Drug sensitivity testing on patient-derived sarcoma cells predicts patient response to treatment and identifies c-Sarc inhibitors as active drugs for translocation sarcomas

Bertha A. Brodin1, Krister Wennerberg2,3, Elisabet Lidbrink4, Otte Brosjö5, Swapnil Potdar2, Jennifer N. Wilson1, Limin Ma1, Lotte N. Moens7, Asle Hesla9, Edwin Porovic1, Edwin Bernhardsson1, Antroula Papakonstantinou6,8, Henrik Bauer5, Panagiotis Tsagkozis5,8, Karin von Sivers9, Johan Wejde6, Päivi Östling8, Olli Kallioniemi8 and Christina Linder Stragliotto4

BACKGROUND: Heterogeneity and low incidence comprise the biggest challenge in sarcoma diagnosis and treatment. Chemotherapy, although efficient for some sarcoma subtypes, generally results in poor clinical responses and is mostly recommended for advanced disease. Specific genomic aberrations have been identified in some sarcoma subtypes but few of them can be recommended with approved drugs.

METHODS: We cultured and characterised patient-derived sarcoma cells and evaluated their sensitivity to 525 anti-cancer agents including both approved and non-approved drugs. In total, 14 sarcomas and 5 healthy mesenchymal primary cell cultures were studied. The sarcoma biopsies and derived cells were characterised by gene panel sequencing, cancer driver gene expression and by detecting specific fusion oncoproteins in situ in sarcomas with translocations.

RESULTS: Soft tissue sarcoma cultures were established from patient biopsies with a success rate of 58%. The genomic profile and drug sensitivity testing on these samples helped to identify targeted inhibitors active on sarcomas. The cSrc inhibitor Dasatinib was identified as an active drug in sarcomas carrying chromosomal translocations. The drug sensitivity of the patient sarcoma cells ex vivo correlated with the response to the former treatment of the patient.

CONCLUSIONS: Our results show that patient-derived sarcoma cells cultured in vitro are relevant and practical models for genotypic and phenotypic screens aiming to identify efficient drugs to treat sarcoma patients with poor treatment options.

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epithelial origin. These are most common in the TP53, RB and ATRX genes.11,12

This information has been useful to identify genetic aberrations in tumours that can be targeted by newly developed drugs.5,10,13 However, to date, only a few of these alterations are matched to approved drugs and less than 5% of the information generated is used to guide patient treatment. The therapeutic efficacy of large-scale genetic profiling on patient tumours is still challenging. Phenotypic or functional screening can be an alternative to overcome this gap. It refers to the identification of molecules with particular biological effects using cell, tissue-based assays or animal models. Phenotypic screening using cancer models such as patient-derived tumour cells (PDCs), organoids and patient-derived xenografts (PDX) is an efficient approach to identify treatments or new therapeutic indications for approved drugs.14,15

The use of PDCs and PDXs in drug screens is gaining considerable interest since these models represent a closer surrogate to the patient tumour. Furthermore, the use of approved drugs for other treatment indications can be accelerated since the preclinical and safety studies in humans have already been performed.

In the present study, we cultured patient-derived sarcoma cell and characterised these cells and the biopsy of origin by analysing gene mutations, cancer driver gene and fusion protein expression. We further determined the sensitivity of the patient-derived sarcoma cells to a library of approved and investigational drugs to evaluate if this approach can be useful to identify targeted inhibitors with potential therapeutic effects for individual sarcoma patients.

MATERIAL AND METHODS

Establishment of sarcoma patient-derived cells (PDC)

Patient-derived cells were dissociated from solid biopsies through enzymatic digestion using a 0.1% collagenase mixture for 1 hour followed by mechanic grinding. Most sarcoma cells were grown in Dulbecco’s modified Eagle’s medium/Ham’s nutrient mixture F12 (DMEM/F12) supplemented with 15% fetal bovine serum (FBS), non-essential amino acids, penicillin and streptomycin. Angiosarcoma cells were cultured in endothelial cell media containing vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and basic fibroblast growth factor (FGF). Muscle cells were grown in skeletal muscle cell media containing 5% fetal calf serum, basic FGF, insulin, and dexamethasone (Promocell, Germany). Ewing’s sarcoma (ES), alveolar rhabdomyosarcoma (ARMS) and angiosarcoma samples were obtained by needle aspiration (FNA) from palpable tumours. The content of tumour cells in the biopsy size, cell viability and histological subtype.

Logarithmic growing patient-derived cells (PDC) were established within a range from 2 days to 18 weeks depending on the biopsy size, cell viability and histological subtype.

Proximity ligation assay (PLA)

Primary cells were cultured in chamber slides for 24 h; thereafter the medium was removed, and the cells were washed twice with phosphate buffered saline (PBS) and fixed for 15 min in 4% paraformaldehyde, following permeabilisation with 0.1% TritonX100 for 15 min. The PLA was performed according to the in situ Fluorescence PLA protocol (Sigma-Aldrich). For detection of fusion proteins, we used two antibodies raised in different species, one recognising the N-terminus and the second recognising the C-terminus of each chimeric protein. Species-specific antibodies conjugated with DNA oligonucleotides were then used to recognise the primary antibody. These DNA probes can be ligated to form a circular probe only in the case that the two antibodies are in the proximity. The DNA probe is subsequently amplified using DNA polymerase and a fluorescent-labelled deoxynucleotide in the amplification reaction allowing the visualisation of the fusion protein in the UV-light range using a confocal microscope.

Cancer driver gene expression

RNA was isolated using AllPrep (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and the quality of the RNA was evaluated using a bioanalyzer (Agilent, Sta Clara CA, USA). The expression of 180 cancer driver genes (Supplementary table 4) was performed using quantitative RT-PCR arrays (RealTimePrimers, Elkin Park PA, USA) and AzuraQuant16 Green Fast qPCR Mix green master mix (Azura Genomics, Rayham, MA USA). Gene expression analysis was calculated using the Livak method, in which the ΔCT values were calculated by normalising values for each gene to the housekeeping genes, ΔΔCT was then calculated by normalising the ΔCT values of either biopsy of patient-derived cells to that of the normal muscle cells and used to determine the fold change (2−ΔΔCT).

Drug sensitivity testing on patient-derived sarcoma cells predicts.

Drug sensitivity and resistance testing (DSRT) developed at the Institute for Molecular Medicine Finland (FIMM).16–18 The FIMM library is composed of 525 agents of which, approximately 40% are drugs approved for treatment of different human cancers and the remaining 60% are being investigated in clinical trials. In this system, drug activity is calculated taking into consideration the response curve and the IC50 for each drug and it is expressed as drug sensitivity score (DSS).19

The compounds were dispensed in 384-well plates (Corning, Tewksbury, MA, USA) at five different concentrations covering a range between 1 and 10,000 nM using acoustic liquid handling (Echo 550, Labcyte Inc, Sunnyvale, CA, USA).

Early passaged (passes 2–5) PDC growing as monolayers were collected by tripinisation. Twenty-five microliters of single-cell suspensions containing 500–2000 cells were dispensed to each well using a Multidrop Combi Reagent Dispenser (Thermo Fisher Scientific, Maltham MA, USA). Plates were subsequently centrifuged, put on an orbital shaker for 10 min followed by additional 10 min incubation at room temperature to allow the cells to plate to the bottom.

The plates were incubated for 72 h at 37 °C, 5% CO2 and a humidified environment. Cell viability was assessed by determining the total adenosine tri-phosphate (ATP) levels in living cells using CellTiter-Glo (CTG) luminescent cell viability assay (Promega, Madison, WI, USA). Luminescence was measured using a multi-mode plate reader (Tecan F-Plex, Switzerland).

The selective drug sensitivity score (sDSS) was calculated by subtracting the DSS of normal cell controls (healthy bone marrow mononuclear cells or normal mesenchymal primary cells) from the DSS of sarcoma cells.

Target enrichment and next-generation sequencing

Target enrichment was performed with the HaloPlex target enrichment system (Agilent Technologies), according to the manufacturer’s instructions. A custom gene panel was designed, covering the coding regions of the 16 target genes listed in Supplementary Table 1. The target genes were captured by 5063 probes, covering a total region of approximately 76 kb. The quality and molarity of the sequencing libraries was assessed on a 2200 TapeStation instrument using a D1000 Screen tape (Agilent Technologies). The enriched and barcoded targets were then sequenced on a MiniSeq or NextSeq instrument (Illumina).

Data analysis

Illumina sequencing adaptors were removed by cutAdapt version 1.8.020 and the trimmed reads were subsequently aligned to the
reference genome (hg19, March 2009 assembly) using BWA mem version 0.7.12 allowing for a maximum of 5% mismatches in a read. Single-nucleotide variation detection was performed using SNPmania version 0.0.7 (Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden) (Ljungström et al, unpublished data, available on request). Insertion and deletion detection was performed using Pindel version 0.2.5a8 with parameters -x 2, allowing the detection of indels up to 512 bp, and -B 100 allowing the detection of structural variants of size \( \sim 100 \) bp. Only positions having a read depth \( \geq 30 \) and variant allele frequency \( \geq 1\% \) were considered. The variants were further filtered to remove all synonymous mutations, as well as variants having a frequency \( > 1\% \) in the 1000Genome project\(^{20,21} \) and the filtered to remove all synonymous mutations, as well as variants having a frequency \( > 1\% \) in the 1000Genome project\(^{20,21} \) and the

RESULTS

Establishment of patient-derived tumour cells (PDC) from sarcoma patients

Soft tissue sarcomas were obtained by surgical excision, or by FNA and were used to establish patient-derived sarcoma cell cultures, hereby denoted as PDC. Normal healthy control cells were also isolated and cultured from three healthy muscle biopsies, one epithelial bladder biopsy, from umbilical cord-derived mesenchymal stem cells (commercial) and from the bone marrow of two healthy donors. Representative samples from the biopsies were dissected for (a) deep freezing, (b) formalin-fixed and paraffin embedding (FFPE), (c) genotypic and transcription profiling and (d) establishment of the PDC (Fig. 1). Fourteen primary cell cultures could be grown in vitro from a total of 24 sarcoma biopsies (58% recovery). The success in establishing the primary cultures was dependent on the viability and size of the biopsy and the treatment of the patient prior biopsy. The time from biopsy to drug screening varied from 2 to 128 days, depending on these conditions (Table 1). To investigate if the outgrowing PDC still resemble the sarcoma tissues from which they were originated, we determined the expression of fusion proteins in sarcomas with translocations by proximity ligation assay (PLA) or RT-PCR. PDCs originating from sarcomas with undefined genetic markers were characterised by gene panel sequencing (Supplementary Table 1 and 2) and cancer-driver gene expression (Supplementary Figure 1 and Supplementary Table 4). The sarcoma subtypes and genetic characteristics of the samples used in this study are presented in Table 1.

Drug sensitivity and resistance testing (DSRT) on patient-derived sarcoma cells (PDC)

The comparison of the DSS values among our sarcoma cohort (14 cases) showed that drug classes such as histone deacetylase (HDAC), cyclin-dependent kinase (CDK), proteasome, mitosis, and mTOR inhibitors were active in most of the sarcoma subtypes tested. However, when normalising the DSS of the sarcoma PDCs to that of healthy cells (bone marrow and mesenchymal controls), to obtain the sDSS, we identified selective inhibitors such as Dasatinib (Supplementary Figure 2).

We therefore correlated the drug responses for individual sarcoma cases in relation to both healthy bone marrow and healthy mesenchymal controls. In this stringent analysis, an sDSS above 5 was considered a potential hit.

In the present study we show the functional and genotypic analysis of six cases of patients affected with sarcomas with translocations consisting of one aRMS, two alveolar soft part sarcomas (ASPS), one synovial sarcoma (SS) and two Ewing sarcoma (ES).

Case 1. Alveolar rhabdomyosarcoma (RMS1)

A 19-year-old male developed a primary tumour in the prostate that was diagnosed as a PAX3-FOXO1-positive aRMS. He underwent treatment according to the Italian Sarcoma Group/
Scandinavian Sarcoma Group protocol III (ISG/SSGIII) consisting of doxorubicin, vincristine and cisplatin (Supplementary Table 3). The patient had a refractory and disseminated disease with multiple metastasis in the lung, sacrum, arm and neck at the time of biopsy. A sample from a palpable neck lesion was obtained by FNA for drug screening ex vivo (Fig. 2a).

We detected the expression of the PAX3-FOXO1A fusion transcript in the PDC (K-RMS1) by RT-PCR confirming the presence of aRMS cells (Fig. 2b). Among the cancer driver genes expressed in K-RMS-1 we found increments in the expression of BAX, RASD1, AKT1, cMYC and the expression of the ASPS1-TFE3 fusion protein was obtained after palliative surgery for the establishment of the PDC (K-ASPS3). The ASPS1-TFE3 was not detected by routine diagnosis in the biopsy (data not shown) nor in the derived PDC; there the ASPS1-TFE3 was not detected by routine diagnosis (K-ASPS3). The ASPS1-TFE3 was not detected by routine diagnosis in the biopsy (data not shown) nor in the derived PDC; there the ASPS1-TFE3 was not detected by routine diagnosis.

Table 1. Origin and characteristics of patient-derived cells (PDC)

| PDC                  | Histological subtype                  | Biopsy method | Biopsy to PDC time (days) | Genetic marker         |
|----------------------|---------------------------------------|---------------|---------------------------|------------------------|
| Sarcoma with translocations |
| K-RMS1              | Alveolar rhabdomyosarcoma             | FNA           | 44                        | PAX3-FOXO1             |
| K-ASPS2             | Alveolar soft part sarcoma            | FNA           | 31                        | ASPSCR1-TFE3           |
| K-ASPS3             | Alveolar soft part sarcoma            | FNA           | 9                         | TP53 mutation (p.R205H) |
| K-ES1               | Ewing sarcoma                         | FNA           | 110                       | EWSR1-FLI              |
| K-ES2               | Ewing sarcoma                         | FNA           | 49                        | EWSR1-FLI              |
| K-SS3               | Synovial sarcoma                      | FNA           | 49                        | SS18-SSX               |
| K-SS2               | Synovial sarcoma                      | S             | 128                       | SS18-SSX not detected  |
| Sarcomas with complex genomes |
| K-MPNST1            | Malignant peripheral nerve sheath tumour | S            | 19                        | TP53 mutation (p.R150W) |
| K-MPNST2            | Malignant peripheral nerve sheath tumour | S            | 47                        | Not analysed           |
| K-MPNST3            | Malignant peripheral nerve sheath tumour | S            | 30                        | No mutations found     |
| K-AS1               | Angiosarcoma                          | FNA           | 45                        | No mutations found     |
| K-UPS1              | Undifferentiated pleomorphic sarcoma   | S             | 32                        | No mutations found     |
| K-MFS1              | Myxoid fibrosarcoma                    | S             | 2                         | TP53 (p.P146S);BRAF (p.Q257H) |
| Healthy controls    |
| K-LMS1              | Leiomyosarcoma                         | FNA           | 77                        | PI4KA (p.R906H)        |
| K MC-1              | Normal muscle                          | S             | 23                        | Not analysed           |
| K MC-2              | Normal muscle                          | S             | 18                        | Not analysed           |
| K MC-3              | Normal muscle                          | S             | 19                        | Not analysed           |
| K MC-4              | Mesenchymal stem cells (commercial)    | UC            | 2                         | Not analysed           |
| K MC-5              | Normal bladder fibroblasts             | S             | 20                        | Not analysed           |

The primary tumour was surgically removed, and the diagnosis was confirmed morphologically as alveolar soft part sarcoma (Fig. 3a). Fresh primary tumour was used for the establishment of the PDC (K-ASPS3). The ASPS1-TFE3 was not detected by routine diagnosis in the biopsy (data not shown) nor in the derived PDC; there the PLA showed cytoplasmic but not nuclear ASPS1-TFE3 signals (Fig. 3b).

Cancer driver gene expression was quantified in the ASPS3 biopsy and PDC. The apoptotic regulator (BAX), the dexamethasone-induced Ras-related (RASD1), the Wilms Tumour 1 (WT1) and mTOR (FRAP1) kinase genes were among the most...
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**Fig. 2**  
Alveolar rhabdomyosarcoma patient-derived cells (K-RMS1).  

**a**  
Giemsa staining of the fine needle aspiration biopsy (FNA) showing high content of rhabdomyosarcoma cells and a light microscopy picture (×10) of the derived PDC. **b** RT-PCR showing the expression of PAX3-FOXO1A in the PDC (K-RMS-1) after 2 and 8 weeks of in vitro culturing. RH30 is an alveolar rhabdomyosarcoma cell line used as a positive control. Primary muscle cells were used as negative control. **c** Heatmap illustrating cancer driver genes expressed in K-RMS-1 at the time of drug screening. Relative expression (normalised to muscle cells) is expressed as log2 fold change. Values were calculated using the Livak method. **d** Plot showing the selective drug sensitivity scores (sDSS) of K-RMS1 in relation to normal bone marrow mononuclear cells (Y-axis), and healthy mesenchymal cell controls (X-axis). The patient treatment at the time of biopsy is highlighted in red.

**Fig. 3**  
Alveolar soft part sarcoma patient-derived cells (K-ASPS2 and K-ASPS3).  

**a** Haematoxylin and eosin staining of formalin-fixed ASPS biopsies and the derived PDC (visualised by light microscopy). **b** Proximity ligation assay showing nuclear expression of the ASPS1-TFE3 fusion protein in the K-ASPS2 cells and cytoplasmic signals in K-ASPS3. Muscle cells as negative control. **c** Expression of cancer driver genes determined by Q-RT-PCR in the K-ASPS3 relative to normal muscle cells. **d** Plot showing the drug activity in K-ASPS2 and **e** K-ASPS3 respectively. Patient treatment at the time of biopsy is highlighted in red.
expressed genes in relation to normal muscle cells (Fig. 3d and supplementary Fig. 1).

Consistent with the activation of mTOR/PI3K pathway in the ASPS3 biopsy and derived PDC, several mTOR/PI3K inhibitors were active in functional screens such as PF04691502, Dactolisib and Belinostat with sDSS values above 10 in relation to healthy bone marrow cell controls. Other selective hits for K-ASPS3 were the purine analogue Fludarabine, the folate anti-metabolite Pemetrexed, the cell division cycle 7 homologue (CD7) kinase inhibitor BMS-863233, and the topoisomerase inhibitor Topotecan. Similar to K-ASPS2 and K-RMS1, Dasatinib was an active drug in K-ASPS3 (Fig. 3e, green dot).

This patient was treated with Sunitinib™, developed progressive disease with brain metastasis that was treated with whole brain radiation 4GyX5; and later developed lung metastases and was treated with doxorubicin. The patient has progressive disease at present, with new cerebellar lesions, underwent surgery and is now on treatment with Dasatinib.

Case 3. Synovial sarcoma
A 33-year-old female presented with a 9.9 x 7 cm tumour in the right scapula of a 26-year-old male, prior to oncologic treatment (Fig. 5a). The EWSR1-FI1 fusion was detected in the biopsy (data not shown) and in 95% of the cells in the PDC confirming the presence of Ewing sarcoma cells (Fig. 5b). Cancer driver gene expression was characterised by the expression of the DNA damage inducible transcript 3 (DDIT3), the mTOR kinase gene FRAP1, the anti-apoptotic gene BCL2 and several autophagy-related genes of the ATG and SEC family, and downregulation of the TP53 gene among others (Fig. 5c).

The patient presented with bone marrow, skeleton and lung metastases at diagnosis and was treated with Scandinavian Sarcoma Group protocol IV (SSGIV) protocol consisting of cisplatin, doxorubicin, etoposide and vincristine with no clinical response. Several mTOR/P13K (Fig.4e, turquoise dots) and the cSrc inhibitor Dasatinib (Fig. 4e, green dot) showed anti-tumour activity in K-SS3. At present, the patient has no evidence of disease.

Case 4. Ewing sarcoma
Two Ewing sarcomas PDC were established from FNA biopsies of two patients: K-ES1 and K-ES2. K-ES1 was obtained from an FNA of a primary tumour in the right scapula of a 26-year-old male, prior to oncologic treatment (Fig. 5a). The EWSR1-FI1 fusion was detected in the biopsy (data not shown) and in 95% of the cells in the PDC confirming the presence of Ewing sarcoma cells (Fig. 5b). Cancer driver gene expression was characterised by the expression of the DNA damage inducible transcript 3 (DDIT3), the mTOR kinase gene FRAP1, the anti-apoptotic gene BCL2 and several autophagy-related genes of the ATG and SEC family, and downregulation of the TP53 gene among others (Fig. 5c).

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![Diagram](image-url)
The patient developed systemic disease and died few months after diagnosis.

K-ES2 originated from a 31-year-old male diagnosed of Ewing sarcoma. The patient received adjuvant treatment with vincristine, doxorubicin and cyclophosphamide (SSG1V) and radiotherapy (1.8 GyX28), followed by autologous stem cell transplantation (Supplementary Table 3). Thereafter, the patient underwent surgery with complete resection of the primary tumour. After a 3-year disease-free survival the patient developed metastasis in the third right rib. An FNA biopsy was taken from this lesion and the tumour cells were cultured in vitro (K-ES2). The EWS-FLI1 fusion protein specific for Ewing's sarcoma was expressed in 95% of the cells in the PDC culture (Fig. 5b). The patient metastasis was treated with docetaxel and gemcitabine before surgery according to the SSG1V protocol and achieved complete response (Fig. 5e). Consistent with the clinical response of the patient, the ex vivo drug sensitivity testing of his tumour cells (K-ES2) showed activity for the taxanes docetaxel, paclitaxel and cabazitaxel (Fig. 5d), but not for gemcitabine suggesting that the complete clinical response could be attributed to the taxane treatment.

The cSarc and ALK inhibitor Dasatinib is active in several sarcoma subtypes. Dasatinib/Sprycel is a tyrosine kinase inhibitor that blocks the activity of several tyrosine kinases including Bcr-Abl and members of the Src kinase family. Dasatinib was active in all PDCs derived from sarcomas with translocations and other sarcoma subtypes (Figs. 2 – 5 and Supplementary Figure 2).

**DISCUSSION**

Phenotypic screening for anticancer drugs has long relied on cytotoxic assays using cancer cell lines, spheroids and xenografts. Although these models have been used extensively for cancer drug development, they have important limitations since serially passaged cell lines lose the genetic characteristics of the primary tumours of origin. Genomic sequencing of cancer cell lines has shown that mutations accumulate during serial passages.

Gene expression is even more affected in cultured cell lines, likely as the result of selection pressure that allows the cells to survive under in vitro conditions. Genes associated with multidrug resistance or with the p53 pathway are reactivated upon in vitro cell resistance. We observed an overexpression of genes associated with autophagy and vesicle formation in the PDCs but not in the biopsy of origin, indicating that in vitro culturing of primary cells activates functions associated with degradation, transport and recycling of cellular components.

Experimental cancer models have been developed for functional screens such as patient-derived tumour xenografts (PDX), ex vivo culturing of tissue slices, and the use of low passaged PDC. All these models are better surrogates of the tumours from which they were generated and are being used in precision medicine in which the genomic and functional signatures of patient tumours are used to find rational treatments for individual patients.

Sarcoma is a highly heterogeneous and aggressive disease in which some histological subgroups lack efficient treatments. Following the breakthrough of the approval of Imatinib™/
Gleevec® for the treatment of gastrointestinal stromal tumours, other specific targets have been identified for sarcomas such as PDGFβ and β, c-Met, PI3K-AKT, HDAC, MDM2 and IGF1R, and inhibitors targeting these pathways have been developed and evaluated in preclinical and clinical trials.25,26

In this study, we cultured and characterised patient-derived sarcoma cells to test their sensitivity towards a comprehensive library of cancer drugs as an approach to identify selective inhibitors for individual cases. Twenty-four sarcoma biopsies were taken but only 14 of them could be grown in vitro. Most of the biopsy donors were affected by progressive disease and had been treated with systemic chemotherapy or radiation at the time of biopsy. Although it is difficult to draw conclusions from a small cohort we observed that poor cell viability in the sample, and adjuvant treatment prior to biopsy, had negative effects on the establishment of PDCs.

Several sarcoma groups are already using functional screens to identify targeted inhibitors for sarcoma. In a recent study, 63 human adult and paediatric sarcoma cell lines were screened against 445 cancer agents including 100 approved drugs. The cell panel consisted of 15 sarcoma subtypes with an overrepresentation of osteosarcoma and Ewing sarcoma cell lines. The drug response results showed that sarcomas are in general poor responders. Ewing sarcoma however was the most responsive group in this series. The drug responses varied within the groups and was associated with specific gene or microRNA expression. Normal healthy controls were not assessed, and several cell lines used in this study have been cultured in vitro for several years.27

Two recent studies have shown that patient-derived tumour xenografts (PDX) and low passage PDCs preserve the intra-tumour heterogeneity and the genomic fingerprint of the tumours of origin.14,15 In this study, whole exome sequencing was performed in a large panel of tumours and demonstrated that patients with advanced cancers carry limited number of targetable genomic aberrations. Similar to our results they show that in vitro drug testing on patient-derived cells can potentially predict patient response to therapy, and it is advantageous when genomic profiling fails to identify targetable genomic alterations.

The power of using patient-derived sarcoma cells to predict patient response to drug treatment has not been evaluated in big cohorts. Recently, patient tumours engrafted in mice were used as a predictive model for response to therapy. In this study, 29 human sarcomas were transplanted in immunodeficient mice (TumorGraft) and 22 (76%) of them engrafted and were subsequently passed in mice for drug sensitivity testing. The most relevant finding was that TumorGrafts could predict response to treatment in 13 of 16 cases of sarcoma patients undergoing treatment; however, time from tumour engraftment to drug sensitivity assay was several months,28 and this represents a limitation if the patient has progressive disease. These results however demonstrate that patient-derived sarcoma cells or xenografts are relevant models that can be used to identify effective treatments for sarcoma patients.

In our study we found several indications that support the use of sarcoma PDC in drug screens as a predictive model of patient treatment response. First, we could confirm that the genetic characteristics of the sarcoma biopsies such as gene mutations and fusion gene expression were preserved in early passed PDCs. Secondly, there was an association between drug sensitivity and the response to the patient’s actual treatment. Poor DSS in drugs used in routine treatment were observed in patients with refractory disease, contrary to a case of a taxane responsive Ewing sarcoma in which taxanes showed activity in vitro.

Thirdly, c-Met inhibitors were active in an ASPS1-TF3-positive alveolar soft part sarcoma consistent with the targeting of the cMET promoter by ASPS1-TF3.

It is also possible that the lack of response of cultured sarcoma cells from patients with recurrent disease is the result of the clonal evolution of their tumour clones resistant to treatment that are preserved in the PDC. The comparison of drug responses between primary and metastatic tumours could shed information and is an issue of interest for further investigations.

In summary, our pilot study shows that patient-derived sarcoma cells can be isolated from surgical and FNA biopsies and expanded in vitro to be used in comprehensive drug sensitivity testing. This is a rapid approach that does not require animal facilities or the reprogramming of cells to establish the PDCs. It can identify selective agents with activity in sarcomas from individual patients and can predict the patient response to standard-of-care treatment. This method can be used as a guide to select treatments for sarcoma patients with otherwise few therapeutic options; however, trials with larger cohorts need to be performed to confirm its clinical value.

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AUTHOR CONTRIBUTIONS

Conception and design: B.B., C.L.S., K.W., E.L., O.K. Development of methodology: B.B., K.W., P.O., L.N.M. Acquisition of data: B.B., C.L.S., S.P., K.W., E.B., E.P., L.M., J.N.W., L.N.M., A.P. Analysis and interpretation of data: B.B., C.L.S., K.W., S.P., P.O., J.N.W., L.N.M., K.v.S., O.B., H.B., A.H., A.P. Writing, review and/or revision of the manuscript: B.B., C.L.S., K.W., J.N.W., E.B., A.P., O.P. Administrative, technical, or material support: O.B., H.B., A.H., P.T., A.P., J.W., K.v.S. Study supervision: B.B., C.L.S.

ADDITIONAL INFORMATION

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Ethical approval and consent to participate: The presented research was conducted with the ethical standards that promote and ensure respect and integrity for all human subjects that participated and justified by the potential benefit to the patient, all in accordance to the Declaration of Helsinki. The collection and use of biopsy material for molecular studies was approved by Central Ethical Review Board in Stockholm (regionala etikprövningsnämnden) No. 2013-1979-31-3. All patients were informed prior to surgery/biopsy and gave consent for using their tumour samples during and after the study.

Availability of data and materials: All data and material are available upon request. Biopsies and cell cultures are accessible provided ethical approval and sample availability.

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