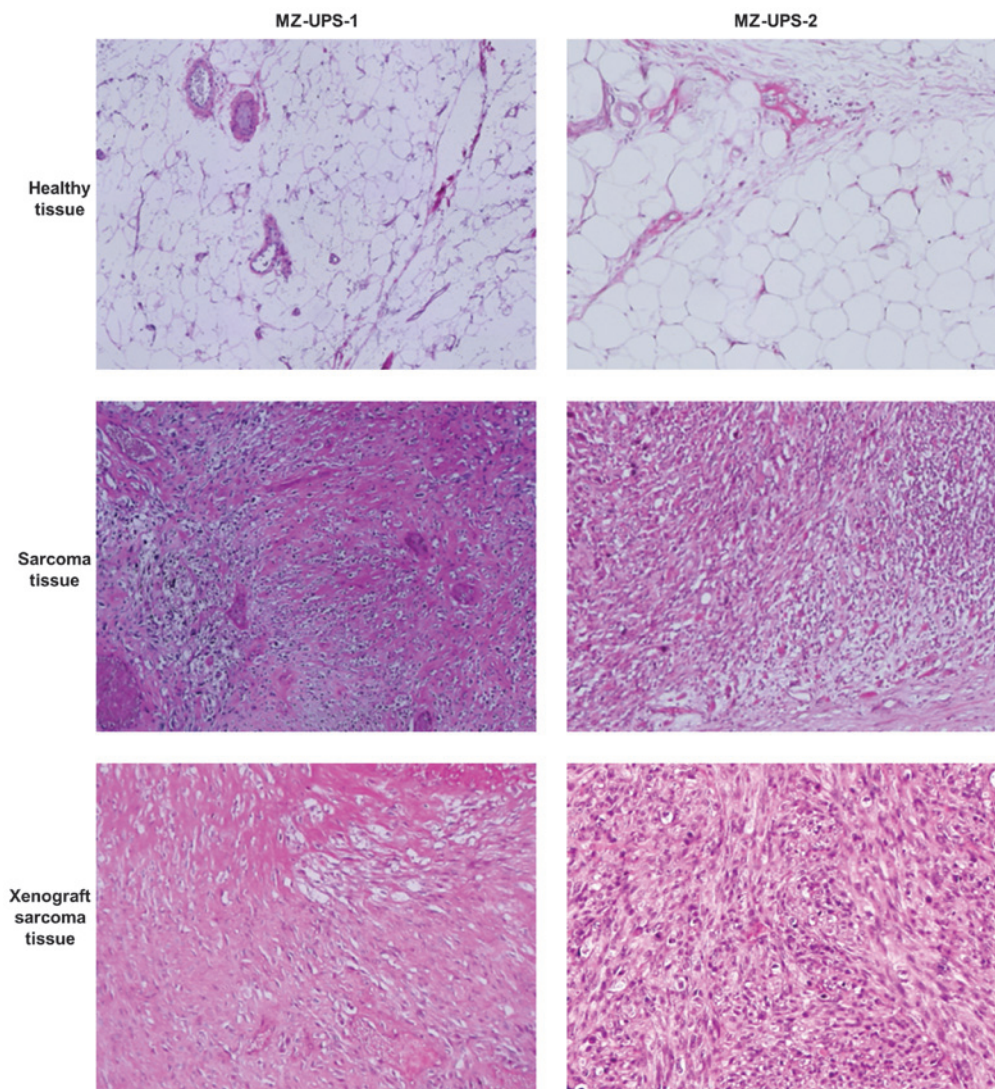


Figure 1. Histology of undifferentiated pleomorphic sarcoma xenograft tumor isolated from NSG mice subjected to xenotransplantation is similar to the original sarcoma tissue resected from human patients. Histological sections of two original human sarcoma tissues (MZ-UPS-1 and MZ-UPS-2), their surrounding healthy tissue and the corresponding xenograft tumors were stained with H&E. Xenograft sarcoma tissue was generated by subcutaneous injection of 1×10^6 cultivated MZ-UPS-1 or MZ-UPS-2 cells into the right flank of NSG mice. Following injection, the solid tumor tissue from the xenograft transplant was isolated and fixed 8 weeks later. The isolated tumor tissue was embedded in paraffin, and sections were stained with H&E. H&E, hematoxylin and eosin; NSG, NOD SCID gamma; NOD, non-obese diabetic; SCID, severe combined immunodeficiency.

increased myxoid regions and a fluid-filled chamber. On the surface of the tumor and the surrounding tissue, neovascularization was clearly observed (Fig. 2). Similarly to the *in vitro* findings, the *in vivo* tumor formation of MZ-UPS-2 cells was slower, compared with MZ-UPS-1 cells. Histologically, the MZ-UPS-2 xenograft exhibited a predominantly storiform growth pattern; however, no myxoid regions or fluid-filled chambers were observed (Fig. 1), contrarily to the original MZ-UPS-2 tumor tissue. Similarly to the xenograft tumor from the MZ-UPS-1 cells, the MZ-UPS-2 tumor exhibited clear neovascularization on the surface.

Chemotherapeutic treatment of undifferentiated pleomorphic sarcoma cell cultures *in vitro*. Doxorubicin is commonly used as the first-line treatment for patients with undifferentiated pleomorphic sarcoma. There is no widely accepted second-line treatment. However, novel therapeutic approaches have been recently identified as second-line treatments for patients with

recurrent or incurable sarcoma, including HDAC inhibitors and tyrosine kinase inhibitors, such as pazopanib, which has been approved in USA and Europe since 2012 for the treatment of distinct STS subtypes (14-19).

The two patients in the present study were administered doxorubicin; however, this treatment did not prevent tumor progression. Pazopanib was also used in the two patients as a second-line treatment, which resulted in the tumor becoming stable. During pazopanib treatment, symptom relief with increased tumor necrosis was observed in one patient (MZ-UPS-2), and in the other patient a mild tumor regression was observed (data not shown).

Prior to the *in vivo* experiments, the fourth passages of the two cell cultures, MZ-UPS-1 and MZ-UPS-2, were incubated with various concentrations of doxorubicin, pazopanib and the HDAC inhibitor SAHA, also known as vorinostat (Fig. 3). The anthracycline doxorubicin is a remarkably potent cytostatic drug, and caused the death of the MZ-UPS-1 cells within

Table II. Two novel undifferentiated pleomorphic sarcoma cell lines.

Features	MZ-UPS-1	MZ-UPS-2
Organism	Human	Human
Ethnicity	Caucasian	Caucasian
Age, years	47	48
Gender	Male	Female
Tissue	Mesenchymal	Mesenchymal
Morphology	Fibroblastic/myofibroblastic	Fibroblastic/myofibroblastic
Cell type	Pleomorphic sarcoma NOS, G3	Pleomorphic sarcoma NOS, G3
Growth properties	Monolayer	Monolayer
Culture medium	DMEM/F12-GlutaMAX™ Supplement with sodium pyruvate and 10% FCS	DMEM/F12- GlutaMAX™ Supplement with sodium pyruvate and 10% FCS
Split ratio	1:3-1:5 every week	1:2 every 2 weeks
Medium renewal	2-3 times weekly	2-3 times weekly
Tumorigenic	Yes, in NSG mice	Yes, in NSG mice

NOS, not otherwise specified; G3, grade 3; DMEM/F12, Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12; FCS, fetal calf serum; NSG, NOD SCID gamma; NOD, non-obese diabetic; SCID, severe combined immunodeficiency.

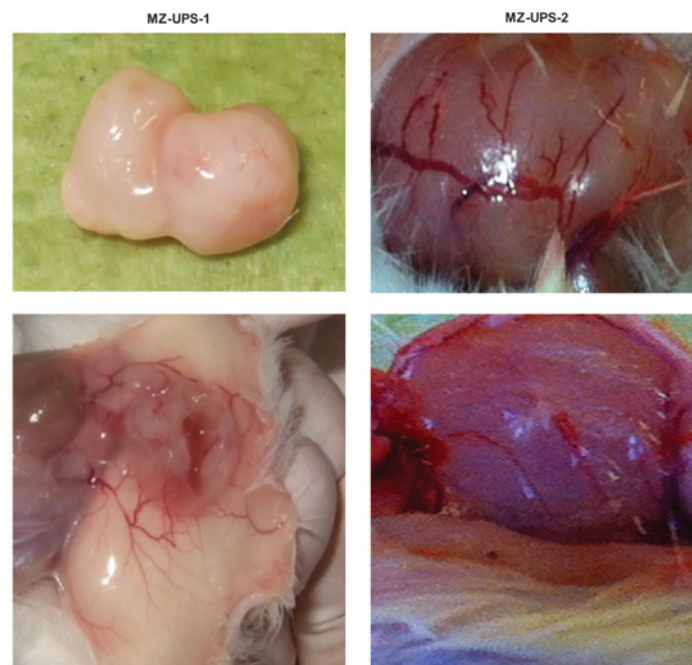


Figure 2. Tumor xenografts observed at 8-12 weeks subsequent to subcutaneous injection of undifferentiated pleomorphic sarcoma cells in NSG mice. Cultivated MZ-UPS-1 or MZ-UPS-2 cells were harvested and washed twice in phosphate-buffered saline. A total of 1×10^6 cells of each culture were injected subcutaneously into the right flank of NSG mice. Solid tumor tissues of $\leq 1.5 \text{ cm}^3$ in size were observed in all three pleomorphic sarcomas at 8-12 weeks following injection. NSG, NOD SCID gamma; NOD, non-obese diabetic; SCID, severe combined immunodeficiency.

14 days, even at a concentration of 50 nM. The viability of the MZ-UPS-2 cells began to decrease on day 7 following incubation with the highest concentration of doxorubicin tested (100 nM). On day 14, the viability of the MZ-UPS-2 cells was considerably decreased, even at a concentration of 10 nM doxorubicin. The viability of the two cell cultures was decreased on day 4 following incubation in the presence of 5 or 10 μM vorinostat. Pazopanib had no inhibitory effect on MZ-UPS-1 cells, and slightly inhibited the growth of MZ-UPS-2 cells. The combination of doxorubicin and pazopanib had no synergistic

effect on MZ-UPS-1 cells, and only caused a small synergistic reduction in the viability of MZ-UPS-2 cells.

Chemotherapeutic treatment in vivo. Immunodeficient NSG mice with xenografts of MZ-UPS-1 or MZ-UPS-2 cells were generated in the present study to compare the efficacy of different chemotherapeutic treatments *in vivo* (which is expected to be comparable to human patient response) with the cytostatic effects demonstrated by these chemotherapeutic drugs *in vitro*.

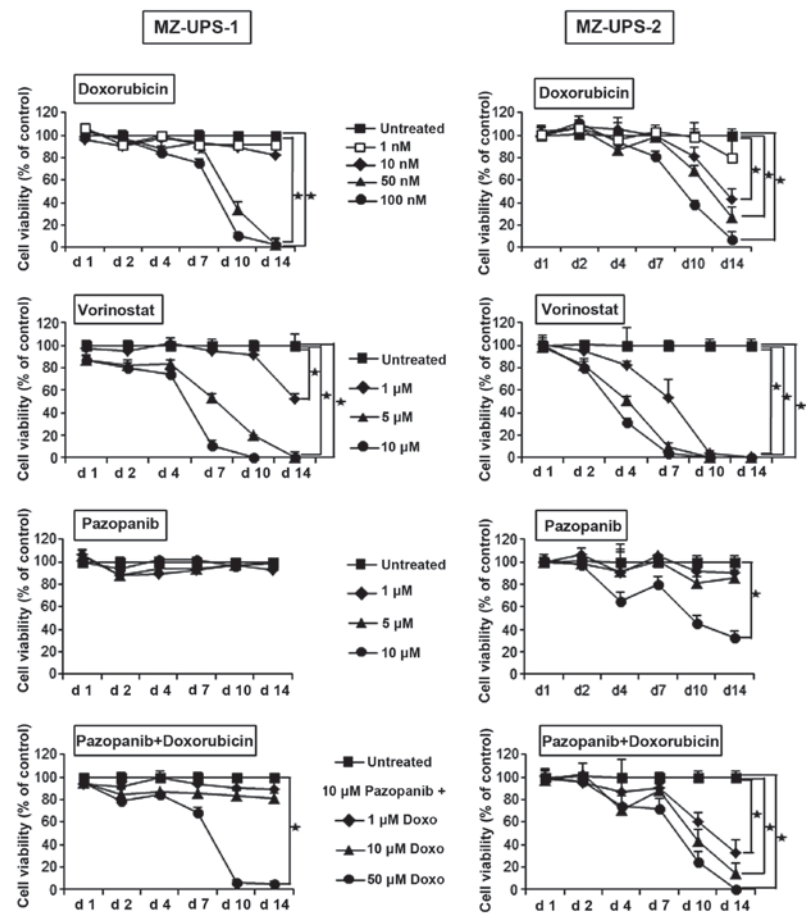


Figure 3. Chemotherapeutic treatment of undifferentiated pleomorphic sarcoma cells *in vitro*. Cultured cells from two patients with undifferentiated pleomorphic sarcoma, MZ-UPS-1 and MZ-UPS-2, were seeded onto 96-well plates at a concentration of 1×10^4 cells/100 μ l medium/well. Doxorubicin was added on day 0 and 7, while vorinostat and pazopanib were added daily. For the determination of cell viability, an alamarBlue™ assay was performed on days 1, 2, 4, 7, 10 and 14. The metabolized substrate was measured photometrically at 570 nm. All experiments were performed in triplicate. Data are presented as the mean \pm standard deviation. * $P \leq 0.01$ vs. untreated cells.

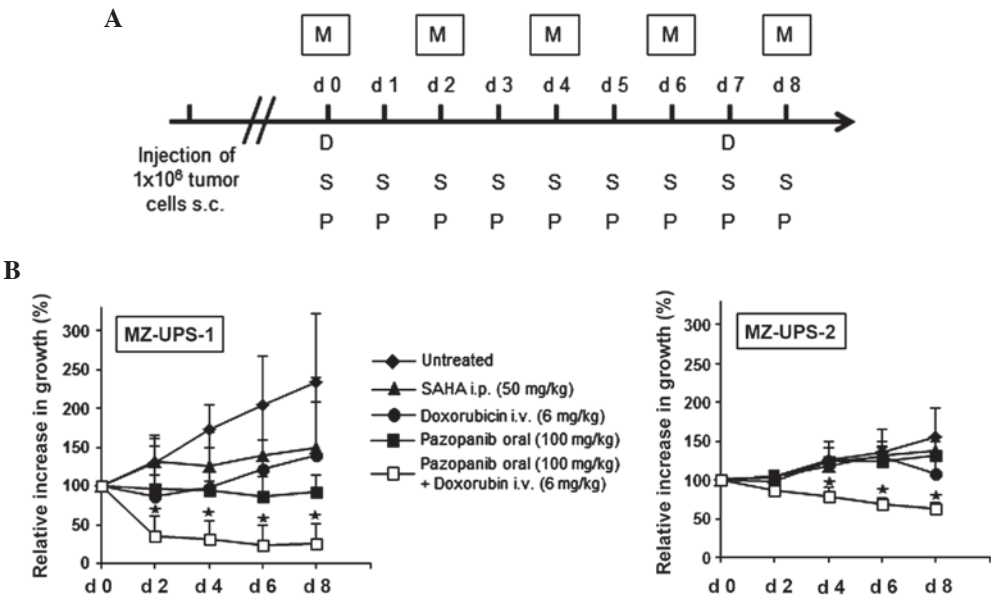


Figure 4. Combined therapy with doxorubicin and pazopanib resulted in a reduction of tumor size in the two xenograft sarcoma mouse models. *In vitro* cultured MZ-UPS-1 and MZ-UPS-2 cells were washed twice in phosphate-buffered saline, and 1×10^6 cells were injected subcutaneously into NSG mice. Chemotherapeutic treatment was started 6-8 weeks following xenotransplantation. (A) The chemotherapeutic regimen administered to the xenograft sarcoma mouse models was as follows: Doxorubicin was injected intravenously, while suberoylanilide hydroxamic acid and pazopanib were administered intraperitoneally and orally, respectively. (B) The initial tumor size of each mouse was normalized to 100%. Tumor size was measured every 2 days, and compared to the initial size. Relative tumor growth was averaged in each group. Data are presented as the mean \pm standard deviation. $n=5-10$ /group. * $P \leq 0.05$ vs. untreated. M, measurement; s.c., subcutaneously; D, doxorubicin; S/SAHA, suberoylanilide hydroxamic acid; P, pazopanib; i.p., intraperitoneally; i.v., intravenously.

Cells from the same passage were isolated and injected into NSG mice. The concentrations of the chemotherapeutic agents used and the frequency of application corresponded to treatment regimens that are normally employed to treat human patients with undifferentiated pleomorphic sarcoma (Fig. 4A), according to the guidelines from the German Society for Hematology and Medical Oncology (www.onkopedia-guidelines.info/en/onkopedia/guidelines). Tumor size from the mouse models was initially measured and normalized to 100% 6-8 weeks subsequent to the injection of the human undifferentiated pleomorphic sarcoma cells. The tumor size of MZ-UPS-1 mice that were not treated with chemotherapy had more than doubled within 8 days. By contrast, the tumor growth of mice treated with doxorubicin or SAHA was markedly decelerated, and treatment with pazopanib resulted in a stabilization of tumor size. Combined therapy with doxorubicin and pazopanib significantly reduced the tumor to <50% of its initial size (Fig. 4B).

The xenograft development of the MZ-UPS-2 cells was slower compared to that of MZ-UPS-1 cells (Fig. 4B), which was comparable to the growth of the MZ-UPS-2 cells *in vitro*. The tumor size of MZ-UPS-2 mice that were not treated with chemotherapy increased ~0.5 times in size 8 days subsequent to the initial measurement of the tumor. Following treatment with doxorubicin, SAHA and pazopanib, the pleomorphic tumor xenograft only marginally decreased in size. The combined therapy of doxorubicin and pazopanib led to a significant reduction in the tumor size, and therefore was the most promising therapeutic treatment *in vivo*. The *in vivo* results obtained in the present study are markedly different to the *in vitro* results, where doxorubicin and the HDAC inhibitor SAHA were the most potent chemotherapeutic agents, while pazopanib only marginally influenced tumor cell viability.

Discussion

Undifferentiated pleomorphic sarcoma is an extremely heterogeneous aggressive subgroup of soft tissue sarcoma (2). Due to the heterogeneity of undifferentiated pleomorphic sarcoma, numerous studies are focused on developing individualized therapeutic strategies for patients, instead of administering a standard chemotherapy to all patients (29). Since there is a limited number of patients affected by each of the undifferentiated pleomorphic sarcoma subgroups, no large clinical trial has been conducted to date to evaluate the efficiency of chemotherapeutic treatment. Therefore, it is crucial to develop preclinical tools that allow the evaluation of individualized therapeutic approaches. Previous studies have demonstrated that there are distinct histopathological differences between the different subtypes of undifferentiated pleomorphic sarcoma, and have revealed a panel of molecular markers that may significantly aid the development of an optimal management regimen for patients with undifferentiated pleomorphic sarcoma (30,31). Consequently, reliable and reproducible preclinical animal models are required, which are similar to the oligoclonal biological diversity observed in human patients, for testing various targeted therapeutic approaches for individual patients.

The present study established two xenograft animal models generated from stable undifferentiated pleomorphic sarcoma

cell cultures to investigate the efficacy of chemotherapeutic regimens for the treatment of undifferentiated pleomorphic sarcoma *in vivo* vs. *in vitro*. The results of the present study demonstrated that there is a clear discrepancy between the *in vitro* cell culture and the *in vivo* xenograft model, which is comparable to a human treatment scenario. The mouse model reflects the local microenvironment of a human tumor, which appears to be crucial to allow a predictive analysis of treatment regimens in addition to monitoring direct cytotoxic effects of drugs (32).

Understanding the various biological sensitivities of the various histological subtypes of undifferentiated pleomorphic sarcoma may lead to the development of individual therapeutic targeted approaches (33).

In contrast to other approaches using solid tumor tissue or silicon chambers to place tumor fragments around the superficial epigastric vessels (26,27), the present study generated stable oligoclonal cell cultures from freshly isolated tumor tissue of two patients with undifferentiated pleomorphic sarcoma, which were similar to the oligoclonal variety exhibited by the original tumors. Additionally, these cultures were subcutaneously injected into immunodeficient mice to establish xenograft animal models. The present study observed that neovascularization was identical between the original tumor and the xenograft tumor. There were no regions of hypoxemia in the xenograft tumor, which is important, as it allows the analysis of anti-angiogenic therapeutic approaches and rules out the possibility of anomalous results that hypoxic conditions may generate during homing and engraftment of the tumor (34).

Furthermore, the present study treated the tumors *in vivo* and *in vitro* with the most common or innovative chemotherapeutic agents currently available. Only in the xenograft mouse model the results observed were comparable to the treatment results of the two patients from whom the original tumors were resected. Therefore, tumor derived cell cultures do not reflect the actual treatment condition that is observed in patients. Notably, the combination of doxorubicin and pazopanib significantly reduced the tumor size with an acceptable toxicity level, in terms of weight loss (<20%), movement disorder and apathy (data not shown).

In addition, the novel xenograft models allow chemotherapeutic analysis at various time points, which may lead to the identification of molecular mechanisms associated with pleomorphic sarcoma development and progression, and other local tumor-tissue interactions.

In conclusion, there is a discrepancy in tumor growth and cell viability between *in vitro* and *in vivo* models concerning chemotherapeutic treatments. The novel and reproducible xenograft animal models generated in the present study have demonstrated that *in vivo* models are required to test potential chemotherapeutic agents for the treatment of undifferentiated pleomorphic sarcoma, since they provide similar results to those observed in human patients, compared with *in vitro* models.

Acknowledgements

The present study was supported by the Internal University Initial Research Founding of the Johannes Gutenberg University of Mainz (Mainz, Germany).

References

- Iwasaki H, Isayama T, Johzaki H and Kikuchi M: Malignant fibrous histiocytoma. Evidence of perivascular mesenchymal cell origin immunocytochemical studies with monoclonal anti-MFH antibodies. *Am J Pathol* 128: 528-537, 1987.
- Katenkamp K and Katenkamp D: Soft tissue tumors: New perspectives on classification and diagnosis. *Dtsch Arztebl Int* 106: 632-636, 2009.
- Poremba C: Soft tissue sarcomas: The role of histology and molecular pathology for differential diagnosis. *Verh Dtsch Ges Pathol* 90: 59-72, 2006 (In German).
- Al-Agha OM and Igbokwe AA: Malignant fibrous histiocytoma: Between the past and the present. *Arch Pathol Lab Med* 132: 1030-1035, 2008.
- Matushansky I, Charytonowicz E, Mills J, Siddiqi S, Hricik T and Cordon-Cardo C: MFH classification: Differentiating undifferentiated pleomorphic sarcoma in the 21st Century. *Expert Rev Anticancer Ther* 9: 1135-1144, 2009.
- Spira AI and Ettinger DS: The use of chemotherapy in soft-tissue sarcomas. *Oncologist* 7: 348-359, 2002.
- Grimer R, Judson I, Peake D and Seddon B: Guidelines for the management of soft tissue sarcomas. *Sarcoma* 2010: 506182, 2010.
- Italiano A, Mathoulin-Pelissier S, Cesne AL, Terrier P, Bonvalot S, Collin F, Michels JJ, Blay JY, Coindre JM and Bui B: Trends in survival for patients with metastatic soft-tissue sarcoma. *Cancer* 117: 1049-1054, 2011.
- Daigeler A, Klein-Hitpass L, Stricker I, Müller O, Kuhnen C, Chromik AM, Steinstraesser L, Goertz O, Steinau HU and Lehnhardt M: Malignant fibrous histiocytoma - pleomorphic sarcoma, NOS gene expression, histology, and clinical course. A pilot study. *Langenbecks Arch Surg* 395: 261-275, 2010.
- Fletcher CDM, Unni KK and Mertens F (eds): World Health Organisation Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. IARC Press, Lyon, 2002.
- Casali PG and Blay JY: ESMO/CONTICANET/EUROBONET Consensus Panel of experts: Soft tissue sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 21 (Suppl 5): v198-v203, 2010.
- Italiano A, Le Cesne A, Mendiboure J, Blay JY, Piperno-Neumann S, Chevreau C, Delcambre C, Penel N, Terrier P, Ranchere-Vince D, *et al*: Prognostic factors and impact of adjuvant treatments on local and metastatic relapse of soft-tissue sarcoma patients in the competing risks setting. *Cancer* 120: 3361-3369, 2014.
- Leahy M, Garcia Del Muro X, Reichardt P, Judson I, Staddon A, Verweij J, Baffoe-Bonnie A, Jönsson L, Musayev A, Justo N, *et al*: SABINE Investigators: Chemotherapy treatment patterns and clinical outcomes in patients with metastatic soft tissue sarcoma. The SARcoma treatment and Burden of Illness in North America and Europe (SABINE) study. *Ann Oncol* 23: 2763-2770, 2012.
- van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, Schöffski P, Aglietta M, Staddon AP, Beppu Y, *et al*: EORTC Soft Tissue and Bone Sarcoma Group; PALETTE study group: Pazopanib for metastatic soft-tissue sarcoma (PALETTE): A randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 379: 1879-1886, 2012.
- Ray-Coquard I and Thomas D: Targeted therapies: Pazopanib for soft-tissue sarcoma: A PALETTE of data emerges. *Nat Rev Clin Oncol* 9: 431-432, 2012.
- Nguyen DT and Shayahi S: Pazopanib: Approval for soft-tissue sarcoma. *J Adv Pract Oncol* 4: 53-57, 2013.
- Ranieri G, Mammi M, Donato Di Paola E, Russo E, Gallelli L, Citraro R, Gadaleta CD, Marech I, Ammendola M and De Sarro G: Pazopanib a tyrosine kinase inhibitor with strong anti-angiogenic activity: A new treatment for metastatic soft tissue sarcoma. *Crit Rev Oncol Hematol* 89: 322-329, 2014.
- Tavallai S, Hamed HA, Grant S, Poklepovic A and Dent P: Pazopanib and HDAC inhibitors interact to kill sarcoma cells. *Cancer Biol Ther* 15: 578-585, 2014.
- Schöffski P, Cornillie J, Wozniak A, Li H and Hompes D: Soft tissue sarcoma: An update on systemic treatment options for patients with advanced disease. *Oncol Res Treat* 37: 355-362, 2014.
- Nakatani T, Marui T, Yamamoto T, Kurosaka M, Akisue T and Matsumoto K: Establishment and characterization of cell line TNMY1 derived from human malignant fibrous histiocytoma. *Pathol Int* 51: 595-602, 2001.
- Krause AK, Hinrichs SH, Orndal C, DeBoer J, Neff JR and Bridge JA: Characterization of a human myxoid malignant fibrous histiocytoma cell line, OH931. *Cancer Genet Cytogenet* 94: 138-143, 1997.
- Mairal A, Chibon F, Rousselet A, Couturier J, Terrier P and Aurias A: Establishment of a human malignant fibrous histiocytoma cell line, COMA. Characterization by conventional cytogenetics, comparative genomic hybridization, and multiplex fluorescence *In situ* hybridization. *Cancer Genet Cytogenet* 121: 117-123, 2000.
- Hakozaki M, Hojo H, Sato M, Tajino T, Yamada H, Kikuchi S and Abe M: Establishment and characterization of a new cell line, FPS-1, derived from human undifferentiated pleomorphic sarcoma, overexpressing epidermal growth factor receptor and cyclooxygenase-2. *Anticancer Res* 26 (5A): 3393-3401, 2006.
- Nishio J, Iwasaki H, Nabeshima K, Ishiguro M, Isayama T and Naito M: Establishment of a new human pleomorphic malignant fibrous histiocytoma cell line, FU-MFH-2: Molecular cytogenetic characterization by multicolor fluorescence *in situ* hybridization and comparative genomic hybridization. *J Exp Clin Cancer Res* 29: 153, 2010.
- Steinstraesser L, Jacobsen F, Schubert C, Gevers K, Stricker I, Steinau HU and Al-Benna S: Establishment of a primary human sarcoma model in athymic nude mice. *Hum Cell* 23: 50-57, 2010.
- Tilkorn DJ, Daigeler A, Hauser J, Ring A, Stricker I, Schmitz I, Steinstraesser L, Steinau HU and Al-Benna S: A novel xenograft model with intrinsic vascularisation for growing undifferentiated pleomorphic sarcoma NOS in mice. *J Cancer Res Clin Oncol* 138: 877-884, 2012.
- Tilkorn DJ, Stricker I, Hauser J, Ring A, Schmitz I, Steinstraesser L, Steinau HU, Daigeler A and Al-Benna S: Experimental murine model of primary high grade undifferentiated pleomorphic sarcoma not otherwise specified. *In Vivo* 26: 559-563, 2012.
- Fletcher CDM, Bridge JA, Hogendoorn P, Mertens F (eds): World Health Organization Classification of Tumours of Soft Tissue and Bone. 4th edition. Volume 5. IARC, Lyon, 2013.
- Radaelli S, Stacchiotti S, Casali PG and Gronchi A: Emerging therapies for adult soft tissue sarcoma. *Expert Rev Anticancer Ther* 14: 689-704, 2014.
- Reichardt P: Soft tissue sarcomas, a look into the future: Different treatments for different subtypes. *Future Oncol* 10 (Suppl 8): s19-s27, 2014.
- Villacis RA, Silveira SM, Barros-Filho MC, Marchi FA, Domingues MA, Scapulatempo-Neto C, Aguiar S Jr, Lopes A, Cunha IW and Rogatto SR: Gene expression profiling in leiomyosarcomas and undifferentiated pleomorphic sarcomas: SRC as a new diagnostic marker. *PLoS One* 9: e102281, 2014.
- Villacis RA, Silveira SM, Barros-Filho MC, Marchi FA, Domingues MA, Scapulatempo-Neto C, Aguiar S Jr, Lopes A, Cunha IW and Rogatto SR: Gene expression profiling in leiomyosarcomas and undifferentiated pleomorphic sarcomas: SRC as a new diagnostic marker. *PLoS One* 9: e102281, 2014.
- Versleijen-Jonkers YM, Vletterie M, van de Luijngaarden AC and van der Graaf WT: Anti-angiogenic therapy, a new player in the field of sarcoma treatment. *Crit Rev Oncol Hematol* 91: 172-185, 2014.
- Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, Arcaroli JJ, Messersmith WA and Eckhardt SG: Patient-derived tumour xenografts as models for oncology drug development. *Nat Rev Clin Oncol* 9: 338-350, 2012.