



Phase II Trial of Gemcitabine and Nab-Paclitaxel for Recurrent Osteosarcoma with Serial Monitoring Using Liquid Biopsy: A Report from the National Pediatric Cancer Foundation

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ABSTRACT

Purpose: The combination of gemcitabine and docetaxel is often used to treat patients with recurrent osteosarcoma. Nab-paclitaxel has preclinical activity against osteosarcoma and is potentially less myelosuppressive than docetaxel. We conducted a prospective multi-institutional phase II trial combining gemcitabine and nab-paclitaxel for patients aged 12 to 30 years with recurrent osteosarcoma and measurable disease.

Patients and Methods: A Simon's two-stage design was used to test a 4-month progression-free survival (PFS-4) of 10% vs. 35%. Patients received nab-paclitaxel 125 mg/m² and gemcitabine 1,000 mg/m² weekly × 3 in 4-week cycles. Immunohistochemical analysis of archival tissue and serial assessment of circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) using ultralow passage whole-genome sequencing were performed to identify potential biomarkers of response.

Results: Eighteen patients received 56 total cycles (median 2, range 1–12). Two patients (11%) experienced confirmed partial response and six (33%) received >2 cycles. The PFS-4 was 28% (95% confidence interval, 13%–59%). Six patients required dose reductions and three patients were removed due to toxicities. All 18 patients had detectable CTCs and 10 had ctDNA identified. All eight patients with *MYC* amplification at study entry experienced disease progression.

Conclusions: Gemcitabine and nab-paclitaxel demonstrated similar clinical activity and toxicity compared to previous retrospective reports utilizing gemcitabine and docetaxel in patients with recurrent osteosarcoma. Serial analysis of CTC and ctDNA was feasible in this prospective multi-institution study and provides preliminary data on the use of these assays in patients with relapsed disease.

Introduction

Osteosarcoma is the most common cancer arising in bone and primarily affects adolescents and young adults. Standard treatment for osteosarcoma includes surgical resection of primary tumor and

chemotherapy consisting of cisplatin, doxorubicin, and high-dose methotrexate. Unfortunately, nearly half of all patients with osteosarcoma relapse after initial therapy, and salvage chemotherapy regimens have not been able to improve outcomes for patients with recurrent unresectable tumors (1–3). Given the overall poor survival

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Clin Cancer Res 2024;30:5314–22

doi: 10.1158/1078-0432.CCR-24-1339

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Translational Relevance

Secreted protein, acidic and rich in cysteine and caveolin-1 expression, is widespread in osteosarcoma and can facilitate intracellular accumulation of nab-paclitaxel. The pharmacokinetics and toxicity profile of nab-paclitaxel in adult studies suggested its combination with gemcitabine may help improve activity and tolerability in pediatric patients with recurrent osteosarcoma, using a regimen established for pancreatic cancer. We prospectively collected serial assessments of both circulating tumor cells and ctDNA. All patients whose tumors had *MYC* amplification by ctDNA analysis experienced disease progression, suggesting its role in identifying very high-risk patients. Overall, the toxicity and activity of the combination was similar to that in previous reports of gemcitabine and docetaxel, with no clear advantage seen for the newer regimen. Our study demonstrated the feasibility of serial blood-based monitoring of relapsed patients in a multicenter clinical trial in patients with relapsed osteosarcoma.

for patients with recurrent osteosarcoma (1, 2), more effective therapies are clearly needed for this population.

The combination of gemcitabine and docetaxel is commonly used to treat patients with soft tissue sarcomas (4) and has also been studied for the treatment of recurrent osteosarcoma (5–7). A larger retrospective study reported an objective response rate of 17% and progression-free survival at 4 months (PFS-4) of 56% with gemcitabine and docetaxel (8), leading to its recommendation as a salvage therapy in the National Comprehensive Cancer Network guidelines (9). However, other studies of this regimen have reported less benefit (7, 10, 11).

Nab-paclitaxel is a nanoparticle albumin-bound (nab) formulation of paclitaxel with more favorable pharmacokinetic properties, less toxicity, and improved intracellular accumulation compared to carrier-based paclitaxel (12, 13). The reduction in toxicity allows for the administration of higher doses, resulting in responses in preclinical models and patients whose tumors were resistant to conventional paclitaxel (12, 14). Nab-paclitaxel has shown single-agent activity against osteosarcoma xenografts (15) and co-treatment with gemcitabine yielded additive benefits (16). The combination has been used in a variety of clinical contexts and is now standard therapy for patients with pancreatic cancer (17).

Previous studies have identified two transport mechanisms for nab-paclitaxel that may have relevance for the treatment of osteosarcoma. Caveolin-1 (CAV-1) facilitates the passage of nab-paclitaxel across the endothelial cell membrane and into the tumor interstitium. The drug then enters the tumor cell via the adhesion protein Secreted Protein, Acidic and Rich in Cysteine (SPARC), which normally mediates the passage of albumin. In some tumor types, SPARC expression on tumor cells or stroma correlates with the activity of nab-paclitaxel (18, 19). SPARC expression is nearly universal in osteosarcoma, and its high expression is correlated with a worse prognosis (20, 21). CAV-1 is also expressed in osteosarcoma, especially in patients with metastatic disease (20). These findings suggest the possibility that expression of one or both proteins may be correlated with the activity of nab-paclitaxel in osteosarcoma, and given the prior experience of combining taxanes with gemcitabine for recurrent

osteosarcoma, provided the rationale for a clinical trial of these agents using a previously established regimen.

One feature of osteosarcoma tumors is their tendency to calcify even when responding to therapy, with no change in measurable size. This can make the assessment of tumor response challenging when using conventional radiological response criteria such as Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (22). Furthermore, tissue biopsies at recurrence are not routinely performed, making investigations into mechanisms of treatment resistance and recurrence difficult. There remains an unmet need for predictive biomarkers in bone sarcomas to guide patient selection and assess response to therapy. “Liquid biopsies,” such as circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in peripheral blood, have been increasingly used as an alternative to tumor biopsies to identify and track disease characteristics (23–26). These liquid biopsies can be repeated at multiple time points during and after treatment to monitor disease burden, tumor heterogeneity, genomic complexity, and evolution throughout treatment (27). In osteosarcoma, there have been several studies that report using next-generation sequencing (NGS)-based platforms to detect ctDNA, including the use of ultralow passage whole-genome sequencing (ULP-WGS) to detect ctDNA, and identify copy number alterations (CNA) within the tumor (27–30). The detection of CTCs has also been reported based on a variety of techniques (31–34). However, the feasibility and utility of using sequential liquid biopsy monitoring of patients with relapsed osteosarcoma undergoing uniform treatment and imaging evaluations on a multi-institutional trial has not yet been reported.

We therefore designed a phase II trial to study the combination of gemcitabine and nab-paclitaxel in pediatric soft tissue and bone sarcomas, utilizing the same dose and administration schedule as approved for pancreatic cancer. The results of the Ewing sarcoma cohort were recently published (35), and the study is ongoing for patients with recurrent rhabdomyosarcoma (NCT02945800). We herein report the results for the osteosarcoma cohort, including correlative studies that included assessment of potential biomarkers of chemosensitivity as well as sequential data for CTC and ctDNA while on therapy.

Patients and Methods

Patient eligibility

Patients aged 12 to 30 years with relapsed or refractory osteosarcoma treated at participating institutions of the National Pediatric Cancer Foundation were included. Patients were required to have measurable disease using RECIST v1.1 (22). Other inclusion criteria were a Karnofsky or Lansky score ≥ 60 , recovery from prior therapy as defined by >3 weeks since myelosuppressive therapy, >7 days since filgrastim and >14 days since pegfilgrastim, >7 days since biological agent, >3 half-lives for monoclonal antibodies, ≥ 2 weeks since completion of local palliative radiation, ≥ 3 months if prior 50% pelvic irradiation, and ≥ 6 weeks for other substantial marrow irradiation. Adequate bone marrow function (absolute neutrophil count $\geq 1,000/\mu\text{L}$ and platelet count $\geq 100,000/\mu\text{L}$), normal serum creatinine or a creatinine clearance ≥ 60 mL/minute/1.73 m² for patients with creatinine levels above normal, and bilirubin/aspartate aminotransferase (AST)/alanine aminotransferase (ALT) $\leq 2.5\times$ upper limit of normal were necessary. Pregnant women, patients with medical or social situations that would limit compliance with study requirements and anyone with $>$ grade 1 neuropathy were

excluded. A summary of representativeness of study participants is listed in Supplementary Table S1. The protocol was approved by the Institutional Review Board for each participating institution, and written informed consent and assent (as appropriate) were obtained according to the local institutional guidelines. The study adhered to key ethical guidelines including the Declaration of Helsinki, Council for International Organizations of Medical Sciences (CIOMS) Guidelines, and Belmont report.

Drug administration

Both nab-paclitaxel and gemcitabine were administered on days 1, 8, and 15 of a 4-week cycle. Nab-paclitaxel was given at the dose of 125 mg/m² as a 30-minute infusion and immediately followed by gemcitabine 1,000 mg/m², given as a 90-minute infusion. All participants received pegfilgrastim beginning on days 16 to 18 of each cycle. As with the pancreatic cancer studies (17), both chemotherapy agents were held on day 15 if the patient experienced grade 4 neutropenia on or prior to that day, and therapy resumed at a reduced dose with subsequent cycles. Nab-paclitaxel was held for the remainder of the cycle for any patients who experienced grade ≥ 3 neuropathy, with dose reduction in subsequent cycles. Patients experiencing febrile neutropenia, failure to meet eligibility criteria on day 29, platelets < 20 000/μL on two occasions during a cycle or need for two platelet transfusions within a 7-day period were subject to dose reduction of gemcitabine (to 675 mg/m²) and nab-paclitaxel (to 100 mg/m²) with subsequent cycles. Patients who experienced the same dose-modifying toxicities even after the appropriate dose reductions, or who did not meet eligibility criteria to start subsequent cycles by day 43, were withdrawn from protocol therapy.

Toxicity and efficacy assessments

Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0 (<http://ctep.cancer.gov>), and any patient receiving at least one dose of either study drug was considered evaluable for toxicity.

Disease evaluations with cross-sectional imaging were performed within 28 days prior to the start of study and at the completion of cycle 2. Patients with stable disease or better continued to have evaluations completed at the end of each even-numbered cycle. Tumor response was reported using RECIST v1.1, including the requirement that responses must be confirmed with additional imaging no sooner than 4 weeks.

Correlative studies

In this study, protein expression of putative markers of chemosensitivity (SPARC, CAV-1, hENT1) was assessed by immunohistochemistry analysis of archival tumor samples, using the most recent tumor sample available. Three commercially available antibodies were optimized and tested to select the most appropriate one for assay development: SPARC (Clone: sc-73472, dilution 1:50; Santa Cruz Biotechnology), CAV-1 (Clone: 7C8, dilution 1:20; Thermo Fisher Scientific), and hENT1 (Clone: SP120, no dilution; Abcam). To quantify protein expression, we used an immunoreactivity score system that incorporates the relative percentage of tumor cells expressing the protein, as well as the relative intensity of staining (36). The score for proportion of tumor cells ranged from 0 to 4 and was based on the proportion of tumor cells expressing the marker (0 = none, 1 ≤ 10%, 2 = 10%–50%, 3 = 51%–80%, and 4 ≥ 80%). The intensity of staining was scored from 0 to 3 and based on the relative extent of color change noted (0 = none, 1 = mild, 2 = moderate, and 3 = dark brown). The

Table 1. Patient characteristics (N = 18).

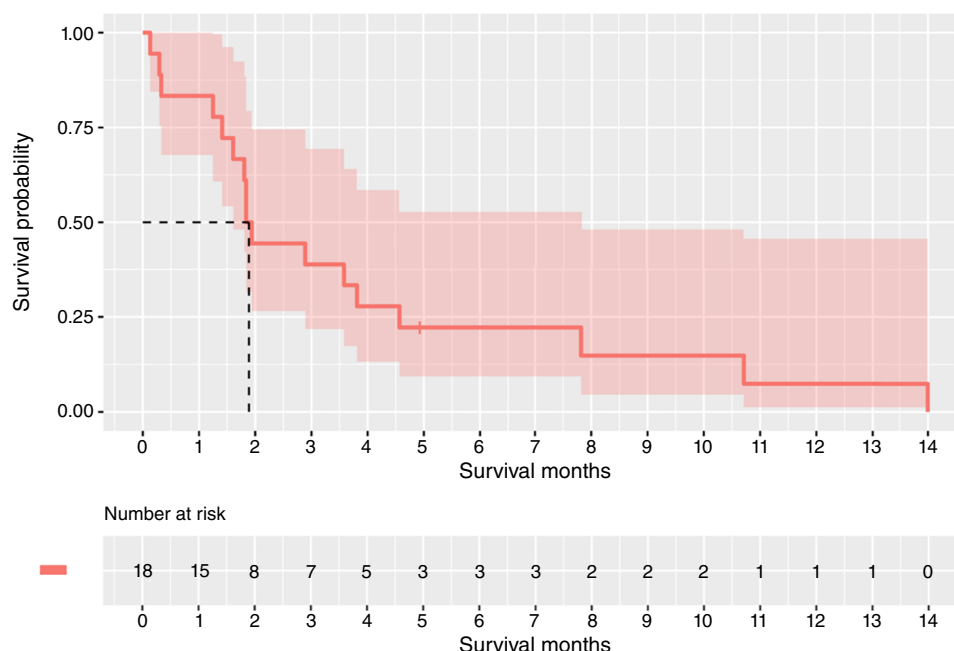
Characteristic	Number of patients (%)
Gender	
Female	9 (50%)
Male	9 (50%)
Age at study entry	
Median (IQR)	16.5 (13.3, 19.8)
Race	
Asian	1 (6%)
Black or African American	4 (22%)
White	11 (61%)
Unknown	2 (11%)
Ethnicity	
Hispanic or Latino	3 (17%)
Non-Hispanic	13 (72%)
Unknown	2 (11%)
Number of prior chemotherapy regimens	
1	3 (17%)
2	8 (44%)
3	4 (22%)
4 or more	3 (17%)
Prior radiation therapy	
Yes	8 (44%)
No	10 (56%)

immunoreactivity score was the product of these two scores, and scores of 0 to 1 were considered negative expression, 2 to 3 were mild, 4 to 8 were moderate, and 9 to 12 were termed strong expression.

A total of 10 mL of peripheral blood was collected in CellSave tubes and 10 mL in Streck DNA BCT at enrollment at cycle 2 day 1, end of cycle 2, and at the end of every two subsequent cycles. Blood collected in CellSave tubes was processed through the CellSieve microfiltration membrane (Creatv MicroTech), and captured cells were enumerated by direct visualization by a trained technician using a BZ-X microscope (Keyence) following immunofluorescence staining. Negative staining for CD45, positive staining for vimentin, and DAPI (4',6-diamidino-2-phenylindole) nuclear stain positivity and morphology were used to define and enumerate CTCs using a custom antibody solution developed by Creatv MicroTech, as previously described (31, 37, 38). When CTC clusters were identified, these were enumerated as a single CTC. Antibody solution quality control was performed using osteosarcoma cell lines OHS and HUVEC as positive controls and MCF-7 cell lines as negative controls, for all lots of antibody solution used and for each assay provided by Creatv MicroTech. Circulating tumor cell enumeration data at baseline and all time points were then compared and correlated to the reported treatment response.

For blood collected in Streck DNA BCT, plasma was isolated by a double centrifugation technique and frozen at −80°C until use (39). Cell-free DNA was extracted from 1 mL of plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen). DNA quantification was performed using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific). Library prep was performed by the NEBNext Ultra II DNA library preparation kit. Resultant 0.5× coverage was analyzed by the ichorCNA algorithm using previously detailed methods (40). According to the ichorCNA copy number prediction, copy number states were mapped to hemizygous deletions (one copy of gene), copy neutral (two copies), copy gain (three copies), amplification (four copies), and high-level amplification (five–seven copies).

Figure 1.
Kaplan–Meier projection of PFS for
all patients ($N = 18$).



Statistical design

Because calcification of tumors can confound response determination, PFS-4 is now a preferred primary endpoint for phase II studies of relapsed osteosarcoma (32). Our trial used a Simon's two-staged design in which the null hypothesis that the PFS-4 was $\leq 10\%$ was tested against a one-sided alternative PFS-4 of $\geq 35\%$ with 80% power. This target response rate was chosen because this level of activity has been achieved by other available salvage regimens (8, 41–43). Eleven evaluable patients were to be accrued in the first stage. If two or more were progression-free at 4 months, then seven additional patients would be added in a second stage for a total of 18 patients. The null hypothesis would be rejected if five or more total patients were progression-free at 4 months. PFS was estimated using Kaplan–Meier methods with confidence interval based on the log-log approach. PFS-4 was compared to the historical 10% rate using the exact binomial test. We also calculated a modified PFS (mPFS) definition to incorporate failure to receive 4 months of protocol therapy for non-progression reasons such as toxicity as an event, in addition to progression and deaths.

Data availability

All relevant data are provided within the article and the accompanying Supplementary data. Because of the Health Insurance Portability and Accountability Act requirements, we are not allowed to share individualized patient genomic data that contain potentially identifying or sensitive patient information. However, we are committed to collaborative data analysis, and more information and mechanisms for data access can be obtained by contacting the corresponding authors or the National Pediatric Cancer Foundation (sunshineproject@moffitt.org).

Results

Patient characteristics are shown in **Table 1**. Eighteen patients with a median age of 16 years (range 12–26) received a total of 56 cycles (median 2, range 1–12) of gemcitabine and nab-paclitaxel. The median

number of prior chemotherapy regimens was 2 (range 1–7). A total of 8/18 patients (44%) also received prior radiation therapy.

Response

Two patients (11%) experienced a confirmed partial response, and six (33%) received up to four cycles (**Fig. 1**). Of the two patients with partial response, one withdrew after six cycles to receive other therapy, and the second came off study after five cycles for recurrent hematologic toxicity. Three patients had stable disease, and one patient remained progression-free after completing 12 cycles of protocol therapy. Median PFS was 1.9 months [95% confidence interval (CI), 1.6–7.8]. Five patients out of the 18 eligible patients were progression-free at 4 months, and therefore the PFS-4 was 28% (95% CI, 13%–59%), with a P value of 0.028 compared to the historical rate of 10%. However, one of the patients with 4-month PFS received less than two cycles of protocol therapy and went off treatment due to an adverse event (AE), and therefore the mPFS-4 was 22% (95% CI, 9%–53%), with a P -value of 0.098 compared to 10% rate. For three patients, this was their first salvage regimen after standard therapy, and none had response or stable disease.

Toxicity

Myelosuppression was the most common grade 3 to 4 toxicity reported. Six patients (33%) required dose reductions for neutropenia ($n = 4$), pleural effusion (1), or neuropathy (1). Two patients were removed from study secondary to neutropenia despite dose reduction and myeloid growth factor support, and one patient came off study due to severe peripheral edema. Grade 3 and above toxicities experienced during this study are listed in **Table 2**.

Correlative studies

Archival tissue samples were available in 10 patients. Strong (3+) expression of SPARC was noted in all the samples, with diffuse expression in nine. In contrast, CAV-1 expression was mostly focal or negative, although two samples had diffuse expression. Expression of hENT-1 was more variable, and results are reported in

Table 2. Toxicity summary ($N = 18$).

AE Category	AE Description	Grade 3		Grade 4	
		<i>n</i>	%	<i>n</i>	%
Blood and lymphatic system disorders	Anemia	11	(61.1)	0	(0)
	Platelet count decreased	3	(16.7)	8	(44.4)
	White blood cell decreased	6	(33.3)	2	(11.1)
	Neutrophil count decreased	4	(22.2)	4	(22.2)
	Lymphocyte count decreased	7	(38.9)	3	(16.7)
Cardiac disorders	Pericardial effusion	1	(5.6)	0	(0)
	Sinus tachycardia	0	(0)	0	(0)
Gastrointestinal disorders	Mucositis, oral	1	(5.6)	0	(0)
	Small intestinal obstruction	1	(5.6)	0	(0)
	Alanine aminotransferase increased	1	(5.6)	0	(0)
	Aspartate aminotransferase increased	1	(5.6)	0	(0)
General disorders and administration site conditions	Edema limbs	1	(5.6)	0	(0)
	Fatigue	1	(5.6)	0	(0)
Infections and infestations	Infections and infestations—Other, specify	0	(0)	0	(0)
	Sepsis	0	(0)	1	(5.6)
Fluid and electrolytes	Dehydration	1	(5.6)	0	(0)
	Hypokalemia	0	(0)	0	(0)
	Hyponatremia	1	(5.6)	0	(0)
	Hypophosphatemia	1	(5.6)	0	(0)
Nervous system disorders	Peripheral motor neuropathy	1	(5.6)	0	(0)
	Peripheral sensory neuropathy	0	(0)	0	(0)
Respiratory, thoracic and mediastinal disorders	Dyspnea	0	(0)	0	(0)
	Epistaxis	1	(5.6)	0	(0)
	Pleural effusion	1	(5.6)	0	(0)
Skin and subcutaneous tissue disorders	Rash, maculo-papular	0	(0)	0	(0)
Vascular disorders	Hypotension	1	(5.6)	0	(0)
	Thromboembolic event	1	(5.6)	0	(0)

Supplementary Table S2. Given that only a few patients experienced sustained benefit, no clinical correlation could be drawn from these results.

All 18 patients had CTCs identified by the CellSieve method during the course of the study, including 16 at the time of study entry. Serial samples collected at study entry and while on therapy were available for 15 patients (Table 3). Three patients had rapid disease progression during the first cycle, and no further samples were collected after study entry. When increasing CTC counts were defined as an increase of >5 increase over two time points, we identified 13 time points in 10 patients who fit this criteria. Of the 13 time points, eight time points correlated with the timing that the patient was found to have progressive disease. In patient 6, an increase of CTC was detected after cycle 1, and the patient was found to have progressive disease following cycle 2 (Table 3).

ctDNA was detected by ULP-WGS in 10 patients (55%) during the course of the study (Supplementary Table S3). While eight patients (44%) had ctDNA detected at baseline study entry (Table 3), there were very few time points with positive ctDNA after treatment started, regardless of treatment response or not (Supplementary Table S3). Osteosarcoma has been recently reported as a disease characterized by recurrent somatic CNA (27). As ULP-WGS analysis by ichorCNA can detect CNAs from the ctDNA, we next probed our dataset to assess whether specific genes previously reported to be commonly amplified or deleted (44, 45) can be detected in ctDNA. Specifically, CNA in *MYC*, *CCNE1*, *CCND3*, *RAD21*, *VEGFA*, *AURKB*, *CDK4*, *PTEN*, *RB1*, *CDKN2A/B*, *NF1*, *NF2*, *MCL1*,

IGF1R, *KIT*, *KDR*, and *PDGFRA* were investigated. Not surprisingly, in the eight patients who had ctDNA detected at baseline study entry, CNA were noted most commonly in *MYC*, *RAD21*, and *CCND3*, corresponding to previously published results from clinical targeted sequencing of osteosarcoma tumors (Fig. 2; ref. 45). All eight patients with *MYC* amplification detected at baseline showed disease progression. Conversely, none of the five patients with either partial response or stable disease noted in our study had any ctDNA identified at baseline.

Discussion

This study provides the first prospective data on patients with recurrent osteosarcoma and measurable disease who received the combination of gemcitabine and nab-paclitaxel. Although there was supporting preclinical information as well as encouraging preliminary responses in patients with soft tissue sarcoma with this combination (46, 47), the results of our study did not show any improvement in activity or toxicity compared to retrospective reports describing the use of gemcitabine and docetaxel for recurrent osteosarcoma (5, 6, 8). While we were able to expand to the second portion of the two-stage Simon design, we ultimately observed a PFS-4 of 28%, which was below our prespecified threshold to warrant further study.

As expected, myelosuppression was the most notable dose-modifying toxicity. As with many sarcoma phase II trials, we administered gemcitabine over 90 minutes to hopefully achieve higher intracellular levels of active metabolites and improve antitumor

Table 3. Correlation of baseline ctDNA and CTC trends with response and timing of progression.

	Entry tumor DNA%	Entry CTC	CTC-1 ^a	CTC-2	CTC-4	CTC-6	CTC-8	CTC-10	CTC-12	Response	>5 increase of CTC
1	5.89%	16	27	200						PD	Yes
2	10.91%	2	3	219						PD	Yes
3	15.45%	6								PD	NE
4	0%	0	0	22						PD	Yes
5	0%	2	4	2						PD	No
6	0%	13	32	30						PD	Yes
7	5.36%	0	0	11						PD	Yes
8	0%	10	26	4	4	48^b				SD	Yes
9	0%	5	5	9	3					PR	No
10	40.44%	14								PD	NE
11	0%	4								PD	NE
12	0%	2	10	0	0	3	108	111	42	SD	Yes
13	0%	13	9	26						PD	Yes
14	9.76%	7	28							PD	Yes
15	25.19%	16	0	4						PD	No
16	0%	8	9	2	2					PR	No
17	17.41%	5	0	53						PD	Yes
18	0%	4	2	0						SD	No

NOTE: Bold value indicates time of disease progression.
Abbreviation: NE, not evaluable.
^aAssessment of CTC after each designated cycle.
^bSample collected at disease progression; patient came off study due to site discretion after four cycles.

activity (48, 49). This longer infusion rate has been associated with greater myelosuppression compared with the 30-minute schedule used for pancreatic cancer, and it is possible that myelosuppression

may have been less if a 30-minute infusion was used, especially in our pretreated population. However, twice as many patients discontinued protocol therapy for progressive disease compared to hematologic toxicity, suggesting that low activity remains the limiting feature of this combination. Toxicity led to dose modifications in 33% and discontinuation in 17% of patients. There were no patient deaths from toxicity, and overall, this outpatient regimen was considered feasible.

The finding of two objective partial responses shows that a subset of patients has tumor reduction with this combination. Unfortunately, no predictive biomarkers are yet available to identify which patients are most likely to respond. On the basis of data regarding SPARC and CAV-1 expression and response to nab-paclitaxel-based therapy in adult carcinomas (18, 19), we explored these possible relationships using immunohistochemistry of archival tumor tissue. However, the small sample size and paucity of responders prevented any formal conclusions. In addition, it is unknown whether SPARC or CAV-1 expression changes throughout the disease course. Similarly, in this small cohort of patients, we could not identify a relationship between the expression of hENT1 and response to this gemcitabine-based regimen, as has been suggested by a larger study in adult soft tissue sarcomas (50).

Other potential biomarkers investigated in this study included serial assessment for CTCs and ctDNA. To our knowledge, our study is the first to prospectively analyze serial samples in patients with relapsed osteosarcoma receiving uniform therapy as part of a multi-institutional clinical trial. This tumor type is well suited for such analyses given the high frequency of micrometastatic disease and hematogenous dissemination. Detection of CTC has been challenging in sarcomas, which lack surface expression of epithelial markers such as EpCAM that are used to detect carcinomas. To overcome this, vimentin has been utilized as a sarcoma-specific marker along with separation based on cellular size (31, 33, 51). In

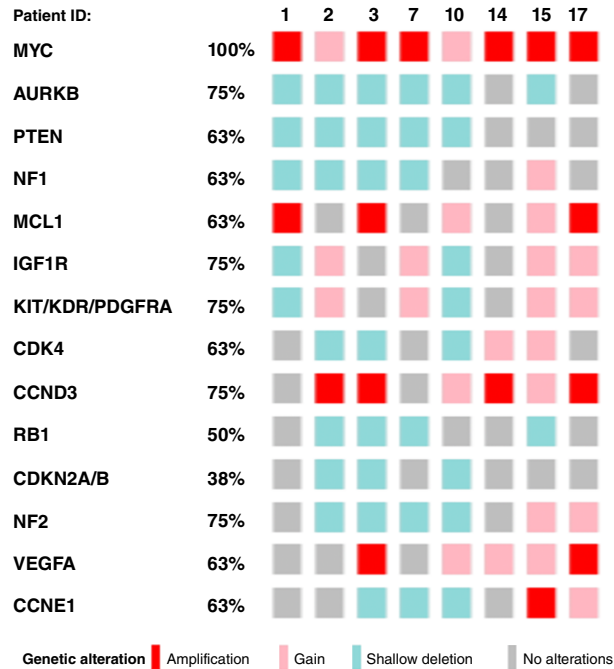


Figure 2. Oncoprint showing genomic alteration from the ctDNA from the eight patients with detectable value, all of whom showed disease progression on study.

this study, to detect CTCs, we utilized CellSieve, a size-based microfiltration platform that we have previously published (31, 37, 38, 52, 53). We were able to identify CTCs in all patients, and in patients who had increasing CTC detected while on study, eight patients were found to have progressive disease at that time point. While these data are promising, there are limitations, as this is from a small pilot cohort, and only one blood sample was drawn at each time point, rather than collecting replicates. Whether CTC detection by CellSieve can be used as a sensitive marker of disease progression requires validation in a large prospective cohort. The ongoing multi-institute study “MCC20320 Blood based biomarkers for minimal residual disease detection in pediatric sarcomas” by the National Pediatric Cancer Foundation (NPCF) has been designed to answer this exact question. CellSieve has been demonstrated to enable identification of specific receptor expression on CTCs (54–56) and to perform molecular analysis on the cells (31). It is possible that further optimization of this methodology, perhaps with the combination of other molecular techniques such as RNA *in situ* hybridization, multiplex immunostaining, and single cell RNA sequencing, can enable further investigations to better characterize these cells, a potential pathway to identifying novel therapeutic targets. Unlike other sarcomas driven by single-nucleotide variants or gene fusions, osteosarcoma is more commonly characterized by large somatic CNAs (44, 45). To approach this unique genomic landscape, there have been approaches using targeted NGS panels (27, 28), as well as ULP-WGS (57). In our study, eight patients (44%) had ctDNA detectable at baseline, and two others had ctDNA detectable after treatment commenced despite being negative at baseline. This rate of positivity is relatively similar to a previous retrospective correlative analysis of 72 patient samples with newly diagnosed localized osteosarcoma, which identified ctDNA in 57% of the samples using ULP-WGS (28). In three-fourths of our positive samples, patients were found to have a gain/amplification of *MYC* by WLP-NGS. In a large genomic analysis of patient samples, *MYC* has been demonstrated to be the most common gain/amplification and has been found to be associated with poor outcomes (44, 45). The patients enrolled in our cohort were all patients with recurrent/refractory osteosarcoma, and the high ratio of patients with *MYC* gain/amplification in our ctDNA results are certainly consistent with these previous reports, with all of them experiencing disease progression. Interestingly, ctDNA remained undetectable for patients with partial response or stable disease, thereby suggesting its utility as a possible marker for treatment response. Furthermore, we identified CNAs in genes with future therapeutic potential, such as gain/amplification of *CDK4* and *AURKB* (44, 58, 59). Whether these gains/amplifications can be impacted by specific inhibitors requires further investigation, but the detection of such CNAs through liquid biopsy points to the potential of using such techniques in the future to guide clinical trial design through non-invasive patient tumor genotyping.

Given that our study simultaneously measured both CTC and ctDNA burden, we hoped to gain preliminary insight into whether one biomarker may be superior or if there were complementary features in a fashion seen in breast cancer (60). While this study was not specifically designed to detect such differences, we did observe that CTC appeared to be better suited to detect disease progression, while ctDNA detection was able to yield molecular disease characteristics that were not available in CTC enumeration. Our findings are preliminary given the relatively small number of patients studied. Ongoing studies such as the previously mentioned NPCF translational research trial are expected to generate data on the ideal

clinical applicability of CTC and ctDNA in pediatric sarcoma patients, including osteosarcoma.

In conclusion, only a small subset of pretreated patients with relapsed osteosarcoma and measurable disease had clinical benefit from the combination of gemcitabine and nab-paclitaxel in this study. When comparing historical retrospective studies, we did not identify an advantage for using nab-paclitaxel versus docetaxel as a partner for gemcitabine in treating patients with relapsed osteosarcoma. The PFS-4 for our patients was lower than that previously reported for other commonly used salvage regimens such as high-dose ifosfamide or tyrosine kinase inhibitors. We conclude that this is a regimen with modest activity and toxicity that is similar to gemcitabine and docetaxel. However, the dynamics of CTC and ctDNA levels seen on this trial may offer insight into how serial collection of blood samples could potentially be used to help monitor response and help identify biomarkers in patients with relapsed osteosarcoma.

Authors' Disclosures

M. Hayashi reports grants from National Pediatric Cancer Foundation during the conduct of the study. A. Bodlak reports grants from NPCF during the conduct of the study. L. Mascarenhas reports personal fees from Gilead and Novartis outside the submitted work. L. Mascarenhas also reports funding for clinical trials from AstraZeneca/MedImmune, Lilly, Bayer, Salaris, Turning Point Therapeutics, Pfizer, Amgen, E.R. Squibb Sons, LLC, Jazz Pharmaceuticals, and Bayer, where all funds were directed to previous institution of employment (ended October 2023) and no personal fees were received; and uncompensated relationships as a Board Member for Children's Oncology Group Foundation and an Emeritus Board Member for The Pablove Foundation. S.C. Borinstein reports personal fees from Merck outside the submitted work. C.A. Pratilas reports grants from Novartis and Kura Oncology, as well as personal fees from Day One Therapeutics outside the submitted work. J. Metts reports previously receiving research funding from Bristol Myers Squibb for research unrelated to this study. D.R. Reed reports other support from Springworks and Eisai outside the submitted work. D.L. Adams reports other support from Creatv MicroTech both during the conduct of the study and outside the submitted work. L.M. Wagner reports personal fees from Jazz Pharmaceuticals during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

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Acknowledgments

This study was generously supported by the National Pediatric Cancer Foundation (nationalpcf.org). We thank the patients, families, and staff at our institutions who helped with all aspects of trial conduct. This work has been supported in part by the Translational Research Core at the H. Lee Moffitt Cancer Center and Research Institute, an NCI-designated Comprehensive Cancer Center (P30-CA076292).

Note

Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Received April 27, 2024; revised July 2, 2024; accepted October 1, 2024; published first October 3, 2024.

References

- Spraker-Perlman HL, Barkauskas DA, Krailo MD, Meyers PA, Schwartz CL, Doski J, et al. Factors influencing survival after recurrence in osteosarcoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer* 2019;66:e27444.
- Gelderblom H, Jinks RC, Sydes M, Bramwell VH, van Glabbeke M, Grimer RJ, et al. Survival after recurrent osteosarcoma: data from 3 European Osteosarcoma Intergroup (EOI) randomized controlled trials. *Eur J Cancer* 2011;47:895–902.
- Ferrari S, Briccoli A, Mercuri M, Bertoni F, Picci P, Tienghi A, et al. Post-relapse survival in osteosarcoma of the extremities: prognostic factors for long-term survival. *J Clin Oncol* 2003;21:710–5.
- Reed DR, Hayashi M, Wagner L, Binitie O, Stepan DA, Brohl AS, et al. Treatment pathway of bone sarcoma in children, adolescents, and young adults. *Cancer* 2017;123:2206–18.
- Navid F, Willert JR, McCarville MB, Furman W, Watkins A, Roberts W, et al. Combination of gemcitabine and docetaxel in the treatment of children and young adults with refractory bone sarcoma. *Cancer* 2008;113:419–25.
- Mora J, Cruz CO, Parareda A, de Torres C. Treatment of relapsed/refractory pediatric sarcomas with gemcitabine and docetaxel. *J Pediatr Hematol Oncol* 2009;31:723–9.
- Fox E, Patel S, Wathen JK, Schuetz S, Chawla S, Harmon D, et al. Phase II study of sequential gemcitabine followed by docetaxel for recurrent Ewing sarcoma, osteosarcoma, or unresectable or locally recurrent chondrosarcoma: results of Sarcoma Alliance for Research Through Collaboration Study 003. *Oncologist* 2012;17:321.
- Palmerini E, Jones RL, Marchesi E, Paioli A, Cesari M, Longhi A, et al. Gemcitabine and docetaxel in relapsed and unresectable high-grade osteosarcoma and spindle cell sarcoma of bone. *BMC Cancer* 2016;16:280.
- National Comprehensive Cancer Network. Bone cancer (version 1.2024). [updated 2023 Aug 7]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/bone.pdf.
- He A, Qi W, Huang Y, Sun Y, Shen Z, Zhao H, et al. Comparison of pirarubicin-based versus gemcitabine-docetaxel chemotherapy for relapsed and refractory osteosarcoma: a single institution experience. *Int J Clin Oncol* 2013;18:498–505.
- Xu J, Guo W, Xie L. Combination of gemcitabine and docetaxel: a regimen overestimated in refractory metastatic osteosarcoma? *BMC Cancer* 2018;18:987.
- Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin Cancer Res* 2006;12:1317–24.
- Henderson IC, Bhatia V. Nab-paclitaxel for breast cancer: a new formulation with an improved safety profile and greater efficacy. *Expert Rev Anticancer Ther* 2007;7:919–43.
- Sparreboom A, Scripture CD, Trieu V, Williams PJ, De T, Yang A, et al. Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clin Cancer Res* 2005;11:4136–43.
- Yang Y, Niu X, Zhang Q, Hao L, Ding Y, Xu H. The efficacy of abraxane on osteosarcoma xenografts in nude mice and expression of secreted protein, acidic and rich in cysteine. *Am J Med Sci* 2012;344:199–205.
- Wagner LM, Yin H, Eaves D, Currier M, Cripe TP. Preclinical evaluation of nanoparticle albumin-bound paclitaxel for treatment of pediatric bone sarcoma. *Pediatr Blood Cancer* 2014;61:2096–8.
- Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013;369:1691–703.
- Desai N, Trieu V, Damascelli B, Soon-Shiong P. SPARC expression correlates with tumor response to albumin-bound paclitaxel in head and neck cancer patients. *Transl Oncol* 2009;2:59–64.
- Desai NP, Trieu V, Hwang LY, Wu R, Soon-Shiong P, Gradishar WJ. Improved effectiveness of nanoparticle albumin-bound (nab) paclitaxel versus polysorbate-based docetaxel in multiple xenografts as a function of HER2 and SPARC status. *Anticancer Drugs* 2008;19:899–909.
- Mayordomo E, Machado I, Giner F, Kresse SH, Myklebost O, Carda C, et al. A tissue microarray study of osteosarcoma: histopathologic and immunohistochemical validation of xenotransplanted tumors as preclinical models. *Appl Immunohistochem Mol Morphol* 2010;18:453–61.
- Zhang L, Marrano P, Kumar S, Leadley M, Elias E, Thorner P, et al. Nab-paclitaxel is an active drug in preclinical model of pediatric solid tumors. *Clin Cancer Res* 2013;19:5972–83.
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
- Ucci A, Rucci N, Ponzetti M. Liquid biopsies in primary and secondary bone cancers. *Cancer Drug Resist* 2022;5:541–59.
- Kjær EKR, Vase CB, Rossing M, Ahlborn LB, Hjalgrim LL. Detection of circulating tumor-derived material in peripheral blood of pediatric sarcoma patients: a systematic review. *Transl Oncol* 2023;34:101690.
- Radovich M, Jiang G, Hancock BA, Chitambar C, Nanda R, Falkson C, et al. Association of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy with disease recurrence in patients with triple-negative breast cancer: preplanned secondary analysis of the BRE12-158 randomized clinical trial. *JAMA Oncol* 2020;6:1410–5.
- Leigh NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clin Cancer Res* 2019;25:4691–700.
- Barris DM, Weiner SB, Dubin RA, Fremed M, Zhang X, Piperdi S, et al. Detection of circulating tumor DNA in patients with osteosarcoma. *Oncotarget* 2018;9:12695–704.
- Shulman DS, Klega K, Imamovic-Tuco A, Clapp A, Nag A, Thorner AR, et al. Detection of circulating tumour DNA is associated with inferior outcomes in

- Ewing sarcoma and osteosarcoma: a report from the Children[R8S2Q1M7]s Oncology Group. *Br J Cancer* 2018;119:615–21.
29. Audinot B, Drubay D, Gaspar N, Mohr A, Cordero C, Marec-Bérard P, et al. ctDNA quantification improves estimation of outcomes in patients with high-grade osteosarcoma: a translational study from the OS2006 trial. *Ann Oncol* 2023;35:559–68.
 30. Peneder P, Stütz AM, Surdez D, Krumbholz M, Semper S, Chicard M, et al. Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden. *Nat Commun* 2021;12:3230.
 31. Hayashi M, Zhu P, McCarty G, Meyer CF, Pratilas CA, Levin A, et al. Size-based detection of sarcoma circulating tumor cells and cell clusters. *Oncotarget* 2017;8:78965–77.
 32. Wu Z-J, Tan J-C, Qin X, Liu B, Yuan Z-C. Significance of circulating tumor cells in osteosarcoma patients treated by neoadjuvant chemotherapy and surgery. *Cancer Manag Res* 2018;10:3333–9.
 33. Satelli A, Mitra A, Cuttrera JJ, Devarie M, Xia X, Ingram DR, et al. Universal marker and detection tool for human sarcoma circulating tumor cells. *Cancer Res* 2014;74:1645–50.
 34. Chinen LT, Mello CA, Abdallah EA, Ocea LM, Buim ME, Breve NM, et al. Isolation, detection, and immunomorphological characterization of circulating tumor cells (CTCs) from patients with different types of sarcoma using isolation by size of tumor cells: a window on sarcoma-cell invasion. *Onco Targets Ther* 2014;7:1609–17.
 35. Oesterheld JE, Reed DR, Setty BA, Isakoff MS, Thompson P, Yin H, et al. Phase II trial of gemcitabine and nab-paclitaxel in patients with recurrent Ewing sarcoma: a report from the National Pediatric Cancer Foundation. *Pediatr Blood Cancer* 2020;67:e28370.
 36. Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. *Pathologe* 1987;8:138–40.
 37. Zhu P, Stanton ML, Castle EP, Joseph RW, Adams DL, Li S, et al. Detection of tumor-associated cells in cryopreserved peripheral blood mononuclear cell samples for retrospective analysis. *J Transl Med* 2016;14:198.
 38. Adams DL, Alpaugh RK, Martin SS, Charpentier M, Chumsri S, Cristofanilli M, et al. Precision microfilters as an all in one system for multiplex analysis of circulating tumor cells. *RSC Adv* 2016;6:6405–14.
 39. Hayashi M, Chu D, Meyer CF, Llosa NJ, McCarty G, Morris CD, et al. Highly personalized detection of minimal Ewing sarcoma disease burden from plasma tumor DNA. *Cancer* 2016;122:3015–23.
 40. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat Commun* 2017;8:1324.
 41. Duffaud F, Mir O, Boudou-Rouquette P, Piperno-Neumann S, Penel N, Bompas E, et al. Efficacy and safety of regorafenib in adult patients with metastatic osteosarcoma: a non-comparative, randomised, double-blind, placebo-controlled, phase 2 study. *Lancet Oncol* 2019;20:120–33.
 42. Gaspar N, Venkatramani R, Hecker-Nolting S, Melcon SG, Locatelli F, Bautista F, et al. Lenvatinib with etoposide plus ifosfamide in patients with refractory or relapsed osteosarcoma (ITCC-050): a multicentre, open-label, multicohort, phase 1/2 study. *Lancet Oncol* 2021;22:1312–21.
 43. Grignani G, Palmerini E, Ferraresi V, D'Ambrosio L, Bertulli R, Asaftei SD, et al. Sorafenib and everolimus for patients with unresectable high-grade osteosarcoma progressing after standard treatment: a non-randomised phase 2 clinical trial. *Lancet Oncol* 2015;16:98–107.
 44. Sayles LC, Breese MR, Koehne AL, Leung SG, Lee AG, Liu H-Y, et al. Genome-informed targeted therapy for osteosarcoma. *Cancer Discov* 2019;9:46–63.
 45. Marinoff AE, Spurr LF, Fong C, Li YY, Forrest SJ, Ward A, et al. Clinical targeted next-generation panel sequencing reveals MYC amplification is a poor prognostic factor in osteosarcoma. *JCO Precis Oncol* 2023;7:e2200334.
 46. Tian Z, Zhang F, Li P, Wang J, Yang J, Zhang P, et al. Albumin-bound paclitaxel and gemcitabine combination therapy in soft tissue sarcoma. *BMC Cancer* 2020;20:698.
 47. Metts JL, Alazraki AL, Clark D, Amankwah EK, Wasilewski-Masker KJ, George BA, et al. Gemcitabine/nab-paclitaxel for pediatric relapsed/refractory sarcomas. *Pediatr Blood Cancer* 2018;65:e27246.
 48. Veltkamp SA, Beijnen JH, Schellens JH. Prolonged versus standard gemcitabine infusion: translation of molecular pharmacology to new treatment strategy. *Oncologist* 2008;13:261–76.
 49. Hensley ML. Update on gemcitabine and docetaxel combination therapy for primary and metastatic sarcomas. *Curr Opin Oncol* 2010;22:356–61.
 50. Vincenzi B, Stacchiotti S, Collini P, Pantano F, Rabitti C, Perrone G, et al. Human equilibrative nucleoside transporter 1 gene expression is associated with gemcitabine efficacy in advanced leiomyosarcoma and angiosarcoma. *Br J Cancer* 2017;117:340–6.
 51. Mu H, Zuo D, Chen J, Liu Z, Wang Z, Yang L, et al. Detection and surveillance of circulating tumor cells in osteosarcoma for predicting therapy response and prognosis. *Cancer Biol Med* 2022;19:1397–409.
 52. Adams DL, Martin SS, Alpaugh RK, Charpentier M, Tsai S, Bergan RC, et al. Circulating giant macrophages as a potential biomarker of solid tumors. *Proc Natl Acad Sci U S A* 2014;111:3514–9.
 53. Adams DL, Zhu P, Makarova OV, Martin SS, Charpentier M, Chumsri S, et al. The systematic study of circulating tumor cell isolation using lithographic microfilters. *RSC Adv* 2014;9:4334–42.
 54. Raghavakaimal A, Cristofanilli M, Tang C-M, Alpaugh RK, Gardner KP, Chumsri S, et al. CCR5 activation and endocytosis in circulating tumor-derived cells isolated from the blood of breast cancer patients provide information about clinical outcome. *Breast Cancer Res* 2022;24:35.
 55. Moran JA, Adams DL, Edelman MJ, Lopez P, He J, Qiao Y, et al. Monitoring PD-L1 expression on circulating tumor-associated cells in recurrent metastatic non-small-cell lung carcinoma predicts response to immunotherapy with radiation therapy. *JCO Precis Oncol* 2022;6:e2200457.
 56. Gardner KP, Cristofanilli M, Chumsri S, Lapidus R, Tang CM, Raghavakaimal A, et al. Beta 2-adrenergic receptor in circulating cancer-associated cells predicts for increases in stromal macrophages in circulation and patient survival in metastatic breast cancer. *Int J Mol Sci* 2022;23:7299.
 57. Klega K, Imamovic-Tuco A, Ha G, Clapp AN, Meyer S, Ward A, et al. Detection of somatic structural variants enables quantification and characterization of circulating tumor DNA in children with solid tumors. *JCO Precis Oncol* 2018;2018:PO.17.00285.
 58. Tavanti E, Sero V, Vella S, Fanelli M, Michelacci F, Landuzzi L, et al. Pre-clinical validation of Aurora kinases-targeting drugs in osteosarcoma. *Br J Cancer* 2013;109:2607–18.
 59. Martin-Broto J, Martinez-Garcia J, Moura DS, Redondo A, Gutierrez A, Lopez-Pousa A, et al. Phase II trial of CDK4/6 inhibitor palbociclib in advanced sarcoma based on mRNA expression of CDK4/ CDKN2A. *Signal Transduct Target Ther* 2023;8:405.
 60. Bortolini Silveira A, Bidard F-C, Tanguy M-L, Girard E, Trédan O, Dubot C, et al. Multimodal liquid biopsy for early monitoring and outcome prediction of chemotherapy in metastatic breast cancer. *NPJ Breast Cancer* 2021;7:115.