Received: 27 December 2022 | Revised: 18 March 2023 | Accepted: 27 June 2023

DOI: 10.1002/cac2.12461

CANCER COMMUNICATIONS Open Access

LETTER TO THE EDITOR

Clinical utility of circulating tumor DNA sequencing with a large panel in patients with advanced soft-tissue sarcomas

To the editor

Soft-tissue sarcomas (STS) represent a very heterogeneous group of rare tumors including more than 100 different subtypes [1]. Surgery and neo/adjuvant radiation therapy represent the cornerstone of treatment for STS. However, despite an optimal resection of the tumor, up to 40% of patients will develop metastatic relapse and will die from the disease [1]. Doxorubicin represents the first-line standard of care for patients with advanced disease since the 1970s, despite several attempts to identify better regimens. The median overall survival (OS) of patients with metastatic disease is < 18 months and has only modestly improved over the past 20 years [2].

We and others have previously reported that next-generation sequencing (NGS) of tumor tissues allows the identification of genomic aberrations with the potential to influence and personalize therapy in up to 50% of patients with advanced STS [3, 4].

Genomic profiling of circulating tumor DNA (ctDNA) is increasingly used to tailor therapy in cancer patients. Indeed, such liquid biopsy has several advantages: non-invasiveness, reduced turnaround times for faster results, and the ability to fully capture the landscape of tumor heterogeneity [5].

The aims of the present study were to investigate the impact of ctDNA profiling in a large cohort of patients with advanced STS included in two prospective precision medicine studies and to decipher the ctDNA molecular landscape of sarcoma. Between December 2020 and August 2021, 98 patients with advanced STS were included in two ongoing institutional molecular profiling studies (Bergonie Institute Profiling, BIP: NCT02534649; Gustave Roussy Profiling, STING: NCT04932525). Their characteristics are described in Supplementary Table S1. Genomic analysis (ctDNA and tissue when available) was performed by using the United States Food and Drug Administration (FDA) approved Foundation One Liquid CDx Assay (324 genes, tumor mutational burden [TMB], microsatellite instability status) [5]. All the results were discussed weekly among a multidisciplinary molecular tumor board (MTB). Actionable targets were classified using the European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT) [6], and matched therapies were proposed based on the molecular profiling results.

The amount of ctDNA at diagnosis correlates with survival in patients with localized Ewing sarcoma and osteosarcoma [6]. Interestingly, we found that a tumor fraction ≥10% was significantly associated with short median OS in our series (13 months [95% CI, 0-26.3 months] vs. 22 months [95% CI, 13.9-30.1 months], $P = 0.021$) (Supplementary Figure S1).

ctDNA NGS allowed the identification of at least 1 genomic aberration in 93 cases (94.9%). A total of 624 aberrations were identified in 186 genes. The proportion of patients with aberrations was higher in those suffering from complex genomics sarcomas (49.2%) than in those suffering from translocation-related sarcomas (21.6%). The distribution of the number of aberrations was also significantly different according to the sarcoma genomics group (complex genomics sarcomas, translocation-related sarcomas, and other sarcomas; $\chi^2 = 0.032$, $P = 0.048$). The 5 genes most frequently altered in the entire cohort were tumor protein 53 (*TP53*), ataxia telangiectasia mutated (*ATM*), tuberous sclerosis 1 (*TSC1*), checkpoint kinase 2 (*CHEK2*), and tyrosine-protein kinase (*KIT*). A total of 289 alterations were identified among all the genes explored in the NGS panel: 256 missense mutations, including substitutions or short indels plus splice variants, 27 truncation mutations/nonsense mutations, 1 gene amplification, 3 gene homozygous deletions, and 2 gene rearrangements. The 26 genes most frequently altered are represented in Supplementary Figures S2-S3.

Abbreviations: ctDNA, circulating tumor DNA; CNV, copy number variation; ESCAT, European Society of Medical Oncology Scale for Clinical Actionability of molecular Targets; MTB, molecular tumor board; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; STS, soft-tissue sarcomas.

[#]These two authors equally contributed to the work

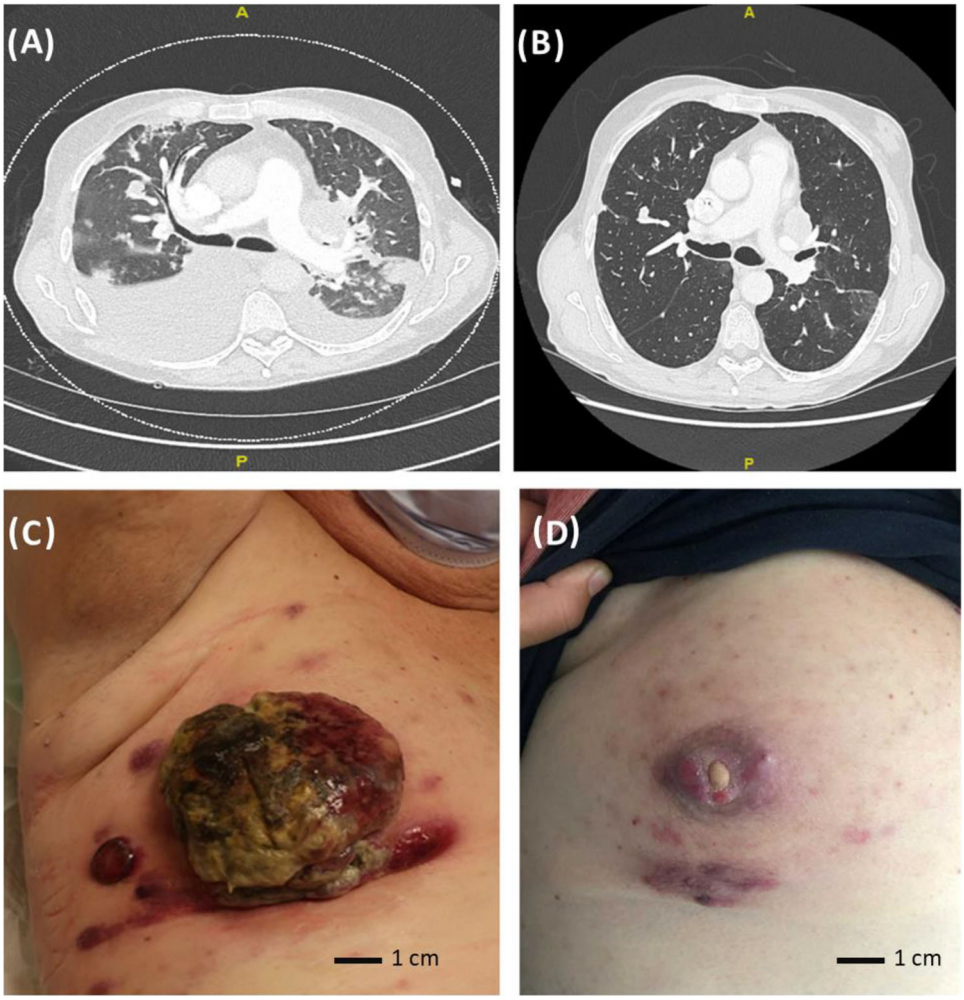

FIGURE 1 FIGURE 1 Objective responses observed in sarcoma patients who received therapy matched to actionable alterations identified through ctDNA sequencing. (A-B) Partial response was observed 6 weeks (B) after treatment onset with mTOR inhibitor in a 63-year-old female patient with advanced leiomyosarcoma harbouring a *PTEN* deletion. (C-D) Partial response was observed 6 weeks after treatment (D) onset in a 73-year-old male patient with advanced angiosarcoma harbouring a *HRAS* and *KRAS* mutation and treated with a combination of HRAS and MEK inhibitors.

Abbreviations: ctDNA, circulating tumor DNA; mTOR, mammalian target of rapamycin; *PTEN*, Phosphatase and TENsin homolog; *HRAS*, Harvey Rat sarcoma virus; *MEK*, mitogen-activated kinase.

At least one actionable aberration that could be used to tailor therapy was identified in 31 patients (32.2%): 14 (31.1%) in the complex genomics sarcoma group, 5 (28.3%) in the translocation-related sarcomas group, and 12 (34.2%) in the other sarcoma group ($P = 0.842$). Targetable genes are presented in Supplementary Figure S4.

Overall, MTB recommended a therapy matched to the genomic profile for 22 patients (22.4%). The 2 main reasons for not recommending a therapy in patients with an actionable alteration were: no clinical trial available for the patient ($n = 6$); the matched therapy was previously received by the patient ($n = 2$).

Ten patients (10.2%) were treated with molecular targeted therapy (Supplementary Table S2, Supplementary Figure S5). The objective response and disease control (objective response and stable disease) rates as per RECIST 1.1 were 20.0% and 40.0%, respectively (Figure 1). The median progression-free survival and OS were 12 weeks and 20 weeks, respectively (Supplementary Figure S6).

Six patients were found to have a high TMB (> 16 MB). The MTB recommended treatment with an immune checkpoint inhibitor (ICI) for 4 patients (4.1%); 2 patients refused the recommended treatment. Two patients (2.1%) received an ICI. The best response was progressive disease in both. The two other patients did not receive ICI as per the physician's decision.

Forty patients also underwent NGS of tissue samples besides ctDNA profiling. Tissue profiling was unsuccessful

for 7 (17.5%) patients. No actionable alteration was found in both ctDNA and tissue profiles for 17 (42.5%) patients. The numbers of actionable aberrations in tissue and ctDNA profiles were similar in 5 (12.5%) patients. The number of actionable alterations was higher in tissue than in ctDNA for 10 (25.0%) patients. The 15 actionable alterations observed in tissue but not in ctDNA are listed in Supplementary Table S3: 10 of them (66.7%) were copy number variants (CNVs). The number of actionable aberrations was higher in ctDNA than in tissue for 1 (2.5%) patient.

Tissue profiling allowed the identification of an ESCAT I, IIIA and IV aberration not presented in ctDNA for 1, 4 and 7 patients, respectively, whereas ctDNA profiling allowed the identification of an actionable aberration (ESCAT IIIA) not presented in tissue for 1 patient.

Thus far, data on ctDNA in STS have been very limited. In patients with localized Ewing sarcoma and osteosarcoma, the amount of ctDNA at diagnosis correlates with survival [7]. Monitoring ctDNA can also have clinical implications particularly to evaluate response to treatment and disease progression [8].

In patients with gastrointestinal stromal tumor, ctDNA and tissue sequencing results were similar, suggesting that liquid biopsy represents a robust approach for tumor genotyping and treatment tailoring [8]. In the present study, we found that 66.7% of ctDNA findings were also identified in tissue profiles. We also found more alterations in tissue than in ctDNA profiling, with the majority (66.7%) being CNVs. This result is in line with a previous study showing lower sensitivity of ctDNA profiling in identifying CNVs [10]. Five patients also had a single nucleotide variant in tissue which was not found through ctDNA profiling. Among them, 3 patients had no detectable ctDNA. ctDNA profiling represents an alternative tool for detecting actionable alterations in STS but is less sensitive than tissue sequencing to detect CNVs (losses or amplifications).

We have previously shown that NGS of tissue allowed the identification of actionable alterations in up to 41% of patients with advanced STS [5]. We demonstrated the capacity of ctDNA profiling to detect actionable aberrations in up to 38% of patients with STS. Future technological development would increase the sensitivity of this approach for CNVs even more.

DECLARATIONS

AUTHOR CONTRIBUTIONS

Conceptualization, Antoine Italiano; methodology, Julie Blanchi, Sofiane Taleb, Arnaud Bayle, Melissa Alame, Yechan Laizet, Paul Dubos; investigation, BV, EK, MT, MS, AI; resources, Antoine Italiano; writing—original draft preparation, Julie Blanchi, Antoine Italiano; writing—review and editing, Julie Blanchi, Sofiane Taleb, Antoine Italiano; supervision, Antoine Italiano; project administration, Antoine Italiano. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors have nothing to report.

CONFLICT OF INTEREST STATEMENT

AI received research grant and honoraria from ROCHE, BAYER, MSD, ASTRAZENECA, MERCK, PHARMAMAR, BMS, PARTHENON, CHUGAI, NOVARTIS. The other authors have no competing interests.

FUNDING INFORMATION

FONDATION MSDAVENIR (HEART grant).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board of Institut Bergonié (IRB 2-323Z). All patients signed informed consent.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

Source data are available on request to the corresponding author.

Julie Blanchi[1,2,#]
Sofiane Taleb[3,#]
Arnaud Bayle[3]
Benjamin Verret[4]
Maud Toulmonde[5]
Mariella Spalato-ceruso[1,5]
Paul Dubos[5]
Yech'an Laizet[5]
Melissa Alame[2]
Emmanuel Khalifa[2]
Antoine Italiano[1,3,5,6]

[1]*INSERM, Unité 1312, SARCOTARGET team, Bordeaux, France*
[2]*Department of BioPathology, Institut Bergonié, Bordeaux, France*
[3]*Drug Developpement Department, Gustave Roussy, Villejuif, France*
[4]*Department of Medicine, Gustave Roussy, Villejuif, France*
[5]*Sarcoma Unit, Institut Bergonié, Bordeaux, France*
[6]*Faculty of Medicine, University of Bordeaux, Bordeaux, France*

Correspondence
Antoine Italiano, Institut Bergonié, 229 cours de l'Argonne, 33000, Bordeaux, France.
Email: a.italiano@bordeaux.unicancer.fr

ORCID

Antoine Italiano https://orcid.org/0000-0002-8540-5351

REFERENCES

1. WHO Classification of Tumours Editorial Board. Soft tissue and bone tumours. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed. Vol. 3).
2. Savina M, Le Cesne A, Blay JY, Ray-Coquard I, Mir O, Toulmonde M, et al. Patterns of care and outcomes of patients with METAstatic soft tissue SARComa in a real-life setting: the METASARC observational study. BMC Med. 2017;15:78.
3. Lucchesi C, Khalifa E, Laizet Y, Soubeyran I, Mathoulin-Pelissier S, Chomienne C, et al. Targetable Alterations in Adult Patients With Soft-Tissue Sarcomas: Insights for Personalized Therapy. JAMA Oncol. 2018;4:1398–404.
4. Gounder MM, Agaram NP, Trabucco SE, Robinson V, Ferraro RA, Millis SZ, et al. Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma. Nat Commun. 2022;13:3406.
5. Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One. 2020;15:e0237802.
6. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol. 2018;29:1895–902.
7. 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's Oncology Group. Br J Cancer. 2018;119:615–21.
8. 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:PO1700285.
9. Serrano C, Vivancos A, López-Pousa A, Matito J, Mancuso FM, Valverde C, et al. Clinical value of next generation sequencing of plasma cell-free DNA in gastrointestinal stromal tumors. BMC Cancer. 2020;20:99.
10. Chan KC, Jiang P, Zheng YW, Liao GJ, Sun H, Wong J, et al. Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem. 2013;59:211–24.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.