

# In-depth molecular profiling of the biphasic components of uterine carcinosarcomas

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## Abstract

Uterine carcinosarcoma is a clinically aggressive malignancy composed of a mix of carcinomatous and sarcomatous elements. We performed targeted next-generation sequencing of 27 uterine cancer and sarcoma genes together with immunohistochemical analyses of selected proteins in 30 uterine carcinosarcomas. This included 13 cases in which the distinct carcinoma and sarcoma components were sequenced separately and 10 cases where the metastatic tumours were analysed in addition to the primary tumours. We identified non-synonymous somatic mutations in 90% of the cases, with 27 of 30 cases (90%) harbouring *TP53* alterations. The PI3K pathway was the most commonly mutated signalling pathway with mutations identified in *PIK3CA*, *PTEN*, *PIK3R1*, and/or *PIK3R2* in two-thirds of the cases. Mutations in *FBXW7*, *PPP2R1A*, *ARID1A* and *KRAS* were demonstrated in a minority of cases. In cases where the carcinomatous and sarcomatous components were separately analysed, most of the mutations identified were present in both components, indicating a common origin for the two components. Furthermore, the same *TP53* alterations and/or PI3K pathway mutations seen in the primary tumours were also identified in the metastatic sites. Overall, carcinosarcomas exhibited heterogeneous molecular features that resemble the heterogeneity seen in endometrial carcinomas, with some showing endometrioid carcinoma-like and others showing serous carcinoma-like mutation profiles. While patients with serous-like tumours presented more frequently with advanced-stage disease compared to patients with endometrioid-like tumours, there was no statistical difference in outcome between the two groups. Our results provide insights into the oncogenesis of uterine carcinosarcoma and identify targetable mutations that represent early oncogenic events. The findings of the different molecular types of uterine carcinosarcoma that parallel the different molecular types in endometrial carcinoma may have future treatment implications with targeted therapies.

**Keywords:** uterine carcinosarcoma; molecular profiles; sequencing; mutations; *TP53*; PI3K pathway; *PIK3CA*; *PTEN*

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## Introduction

Uterine carcinosarcoma [1] is a malignant mixed epithelial–mesenchymal tumour that accounts for about 3–4% of all uterine malignancies [2,3]. Although

uncommon, this tumour follows an aggressive clinical course and accounts for 16% of deaths caused by uterine malignancy [4,5]. Patients with International Federation of Gynecology and Obstetrics (FIGO) stage 1–2 disease have a 5-year disease-specific survival of 59%, while those with stage 3 and 4 disease

have a 5-year disease-specific survival of 22% and 9%, respectively [4]. Histologically, uterine carcinosarcoma is composed of carcinoma and sarcoma elements that impart a biphasic appearance. Both the carcinoma and sarcoma components are typically high grade. The carcinoma component may resemble known histologic subtypes of endometrial cancer such as endometrioid or serous carcinoma in some cases [6], while the sarcoma component can display homologous or heterologous differentiation [7].

Although initially considered as a subtype of uterine sarcomas [8], it is now thought that most uterine carcinosarcomas represent sarcomatous transformation/transdifferentiation of endometrial carcinoma (metaplastic carcinoma) rather than a collision between two biologically distinct tumour types [9]. The clinical behaviour of carcinosarcomas supports the carcinoma origin hypothesis [10], as carcinosarcomas like most carcinomas preferentially follow lymphatic dissemination [4,11]. A number of prior studies compared patterns of X-chromosome inactivation, microsatellite instability (MSI), types of *TP53* and *KRAS* mutations and loss of heterozygosity (LOH) status between the carcinoma and sarcoma elements and demonstrated shared molecular features in the majority of cases [12–15]. However, these results also suggest a biclonal nature (collision tumour) in a minority (10–20%) of uterine carcinosarcomas [13,14].

Our group has previously performed mutational analysis on a series of uterine carcinosarcomas using a nine gene panel, though we did not analyse the carcinoma and sarcoma elements separately [16]. While prior studies have shown evidence for the monoclonal nature in most uterine carcinosarcomas, there has not been a comprehensive molecular study of the carcinoma and sarcoma components of carcinosarcomas. In this study, we employed next-generation targeted gene sequencing to decipher and compare the mutational profiles between the different components of uterine carcinosarcomas, as well as between primary and metastatic tumours.

## Methods

### Study samples

This study examined tissues from 30 uterine carcinosarcomas with available formalin-fixed paraffin-embedded (FFPE) tumour and normal tissue, fresh frozen tumour (FFT) samples obtained from resection specimens, as well as matched normal tissue (buffy coat). The tissue samples were obtained from the Vancouver General Hospital pathology archives (FFPE tumour and normal tissue) and the BC Cancer

Agency OvCaRe Tumour Biobank (FFT tissue and buffy coat). This study has received institutional research board approval. All patients were approached for written informed consent, before undergoing surgery, to donate tissue surplus to diagnostic requirements plus a blood sample. The histology slides from the 30 hysterectomy specimens were reviewed by the study pathologists (LH and CHL). FFPE block(s) that contained the areas of interest were identified for DNA extraction.

### DNA extractions

For FFPE tissue blocks, the tumour containing areas in the primary and metastatic tumours were marked and cored (3–4 cores at 0.6 mm diameter). DNA was extracted from the tissue cores using the Qiagen FFPE DNA kit (Valencia, CA, USA) as per manufacturer's protocol. In 14 of the 30 cases, the carcinoma and sarcoma elements formed spatially distinct areas in the primary tumour, such that DNA from the separate components was extracted for comparison. The carcinoma and the sarcoma elements in the remaining 16 cases were closely admixed in the primary tumour and the extracted DNA represented a mixture of the two components. In 10 cases, DNA from the metastatic tumour was also extracted.

For FFT samples, all tumours were verified by frozen section to ensure adequate viability and cellularity of tumour tissue. The FFT tissue was then cryosectioned for DNA extraction using the Genra Puregene kit (Qiagen) (Valencia, CA, USA) as per manufacturer's protocol. Germline DNA was extracted from buffy coat. In the cases where matched buffy coat was not available, DNA was extracted from normal FFPE tissue. All DNA was quantified using the Qubit fluorometer using Molecular Probes broad range Qubit quantification kit (Life Technologies) (Carlsbad, CA, USA).

### Discovery targeted sequencing and analysis

An Illumina custom Truseq amplicon panel (version 1) (Illumina, San Diego, CA, USA) was designed using the Illumina Design studio software to amplify 175bp libraries. This included 1519 amplicons in 27 genes that were previously found to be recurrently mutated in endometrial cancer and/or uterine sarcoma [16–21]: *ABCC9*, *AKT1*, *AKT2*, *AKT3*, *ARHGAP35* (*GRLF1*), *CCND1*, *CHD4*, *CTCF*, *CSMD3*, *EP300*, *FGFR2*, *KRAS*, *MAP3K4*, *MED12*, *ARID1A*, *CTNNB1*, *PTEN*, *PIK3CA*, *PIK3R1*, *PIK3R2*, *POLE*, *PPP2R1A*, *FBXW7*, *SPOP*, *TP53*, *TSPYL2*, *ZFXH3*. The Illumina Truseq custom library preparation was

utilized using 250 ng of DNA for FFPE DNA. The Illumina protocol was followed for library preparation (includes sample PCR and barcoding); however, the protocol was modified for pooling and normalization. Before library pooling, libraries were quantified using the Qubit fluorometer, then pooled at equal concentrations. Each library pool was then quantified for amplifiable products using the KAPA Illumina SYBR qPCR quantification kit using the ABI7900 fast real-time instrument. Each pool was run on the Illumina MiSeq using 300 cycle version 2 kits, and all bam and VCF files were generated using Illumina MiSeq reporter. Analysis was performed using the VCF files generated by the somatic variant caller 3.2.3.0, then filtered based on reads passing filter, non-synonymous, somatic mutations with >5% variant allele frequency. All potential mutations were then manually interrogated using the Integrated Genome Viewer (IGV).

#### Mutation validations

All non-synonymous somatic mutations (except for *CSMD3*) underwent secondary validation using either Fluidigm 48X48 Access Arrays (Fluidigm, Markham, ON, Canada), then barcoded and sequenced on a MiSeq, or by Sanger sequencing. In brief, primer sets were designed using Primer 3 to amplify the specific mutations, tagged with CS1 (5'-ACACTGACGACATGGTTCTACA-3') and CS2 (5'-TACGGTAGCAGAGACTTGGTCT-3') sequencing tags, and synthesized by IDT Technologies (Coralville, IA, USA). PCR products (150–200bp) were generated using the Fluidigm 48X48 Access Arrays, as per manufacturer's protocol, with input of 100 ng for FFPE derived DNA, and 50 ng for high-quality DNA from buffy coat or frozen tumour DNA. DNA barcodes (10bp) with Illumina cluster-generating adapters were added to the amplified libraries as previously described [22], then purified using Agencourt AMPure XP beads (Beckman Coulter, Mississauga, ON, Canada), quantified and pooled as described by our methods for the custom discovery targeted panel sequencing. In total, 96 libraries were sequenced using a MiSeq 300 cycle V2 kit on the Illumina MiSeq for ultra-deep validations. Analysis was performed as described for discovery targeted panel sequencing. Sanger sequencing was utilized if the amplicon of interest failed MiSeq validation sequencing, and was performed as previously described [23]; however, CS1 and CS2 primers were used as a universal sequencing primers on the ABI 3130xl Genetic Analyzer (Applied Biosystems). In the case of *TP53*, the Truseq panel sequencing did not cover the entire region of exons 4 and 5. Therefore, Sanger sequencing of these exons

was performed when the immunohistochemistry and sequencing results did not concur. These included re-sequencing for cases 1, 13, 16, 17, 18, 21 and 29.

#### Immunohistochemistry and analysis

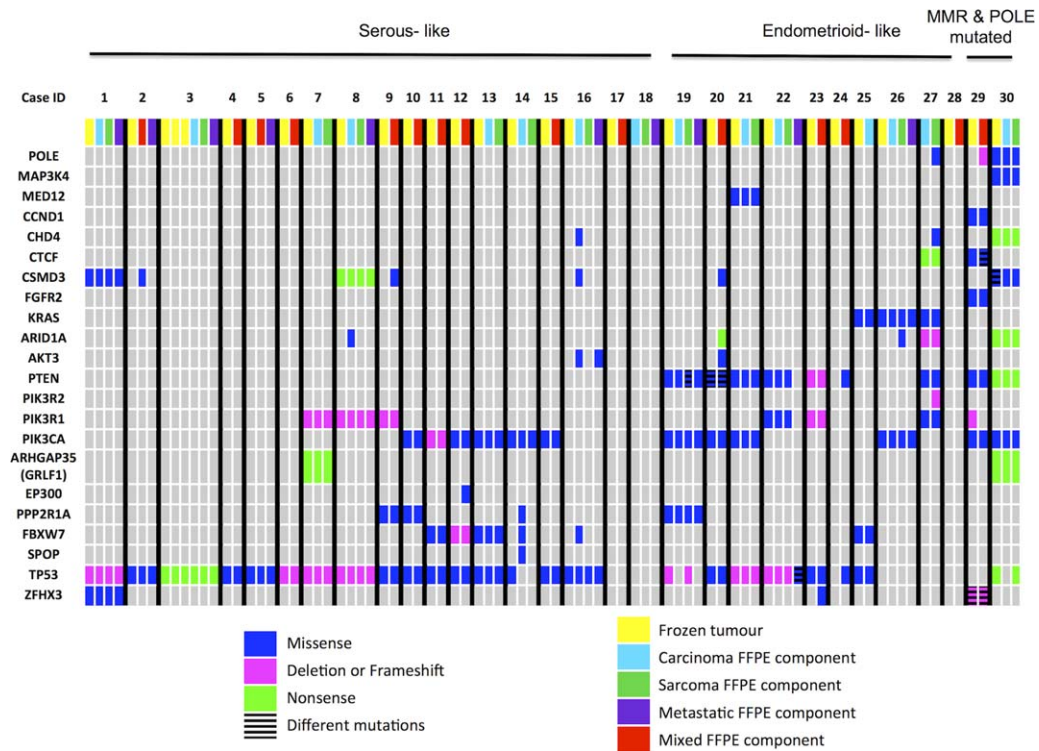
All immunohistochemistry was performed on tissue microarrays and/or whole sections using the Ventana Discovery XT and the Ventana Benchmark XT automated systems (Ventana Medical Systems, Tucson, AZ, USA). Immunohistochemical stains for mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6) were performed as previously described [24]. Primary incubations were performed as follows: p53 (Dako, mouse monoclonal antibody, clone DO-7) at 1:400 dilution, 32 minutes, 37°C, with heat induced antigen retrieval;  $\beta$ -catenin (Cell Marque, mouse monoclonal antibody, clone 14) at 1:100 dilution, 32 min, 37°C, with heat induced antigen retrieval; BAF250 (*ARID1A*) (Sigma, rabbit polyclonal antibody) at 1:100 dilution, 64 min, 37°C, WT1 (Dako, mouse monoclonal antibody, clone 6F-H2) at 1:100 dilution, 32 min, 37°C. The Ventana Universal Secondary Antibody was used for 32 min at 37°C. The detection systems were the Ventana OptiView DAB kit (p53,  $\beta$ -catenin) and Ventana ChromoMap DAB kit (BAF250/*ARID1A*).

## Results

#### Clinical and pathologic features of study samples

We performed targeted sequencing of 30 uterine carcinosarcomas with matched FFT and FFPE tumour DNA samples. The mutation findings in this study are summarized in Figure 1, and all mutations (except *CSMD3*), were orthogonally validated and confirmed to be somatic (supplementary material Tables 1 and 2). We performed sequencing in both matched frozen and FFPE primary tumour samples in 28 of 30 cases, as two cases did not have sufficient frozen tumour tissue available for analyses. In 14 of 30 tumours, the carcinoma and sarcoma components formed spatially distinct tumour areas such that the separate components were sequenced separately. In addition to the analysis of the primary tumour, extrauterine metastases were analysed separately in 10 cases.

The clinical features of the study cohort are summarized in Table 1 (detailed information in supplementary material Table 3). Pre-operative biopsy was performed in 28 of 30 patients and the diagnoses were carcinosarcoma in 24 patients, high-grade serous carcinoma in three patients and grade 3



**Figure 1.** Mutation profiles of all uterine carcinosarcomas. Uterine carcinosarcoma patients are grouped into columns and are separated by black lines. The genes that were sequenced are found in rows. Coloured boxes indicate the presence of a DNA alteration (point mutations, frameshifts, deletions). The grey boxes indicate that no alteration was identified in the specific gene and specific patient. The cases are grouped into serous-like molecular subtype and endometrioid-like molecular subtype based on the pattern of mutations. The last two cases (ID 29, 30) are tumours with MMR and POLE mutated, respectively.

endometrioid carcinoma in one patient. Half of the patients presented with uterine confined disease, while the other half presented with extrauterine tumour spread. The sarcoma component was high-grade in all except one case (case 30; Figure 2A–D). Heterologous differentiation was present in 16 of 30 primary tumours with rhabdomyoblastic differentiation being the most common. In the primary tumour, the proportion of the carcinoma component varied from <5% to >95% (average of 50%). The extrauterine (metastatic foci) tumours (10 patients) consisted only of carcinoma in six cases, sarcoma in one case, and a mix in three cases. The carcinoma elements of the metastatic tumour resembled the carcinoma component of the primary in all except one case (case 22; Figure 2E–F).

#### Targeted sequencing shows frequent TP53 and PI3K pathway mutations in primary uterine carcinosarcoma samples

We identified non-synonymous somatic mutations in 27 of 30 (90%) cases (Figure 1). There were 28 cases where both frozen and FFPE tumour samples were

available for analysis. The mutation profiles were completely concordant in 25 samples after excluding low-frequency findings (mutations found in <5% of the reads) as likely minor clonal changes (intratumoural heterogeneity). Three cases (14, 20 and 24) showed some differences between the mutations identified in the frozen or FFPE tumour sample. Overall, there appeared to be good concordance in the detection of mutations in FFPE tumour tissue.

*TP53* mutations were present in the majority of carcinosarcoma (24 of 30) (80%). Most of the tumours harbouring missense *TP53* mutations showed diffuse nuclear p53 immunostaining, with the exception of two cases (7 and 21) that harboured *TP53* deletions and showed diffuse nuclear p53 immunostaining (Table 2). Cases 19 and 30 both demonstrated *TP53* mutations (frameshift and nonsense) in the sarcoma component but not the carcinoma component of the tumour, and both displayed a wild-type pattern of p53 immunostaining in both components of the tumour. Loss of nuclear p53 immunohistochemistry was detected in 5 cases with indel or nonsense mutations, and 3 additional tumours (cases 17, 18 and 29) displayed abnormal patterns of staining

**Table 1.** Summary of the clinicopathologic features of the study series (n = 30)

Age (range)	65 years (50–83 years)
Menopausal status	
Pre-menopausal	1
Post-menopausal	29
Tumour size (range)	6.0 cm (2.3 – 15.0 cm)
Sarcoma component	
Homologous	14
Heterologous	16
Rhabdomyosarcoma	5
Chondrosarcoma	3
Osteosarcoma	3
>1 sarcoma type	5
Metastases	
Absent	19
Present	11
Pelvic metastases	6
Carcinoma only	3
Sarcoma only	1
Carcinoma and sarcoma	2
Distant metastases (beyond pelvis)	5
Carcinoma only	5
Sarcoma only	0
Carcinoma and sarcoma	0
Lymph nodes	
Benign	12
Positive for metastases	3
Not taken	15
Pelvic washings	
Negative	17
Positive	9
Not taken	4
Clinical Stage	
I	15
II	0
III	10
IV	5

with no detectable mutations (Table 2 and supplementary material Table 2). In total (27 of 30) 90% of all tumours harboured *TP53* aberrations as assessed by either sequencing or immunohistochemistry.

In the primary tumours, mutations involving genes that encode the kinase or regulatory proteins of the PI3K pathway were identified in 20 of 30 tumours (67%), where multiple PI3K pathway proteins were mutated in 6 tumours. *PIK3CA* mutations were found in 12 of 30 (40%) tumours, which were scattered throughout the different functional domains of *PIK3CA* (Figure 3). *PTEN* mutations, most commonly missense in type, were observed in eight tumours (27%). *PIK3R1* mutations were found in five tumours (17%), while only one tumour demonstrated a *PIK3R2* mutation. Of note in this cohort, *PIK3CA* mutations were rarely found with concurrent *PIK3R1* or *PIK3R2* mutations. One tumour (case 20) with a *PIK3CA* mutation also harboured an *AKT3* (E167D) mutation, while case 18 harboured an *AKT3* (M336I) mutation

at low frequency (5%) without concurrent PI3K pathway mutations. No mutations involving *AKT1* or *AKT2* were identified in this series.

Other recurrently mutated genes included *FBXW7* (six tumours, 20%), *PPP2R1A* (four tumours, 13%), *KRAS* (three tumours, 10%), *ARID1A* (three tumours, 10%), *ZFHX3* (two tumours, 7%) and *CSMD3* (seven tumours, 23%). By immunohistochemistry, only two cases showed loss of BAF250 (*ARID1A*), which corresponded to the presence of frameshift and nonsense mutations (Table 2). There was no evidence of *CTNNB1* mutations by sequencing or by immunohistochemistry. A somatic *MED12* mutation (D23Y) was identified in one tumour, which has been previously documented in a case of endometrial carcinoma [27]; however, it was not the typical hotspot *MED12* mutation found in uterine smooth muscle tumours [20,21].

The expression of mismatch repair (MMR) proteins (*MLH1*, *PMS2*, *MSH2* and *MSH6*) was evaluated by immunohistochemistry in all 30 primary tumours (Table 2). Only 1 of 30 tumours (3%) (case 29), showed MMR protein deficiency (*MLH1* and *PMS2* loss) while the remaining 29 tumours demonstrated intact expression of MMR proteins.

A somatic *POLE* exonuclease domain mutation (M444K) was identified in one tumour (case 30), which was reported in an ultra-mutated endometrial carcinoma in a prior study [17]. In keeping with the ultra-mutator phenotype, this tumour also possessed 11 point mutations involving eight other genes. In retrospective histologic review, this tumour was reclassified as a FIGO grade 2 endometrioid-type carcinoma with spindle cell elements (and focal chondroid differentiation) (Figure 2A–D). This was the only histologic outlier in our present series as all other cases were confirmed to be carcinosarcoma. Therefore, in histologically confirmed uterine carcinosarcomas (n = 29 tumours), none of the tumours harboured *POLE* exonuclease domain mutations.

#### Carcinoma and sarcoma components of uterine carcinoma show concordant mutation profiles

In this series, 13 of 29 uterine carcinosarcomas displayed spatially distinct carcinoma and sarcoma components such that these paired components were amenable to separate analyses. Twelve of the 13 pairs demonstrated at least one mutation in common with eight cases being 100% identical, while one case (case 18) showed no detectable mutations in either component (Figure 1). Two cases exhibited additional mutation(s) in the sarcoma component compared to the carcinoma component: case 19 with a *TP53* missense mutation, and case 27 with *PIK3R2*,

**Table 2.** Immunohistochemical features of 30 uterine carcinosarcomas

Case No.	p53	MMR	ARID1A	B-catenin
1	Abnormal (loss)	Normal	Normal	Membranous
2	Abnormal (diffuse)	Normal	Normal	Membranous
3	Abnormal (loss)	Normal	Normal	Membranous
4	Abnormal (diffuse)	Normal	Normal	Membranous
5	Abnormal (diffuse)	Normal	Normal	Membranous
6	Abnormal (loss)	Normal	Normal	Membranous & cytoplasmic
7	Abnormal (diffuse)	Normal	Normal	Membranous
8	Abnormal (loss)	Normal	Normal	Membranous
9	Abnormal (diffuse)	Normal	Normal	Membranous
10	Abnormal (diffuse)	Normal	Normal	Membranous & cytoplasmic
11	Abnormal (diffuse)	Normal	Normal	Membranous
12	Abnormal (diffuse)	Normal	Normal	Membranous
13	Abnormal (diffuse)	Normal	Normal	Membranous
14	Abnormal (diffuse)	Normal	Normal	Membranous
15	Abnormal (diffuse)	Normal	Normal	Membranous
16	Abnormal (diffuse)	Normal	Normal	Membranous
17	Abnormal (diffuse)	Normal	Normal	Membranous & cytoplasmic
18	Abnormal (loss)	Normal	Normal	Membranous
19	Normal	Normal	Normal	Membranous
20	Abnormal (diffuse)	Normal	Normal	Membranous & cytoplasmic
21	Abnormal (diffuse)	Normal	Normal	Membranous
22	Abnormal (loss)	Normal	Normal	Membranous
23	Abnormal (diffuse)	Normal	Normal	Membranous
24	Abnormal (diffuse)	Normal	Normal	Membranous
25	Abnormal (diffuse)	Normal	Normal	Membranous
26	Normal	Normal	Normal	Membranous & cytoplasmic
27	Normal	Normal	Loss	Membranous
28	Normal	Normal	Normal	Membranous
29	Abnormal (diffuse)	Abnormal (Loss of MLH1 & PMS2)	Normal	Membranous
30	Normal	Normal	Loss	Membranous

MMR: mismatch repair protein (MLH1, MSH2, MSH6 and PMS2).

*CHD4* mutations, and a *POLE* non-exonuclease domain mutation. In contrast, two cases displayed additional mutation(s) in the carcinoma component compared to the sarcoma component: case 8 with an *ARID1A* mutation, and case 14 with *PPP2R1A*, *FBXW7* and *SPOP* mutations. Overall, there was complete concordance or partial overlap in mutations identified in the carcinoma and the sarcoma components in 11 of 12 cases, hence indicating clonal relatedness between the carcinoma and the sarcoma components.

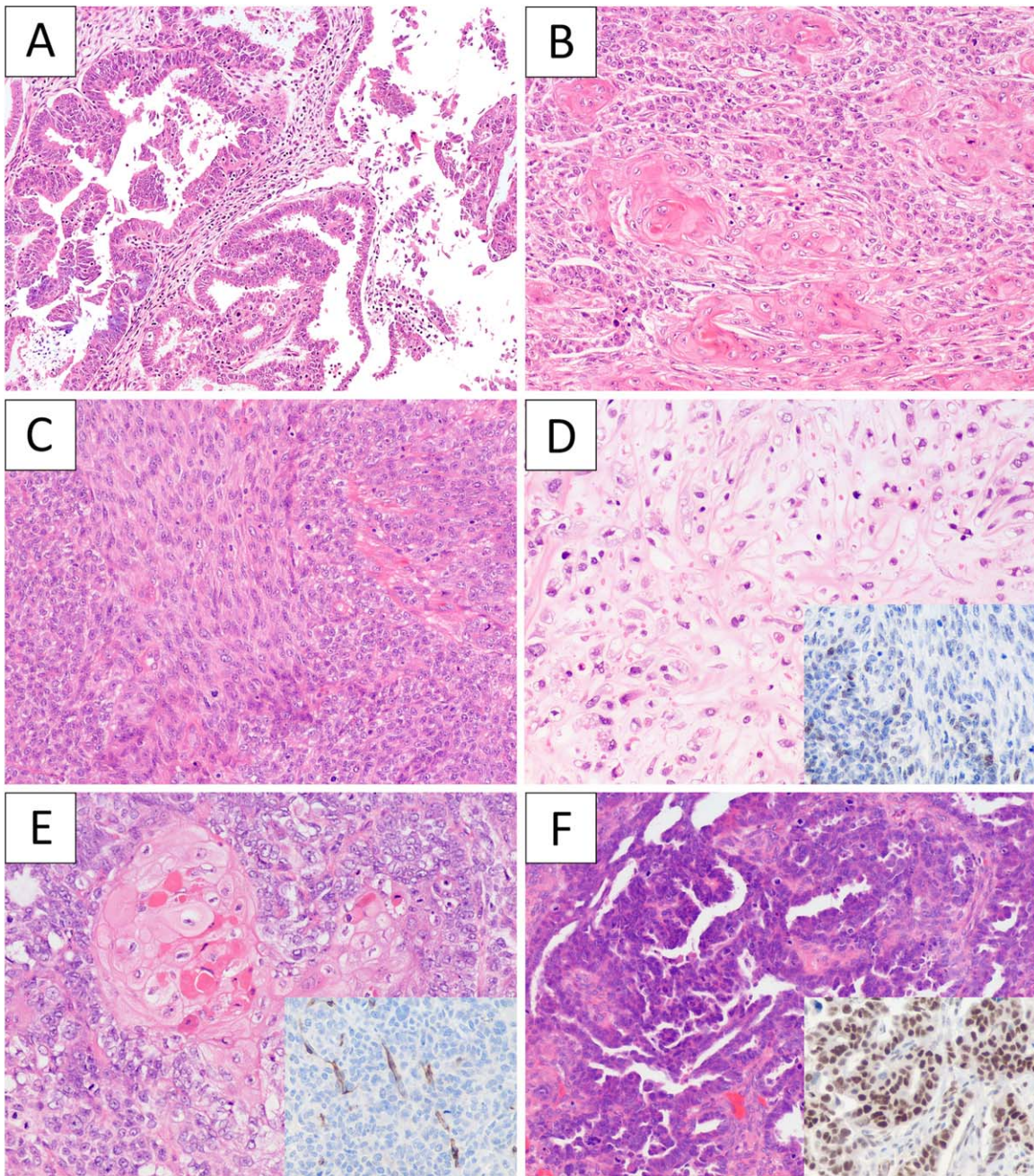
#### Primary and metastatic tumours show concordant mutation profiles

Matched uterine primary and metastatic tumour samples were analysed in 10 cases, in one of which no mutations were identified. Of the nine cases with

validated somatic mutations, five cases demonstrated identical mutations in the metastatic tumour compared to the primary. Three showed partially overlapping mutations but with fewer mutations identified in the metastatic tumour compared to the respective uterine primary. For case 22, the carcinoma and the sarcoma components of the uterine primary all harboured the same *PTEN*, *PIK3R1* and *TP53* (frameshift) mutations with loss of p53 immunostaining. In contrast, the metastatic tumour (bilateral ovaries and peritoneal deposits) displayed no evidence of *PTEN*, *PIK3R1* mutations, and harboured a different *TP53* missense mutation with diffuse nuclear p53 immunostaining (Figure 2E–F). STR testing confirmed that the DNA of the carcinosarcoma and the apparent metastatic tumour were derived from the same patient (supplementary material Table 4). Histologically, the carcinoma component of the primary carcinosarcoma displayed typical endometrioid features with squamous differentiation, while the ovarian/peritoneal tumour consisted purely of carcinoma with features consistent with ovarian high-grade serous carcinoma. Additional immunohistochemistry analysis demonstrated nuclear WT1 positivity in the ovarian tumour but not the uterine tumour (Figure 2E–F) [28]. These findings strongly indicate a synchronous uterine carcinosarcoma and ovarian high-grade serous carcinoma in this patient. Further review of annotated clinical data revealed a personal history of bilateral high-grade breast carcinomas and a strong breast cancer family history, thus raising the possibility of *BRCA1/2* germline background.

#### Clinical significance of molecular profiles in uterine carcinosarcoma

We have previously identified different molecular types of uterine carcinosarcomas with some tumours showing endometrial serous carcinoma-like mutation profiles (characterized by the presence of *TP53* mutation with *PPP2R1A* and/or *FBXW7* mutations and in the absence of *PTEN*, *CTNNB1*, *KRAS* or *ARID1A* mutations) and other tumours showing endometrioid carcinoma-like mutation profiles (characterized by the presence of *PTEN*, *CTNNB1*, *KRAS* and/or *ARID1A* mutations) [16]. Based on the combined genetic and immunohistochemical profiles in the present cohort, 18 tumours showed serous-like while 11 tumours showed endometrioid-like molecular profiles (Figure 1 and Table 2). There was good agreement between histologic subtyping (based on the morphology of the epithelial component) and



**Figure 2.** The histological features of selected carcinosarcoma cases. (A) Case 30 showed a low-grade endometrioid component (A), solid areas with abundant squamous differentiation (B), low-grade spindle cell component (C) and focal chondroid differentiation (D). p53 immunohistochemistry (D, inset) was focal in both the endometrioid and spindle cell components. Case 22 had uterine, bilateral ovarian and peritoneal tumour. The uterine tumour showed a carcinosarcoma with prototypical endometrioid histology with squamous differentiation (E) and negative staining for WT1 (E, inset). The ovarian tumour as well as the peritoneal tumour deposits were all pure carcinoma with high-grade serous carcinoma histology (F) and strong nuclear staining for WT1 (F, inset).

molecular subtyping in 27 of 29 uterine carcinosarcomas (93% agreement; Figure 4).

Clinically, the majority of patients with serous-like tumour presented with advanced stage disease as 12 of 18 (67%) patients had stage III-IV disease, while

8 of 11 (73%) patients with endometrioid-like tumour presented with stage I disease (Table 3). Despite the differences in presenting disease stage, there was no significant difference in disease-specific survival between patients with endometrioid-type

Table 3. Clinical feature stratified by molecular subtypes

Molecular subtype	Number of cases	Age (years)	Tumour size (cm)	Sarcoma type	Stage
Serous-like	18	63.1 (50–80)	6.5 (2.3–15)	Homologous: 7 Heterologous: 11	Low (stages 1 and 2): 6 High (stages 3 and 4): 12
Endometrioid-like	11	70.2 (54–83)	5.4 (2.5–11)	Homologous: 6 Heterologous: 5	Low (stages 1 and 2): 8 High (stages 3 and 4): 3
p-value		0.0521*	0.452*	0.129**	0.0376**

\*p-value determined by student's t-test.

\*\*p-value determined by chi-squared test.

tumour and patients with serous-type tumours ( $p = 0.98$ ) (Figure 5).

## Discussion

Uterine carcinosarcoma is a histologically intriguing biphasic tumour that contains both carcinoma and sarcoma within the same tumour mass. While the carcinoma and the sarcoma elements can be intimately admixed, they can also form spatially distinct components. This prompted different theories regarding the histogenesis of uterine carcinosarcomas (collision tumour versus metaplastic carcinoma). The prevailing view at the present is that the carcinoma and sarcoma components share a common origin in the majority of uterine carcinosarcoma [12–15]. Our findings here provide further objective and unambiguous support for the common origin hypothesis. This is evident in the cases where the two histologic components were separately analysed; all cases with detectable mutations shared at least one somatic mutation in common between the carcinoma and the sarcoma components.

While our results clearly demonstrated genetic similarities between the carcinoma and the sarcoma components, it did not offer definitive insights into the

directionality of tumour progression for the different histologic components. Most of the cases analysed showed identical mutations profiles between the carcinoma and the sarcoma components. There were four tumours that showed differences in mutations in addition to shared mutations between the two components. These included two cases with additional mutations found in the sarcoma component and two cases with additional mutations found in the carcinoma component. These differences, however, may represent intratumoural genetic heterogeneity and it would not be prudent to infer directionality solely based on the presence of additional mutations in this small number of cases. Even though the directionality of progression cannot be concluded from these results, the overall mutation profiles seen in the present series and our previously published series of uterine carcinosarcoma resembles the mutation profiles of endometrial serous and endometrioid carcinomas [16]. This provides indirect evidence that uterine carcinosarcomas most likely develop through sarcomatous transdifferentiation of the underlying endometrial carcinoma, as suggested by earlier studies [12–15]. Moreover, it is equally likely sarcomatous transdifferentiation represents a loose state of control in cell differentiation, which reflects a highly disrupted genetic/epigenetic cellular milieu of the tumour. As such, sarcomatous transdifferentiation can

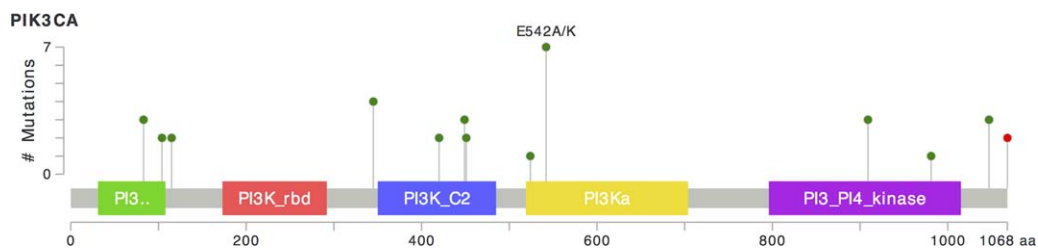
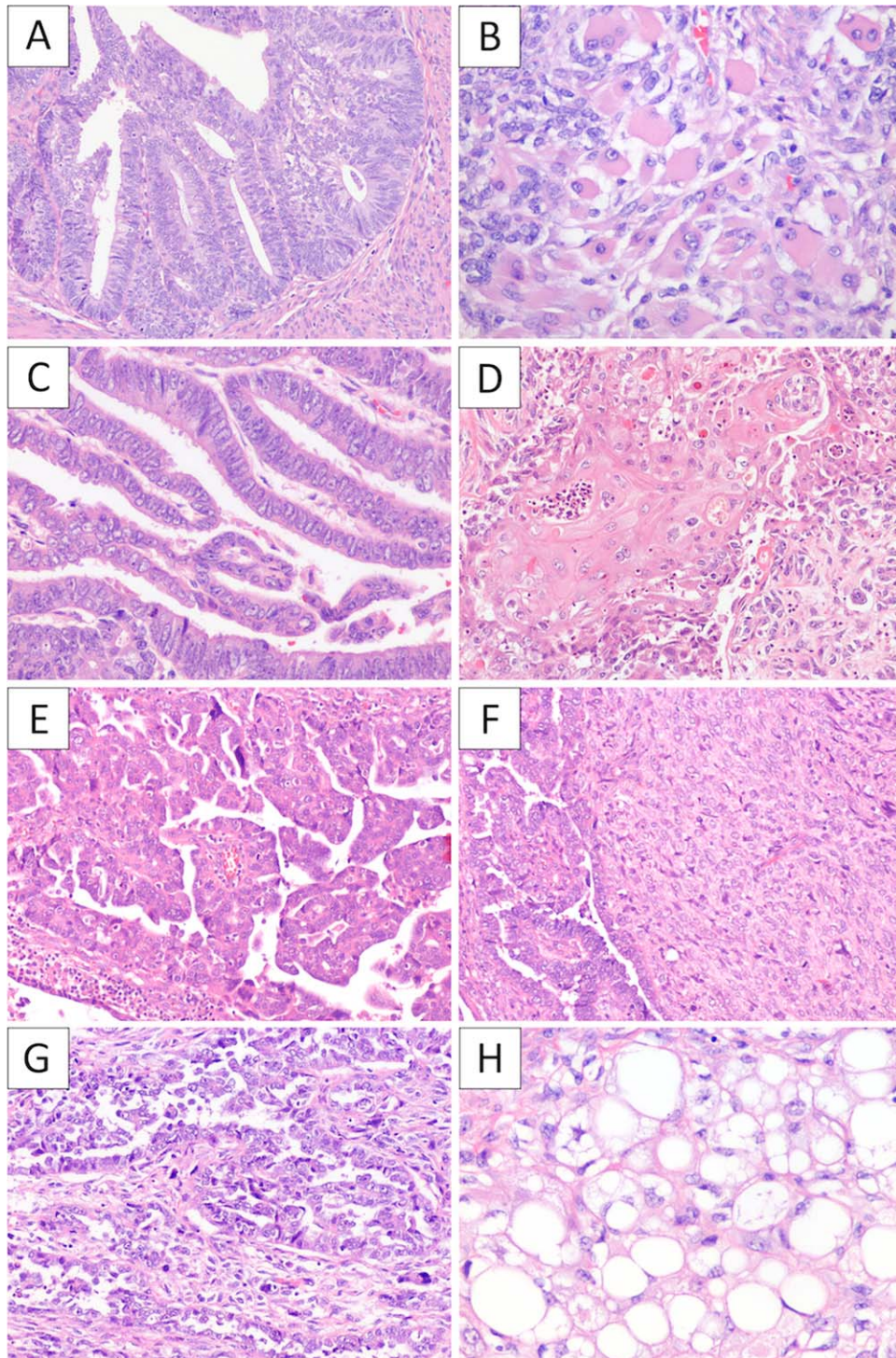
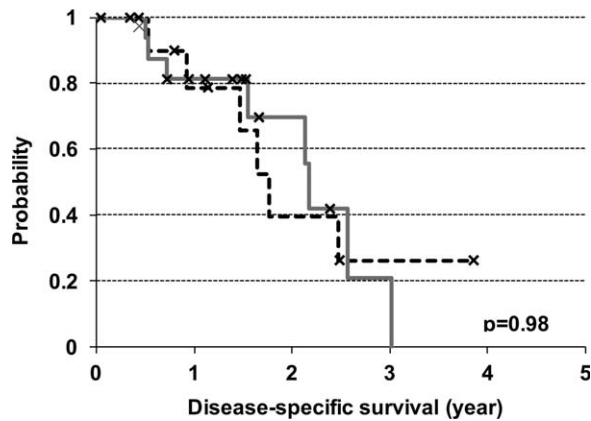


Figure 3. Schematic depiction of the PIK3CA mutations identified in uterine carcinosarcoma. Each identified PIK3CA mutation is indicated as a lollipop plot along the protein domains of PIK3CA. The number of mutations for all cases is indicated on the x-axis. Green dots indicate point mutations, and red dots indicate frameshift mutations. Mutations can be identified along all regions of the PIK3CA gene and are not restricted to only the classical hotspot regions. The mutation plot was constructed using the cBioPortal [25,26] visualization tool MutationMapper v1.0.





**Figure 4.** Representative histology of uterine carcinosarcoma with endometrioid-like and serous-like molecular features. (A) Tumours with endometrioid-like molecular features typically displayed endometrioid-type histology in the carcinoma component, as illustrated by case 27, which showed low-grade endometrioid carcinoma component (A) with rhabdomyoblastic (B), osteogenic and chondroid differentiation (not shown), and by case 21 that showed low-grade endometrioid carcinoma component (C) with squamous differentiation (D). Tumours with serous-like molecular features typically displayed serous-type histology in the carcinoma component, as illustrated by case 3, which showed high-grade serous carcinoma component (E) and homologous sarcoma (F), and case 9 that showed high-grade serous areas (G) with lipogenic (H) and rhabdomyoblastic differentiation (not shown).



**Figure 5.** Disease-specific survival analysis stratified by carcinosarcoma molecular subtypes. The Kaplan–Meier survival analysis for disease specific survival where the dotted black line represents the endometrioid-like molecular subtype and the solid grey line represents the serous-like molecular subtype.

be regarded as a morphologic surrogate marker for more aggressive clinical behaviour of the endometrial carcinoma, rather than a sequential state of tumour progression with the sarcoma component representing the more biologically aggressive component of the tumour. This notion is in keeping with the clinical observation that the metastatic tumour in the great majority of cases is comprised either exclusively or predominantly of the carcinoma elements [4,5].

We have identified mutations involving PI3K pathway in two-thirds of uterine carcinosarcoma examined, most commonly involving *PIK3CA* and *PTEN*. In contrast to prior studies on uterine carcinosarcomas [29,30], our present analyses identified both traditional *PIK3CA* hotspots (exons 9 and 20) mutations as well as mutations in the adaptor binding domain, helical domain, and C2 domain which can also enhance kinase enzymatic activity [31,32]. When present, these PI3K pathway mutations were found in the carcinoma and sarcoma components of the primary tumour, as well as in the metastatic tumour. This indicates that these PI3K pathway mutations occur relatively early in the tumourigenesis of carcinosarcoma and likely represent important oncogenic driver events that could be targeted with PI3KCA/mTOR inhibitors [33–35]. We also observed a very high frequency of *TP53* alterations (90%) in this series of uterine carcinosarcomas, which is supported by previous literature [5,12,16]. The same *TP53* aberrations were present in both carcinoma and sarcoma components of uterine carcinosarcoma, which also implicates an early event in tumourigenesis. In keeping with prior studies, MMR protein deficiency that suggests underlying microsatellite instability was

rarely observed in our present series of uterine carcinosarcoma [12,24]. A recent study demonstrated frequent mutations in chromatin remodelling genes (*ARID1A*, *ARID1B*, *MLL3*, *SPOP*) with the identification of 4 mismatch repair deficient tumour in a cohort of 17 uterine and five ovarian carcinosarcomas [36]. While these results are overall different from our present cohort, a closer examination reveals that nearly all of the identified *ARID1A*, *ARID1B* and *MLL3* mutations were found in the four MMR-deficient uterine carcinosarcomas and in four of the five ovarian carcinosarcomas. We did, however, identify a few cases in our cohort with *ARID1A* mutations and one with an *SPOP* mutation. Lastly, *POLE* exonuclease domain mutations do not appear to occur in *bona fide* uterine carcinosarcoma.

About two-thirds of the present series of uterine carcinosarcoma demonstrates a serous carcinoma-like mutation profile, while the remaining third demonstrated an endometrioid carcinoma-like mutation profile. This apparent molecular heterogeneity is similar to what we have observed in a previous cohort of uterine carcinosarcomas [16]. In addition to the serous carcinoma-like uterine carcinosarcoma (all of which harboured *TP53* aberrations), half of the uterine carcinosarcoma that displayed an endometrioid carcinoma-like mutation profile also harboured *TP53* aberrations. Based on the proposed molecular types of endometrial carcinoma by the recent TCGA (The Cancer Genome Atlas) study [17], our findings here indicate that a significant majority of uterine carcinosarcoma likely belong to the copy number high serous-like molecular type, with a minor subset representing copy number low endometrioid molecular type and more rarely the microsatellite-unstable hypermutated molecular type. With regards to clinical features, we did not observe a significant difference in patient survival between the two apparent molecular types of uterine carcinosarcoma, however our study series is relatively small. In contrast, De Jong *et al* found that uterine carcinosarcomas with non-endometrioid carcinoma components showed a trend for worse disease-specific survival compared to carcinosarcomas with endometrioid carcinoma components in 40 tumours, although the difference was not significant [5]. Irrespective of the prognostic significance, there are clearly differences in the underlying tumourigenic mechanisms between the two apparent molecular types and this may have treatment implications with emerging therapies.

With regards to the *POLE*-mutated cases, endometrioid carcinoma with spindle cell elements is a recognized histologic mimic of carcinosarcoma, as both tumour types display biphasic appearance and can

include foci of heterologous differentiation [37,38]. Clinically, endometrioid carcinoma with spindle cell elements is associated with a much more favourable prognosis than uterine carcinosarcoma [37]. Interestingly, endometrial carcinoma of *POLE* ultra-mutated molecular type is also associated with excellent prognosis [17,39]. This patient with *POLE* mutated endometrioid carcinoma presented with stage 1B disease and underwent chemotherapy treatment, and is alive with no evidence of disease recurrence nearly seven years later. Further studies are needed to investigate whether there is increased propensity for *POLE*-mutated endometrioid carcinoma to display spindle cell elements.

In summary, we performed targeted gene sequencing on a series of uterine carcinosarcomas, and identified frequent aberrations involving *TP53* and *PI3K* pathway that may be therapeutically targeted. Our analyses demonstrate that the carcinoma and the sarcoma components of uterine carcinosarcoma are similar if not identical genetically; hence they are both parts of the same tumour. We also confirmed previous observations that most uterine carcinosarcomas display either endometrial serous carcinoma-like or endometrioid carcinoma-like molecular profiles. These findings altogether support the notion that uterine carcinosarcoma represents sarcomatous transdifferentiation of endometrial carcinoma, and the identification of the different molecular types of uterine carcinosarcoma (reflecting the underlying endometrial carcinoma molecular types) may have future clinical management implications.

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### Author contributions

MKM, LNH, BAC, CHL conceived and designed the study. MHC, LNH, JNM, BAC, CBG, CHL assisted in accruing samples for the study. MKM, LNH, NR,

JS, WY, RM carried out the technical experimentation. LNH, CBG, CHL performed pathological reviews. MKM, LNH, CHL, BAC, JNM, CBH, DGH assisted in the interpretation of the data. MKM, LNH and CHL wrote the manuscript and created the figures. All authors have reviewed and approved the manuscript.

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**SUPPLEMENTARY MATERIAL ON THE INTERNET**

Additional Supporting Information may be found in the online version of this article.

Detailed materials and methods for STR analysis and statistical analysis.

**Table S1.** Details for all mutations identified in all samples including: genomic position, nucleotide changes, amino acid changes, depth, and frequency.

**Table S2.** A simpler version of Supplemental Table 2 with condensed amino acid changes, IHC data, histology and cellularity for each case.

**Table S3.** Includes all clinical data for each uterine carcinosarcoma case.

**Table S4.** Detailed results for the carcinosarcoma cases tested by STR analysis.