molecular tumor board

Olaparib in the Setting of Radiotherapy-Associated Sarcoma: What Can Precision Medicine Offer For Rare Cancers?

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JCO Precis Oncol 7:e2200582. © 2023 by American Society of Clinical Oncology

Introduction

In the era of precision medicine, trials testing the strategy of matching patients with molecularly guided anticancer treatments have evidenced an improvement in progression-free survival compared with standard therapy, but with several limitations preventing a generalization of this strategy.[1-4] Indeed, these trials are underpowered on account of intratumor heterogeneity, which can be spatial and temporal.[5] They can also be affected by the sample conservation method, since formalin fixation can cause DNA damage, leading to false-negative results.[6,7] The sequencing technologies actually available can also have considerable impact, as sequencing is usually limited to coding genomes, and only 15%-20% of tumors harbor a predominant or a targetable molecular event.[8] The strategy is even more complex when epigenetic and/or transcriptomic abnormalities are added.[9] Last but not least, the data obtained requires substantial efforts in terms of biocomputing and literature analyses. Computer algorithms are becoming more and more complex to efficiently approach the functional value of the abnormalities identified.

Soft tissue sarcomas are heterogeneous with more than 100 different subtypes according to the latest WHO classification.[10] Large tumor genome sequencing programs failed to identify recurrent somatic driver point mutations in most of sarcoma subtypes while several oncogenic gene fusion translocations were identified in specific sarcoma subtypes. Most of these large studies discussed tumor type–specific molecular features but did not provide molecular reclassification for therapeutic targets.[11] In addition, sarcoma translocations have a limited therapeutic impact.[12] More personalized genomic approaches could provide benefit to patients with rare tumors.

We report here the case of a radiation-associated sarcoma[13] in a young woman included in the 2025 French Genomic Program, a national initiative that aims to use the genomic characterization of tumors for the personalized treatment of patients with rare cancers, with a dedicated multidisciplinary meeting. As part of the French Genomic Program, the Sequencing Omics Information Analysis (SeqOIA) platform performs tumor and germline whole-genome sequencing, and tumor whole-exome and RNAseq sequencing using frozen tissue samples.

The Clinical Case

A 34-year-old woman, nonsmoker, without a notable medical history, was diagnosed with a 6-cm left breast cancer. The histologic analysis found an invasive ductal carcinoma, Scarff-Bloom-Richardson grade III, estrogen receptor (ER)–positive, progesterone receptor–positive Ki67 at 30% and overexpressing HER2. The tumor was staged as T2N2M0 according to the eighth edition of the American Joint Committee on Cancer TNM Staging System for Breast Cancer. No germline mutation was identified in a panel of genes predisposing to breast cancer, including *BRCA1/2*, *PALB2,* and *TP53*. She received neoadjuvant chemotherapy using four cycles of epirubicin 100 mg/m^2 with cyclophosphamide 500 mg/m^2 every 3 weeks, then four cycles of docetaxel 100 mg/m^2 with trastuzumab 8 mg/kg for the loading dose and 6 mg/kg every 3 weeks, followed by total mastectomy, and radiotherapy of the chest wall and the regional lymph nodes. Trastuzumab was maintained for 1 year thereafter at 6 mg/kg every 3 weeks, along with ovarian suppression for 2 years and an oral hormone therapy for 5 years (Fig 1A).

Six years after radiotherapy, she experienced pain in the left thoracic wall caused by a 10-cm hypermetabolic mass, identified using 18-fluorodeoxyglucose-positron emission tomography-computed tomography (Fig 1B).

A biopsy of the mass enabled the diagnosis of a radiotherapy-induced, poor-prognosis, high-grade osteosarcoma according to the FNCLCC classification, ER-negative, progesterone receptor–negative Ki67 at 80%, and no overexpression of HER2. The patient then received a combination of chemotherapy with doxorubicin 60 mg/m^2, ifosfamide 1.5 g/m^2, and cisplatin 100 mg/m^2 every 3 weeks. After six cycles, she had an excellent partial response according to PERCIST criteria 1.0 (Fig 1B). No standard treatment was recommended thereafter.

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on January 10, 2023 and published at ascopubs.org/journal/po on February 27, 2023: DOI https://doi.org/10.1200/PO.22.00582

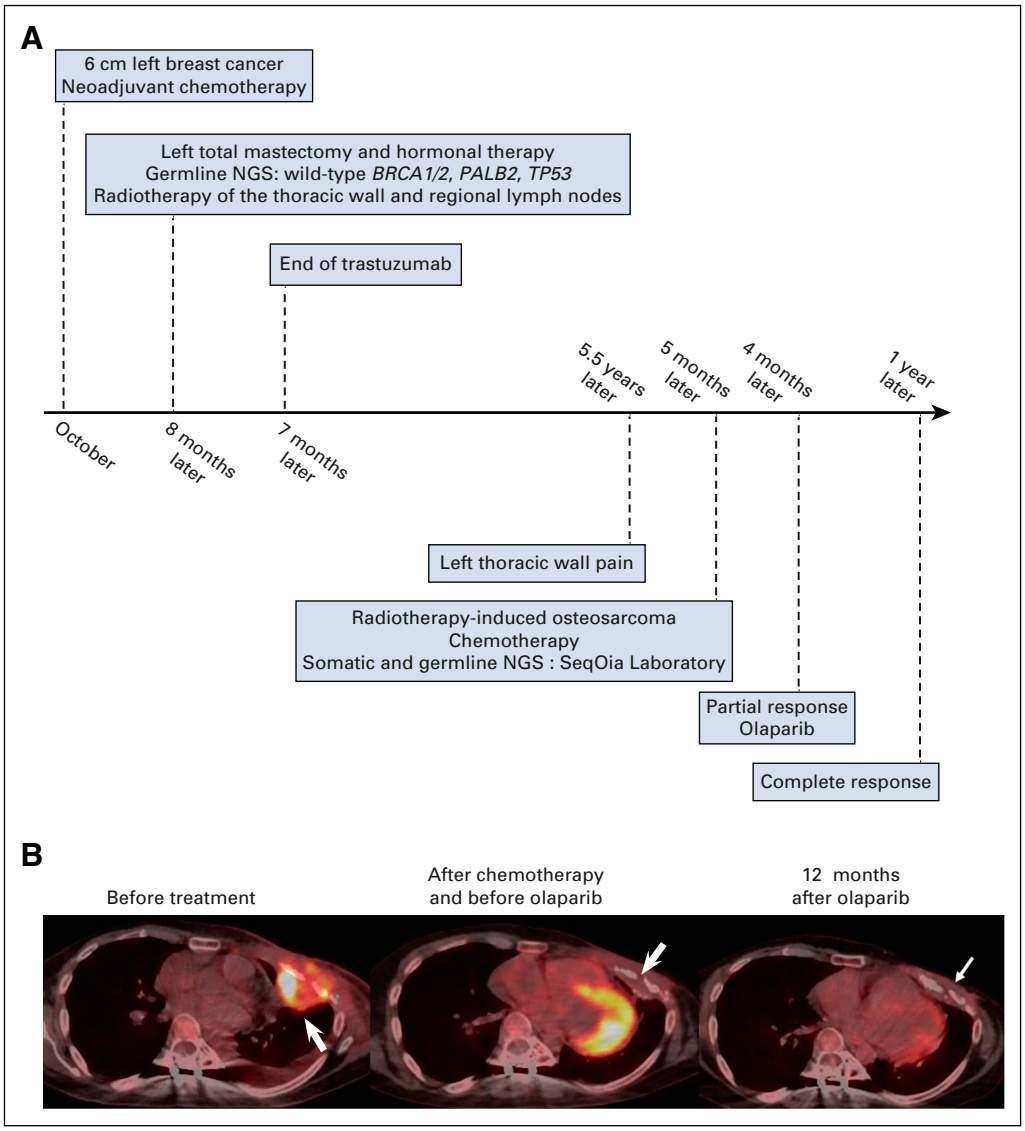

FIG 1. (A) Clinical timeline. (B) 18-fluorodeoxyglucose-positron emission tomography evaluation showing the hypermetabolic left thoracic wall lesion before treatment at a lean body mass–corrected (SUL peak) of 13 (left panel); after five cycles of doxorubicin, ifosfamide, and cisplatin, the SUL peak was 2 (middle panel); 3 months after olaparib, the SUL peak was 1.8 (right panel). NGS, next-generation sequencing; SUL, standard uptake value.

Concomitantly with the sarcoma diagnosis, germline and somatic whole-genome and whole-exome sequencing were carried out on the SeqOIA platform.[14] After quantification and qualification of the nucleic acids, libraries were prepared, and then sequenced in 'pair-end' using single base substitution (SBS) technology (Flow Cell S4, NovaSeq 6000, Illumina). For whole-exome sequencing, exome capture was performed by single-plex hybridization (Twist Human Core Exome Kit + IntegraGen Custom v1, Twist BioScience). Because of the insufficient quality of the nucleic acids, RNAseq was not performed.

After alignment with the reference human genome (GRCh38.92.fa [GRCh38, release-92, Jul 02 2018]),[15] and quality control, variant calling was performed using Haplotype Caller (GATK4, v4.1.0.0) and Mutect 2 (GATK4, v4.1.4.1). Variant annotation was performed using SNPeff (4.3t) and SnpSift (4.3t). Somatic variants were used to calculate the mutational load (pyTMB [v1.3.0dev]) and to extract mutational signatures (COSMIC [v3] databases integrated with SigProfiler [v1.0.9]; Appendix 1).

We found a low tumor mutational burden, with two mutations per mega base, including two probably pathogenic mutations in *TP53* and *EIF4A2* genes (Table 1). This was associated with the SBS3 mutational signature of homologous recombination deficiency (HRD),[16] and a genomic profile showing several HRD-specific events[17] (Fig 2A). We did not identify the usual radiation-associated mutational signature profile, such as SBS18, and SBSs 1, 2 and 3,

TABLE 1. List of Somatic Point Variations, Small Insertions/Deletions, and CNV

List of Somatic Point Variations, Small Insertions/Deletions

Gene	Transcript	Coding Nomenclature	DNA-Tum VAF (alt/tot)	CNV Status	Census	Classification[a]
FANCM	ENST00000267430.9	ENST00000267430.9 (FANCM):c.1667A>G p.(Asp556Gly)	0.72 (263/363)	Gain (AAAB)		Variant of uncertain significance
TP53	ENST00000359597.8	ENST00000359597.8 (TP53):c.919 + 1_919+3delGTA	0.48 (109/229)	Loss (AA)	tier 1l oncogene, TSG, fusion	Probably Pathogenic
EIF4A2	ENST00000440191.6	ENST00000440191.6 (EIF4A2):c.670G>A p.(Glu224Lys)	0.21 (50/238)	Gain (AAB)	tier 1lfusion	Probably pathogenic
GLI3	ENST00000479210.1	ENST00000479210.1 (GLI3):c.71C>A p.(Ala24Asp)	0.13 (45/335)	Gain (AAABB)		Variants of uncertain significance
SWAP70	ENST00000318950.10	ENST00000318950.10 (SWAP70):c.866C>T p.(Ser289Leu)	0.17 (34/203)	Gain (AAA)		
DSG2	ENST00000261590.12	ENST00000261590.12 (DSG2):c.2951C>A p.(Thr984Lys)	0.35 (88/253)	Gain (AAB)		
CHFR	ENST00000432561.6	ENST00000432561.6 (CHFR):c.1102+1G>T	0.11 (18/170)	Gain (AAA)		
CHL1	ENST00000256509.6	ENST00000256509.6 (CHL1):c.2191C>A p.(Pro731Thr)	0.21 (39/187)	Gain (AAAAB)		
PRKAG2	ENST00000287878.8	ENST00000287878.8 (PRKAG2):c.992A>G p.(Tyr331Cys)	0.41 (91/224)	Loss (AA)		
USF1	ENST00000368020.5	ENST00000368020.5 (USF1):c.672G>C p.(Lys224Asn)	0.16 (42/268)	Gain (AAABB)		
PTPN21	ENST00000328736.7	ENST00000328736.7 (PTPN21):c.1407C>A p.(Ser469Arg)	0.16 (68/434)	Gain (AAAB)		
SOX2	ENST00000325404.2	ENST00000325404.2 (SOX2):c.816C>A p.(Asp272Glu)	0.11 (34/305)	Gain (AAB)	tier 1loncogene	
TOP2B	ENST00000435706.6	ENST00000435706.6 (TOP2B):c.1028A>T p.(Asp343Val)	0.35 (74/214)	Gain (AAB)		
DNAJC9	ENST00000372950.4	ENST00000372950.4 (DNAJC9):c.322-6C>T	0.54 (80/147)	Loss (AA)		
MY O 18A	ENST00000527372.6	ENST00000527372.6 (MYO18A):c.6133A>C p.(Thr2045Pro)	0.33 (66/203)	Loss (AA)		

List of CNV; Inferred Ploidy: 2.9; Inferred Tumor Cellularity: 53%.

Chr	Start	End	Size (Mb)	No. of Copies	Genotype	Status	Genes	Census
X	39619410	40284940	0.67	0	—	Loss	*BCOR*	tier 1ITSGIF
19	35667791	36089613	0.42	0	—	Loss	*KMT2B*	
11	43538914	44472181	0.93	27	27A	Gain	*EXT2* *ACCS*	tier 1ITSG
11	47678186	47906075	0.23	26	26A	Gain	*FNBP4*	
11	44472555	45417138	0.95	18	18A	Gain	*CD82*	
11	45417545	46309400	0.89	16	16A	Gain	*CREB3L1* (txstart- intron3)	tier 1ITSGIF
11	46309811	46368140	0.058	7	AAAAAAA	Gain	*CREB3L1* (intron 3-txstop)	tier 1ITSGIF

NOTE. Gene: name of the mutated gene; Transcript: Ensembl transcript identifier (ENST); nomenclature: HGVS nomenclature (c. and p.); tumor DNA: VAF = allelic frequency, alt = number of reads supporting the mutated sequence, tot = total number of reads supporting the reported position in tumor DNA; CNV status: gain and loss of genetic material; census: indicates if the mutated gene is present in the cancer gene census list of the COSMIC database. This list is divided into two sublists (Tier 1 = validated oncogenic role of the mutations in cancer; Tier 2 = probable oncogenic role but still under validation); role of the gene according to this list: F, fusion; OG, oncogene; TSG, tumor suppressor gene.

CNV: Each altered region is characterized by its genomic location (chromosome [Chr], genomic position of the beginning [Start] and end [End] of the region), its size in mega base, the absolute copy number (number of copies), the associated genotype (A2:B1 means 2 copies of the A allele and 1 copy of the B allele), the status of the region (homozygous deletion, amplification), and the names of the genes present in the region. The genes of the cancer gene census are indicated in the last column as well as their role according to this list: F, fusion; OG, oncogene; TSG, tumor suppressor gene.

Abbreviations: CNV, copy-number variation; ENST, Ensembl transcript identifier; F, fusion; HGVS, Human Genome Variation Society; OG, oncogene; TSG, tumor suppressor gene; VAF, variant allele frequency.

[a]Classification: Classification of the selected variant according to the American College of Medical Genetics and Genomics (ACMG) criteria (PMID: 2,5741868), the recommendations of the French NGS-DIAG network (19/12/2017), and the recommendations of the French Group of Oncology Cytogenomics—CGFCO (under publication).

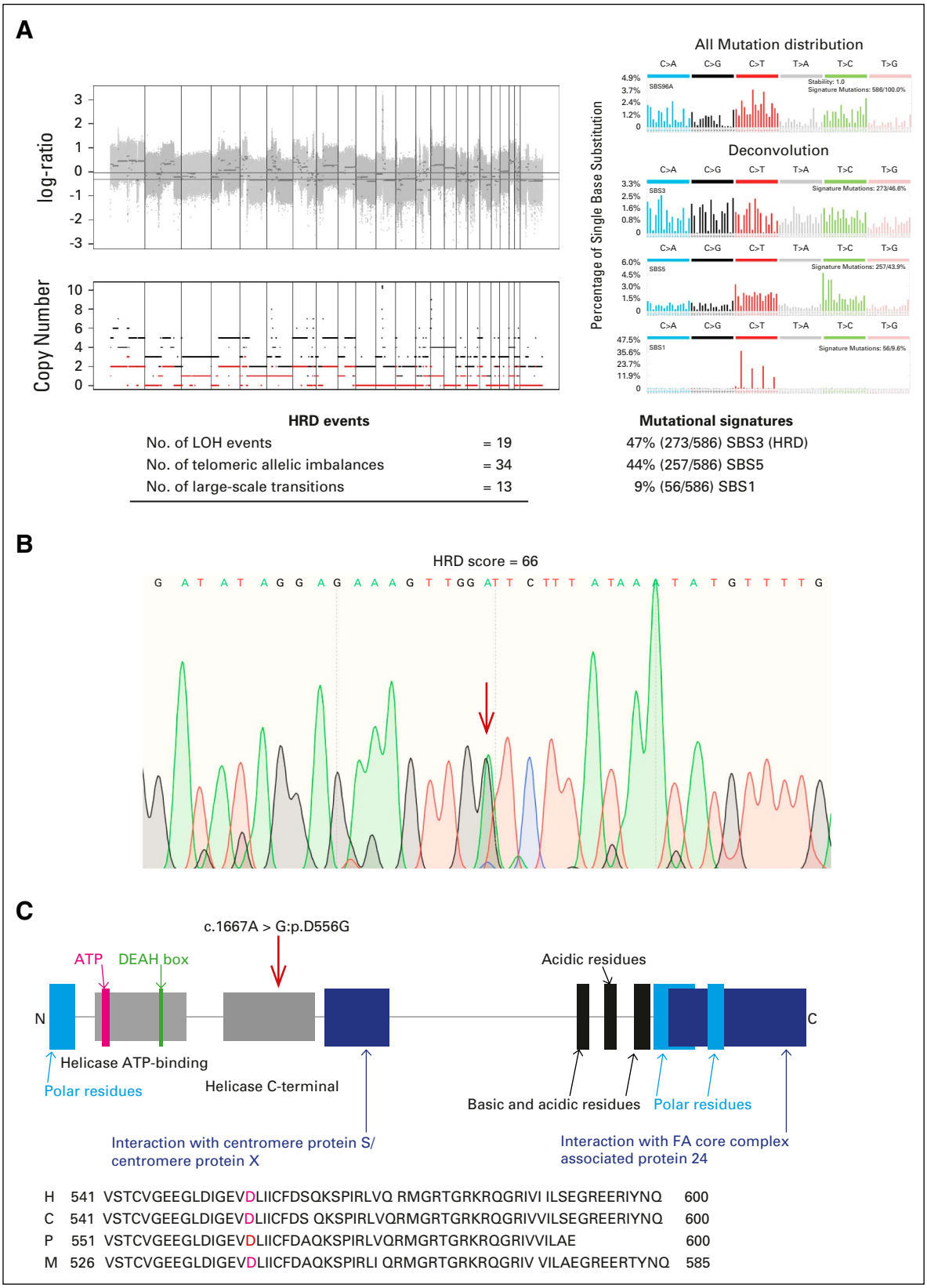

FIG 2. (A) Homologous recombination deficiency signature. (B) *FANCM* gene germline mutation using Sanger sequencing (red arrow). (C) FANCM protein structure showing the mutation c.1667A>G:p.D556G, and sequence homology between human, chimpanzee, pig, and mouse (*E*-value 0.0, percent identity/score: chimpanzee 98.78%/3,982, pig 77.49%/3,021, mouse 64.01%/2,398). The amino acid in position 556 is very well preserved. HRD, homologous recombination deficiency.

which reflect the direct effect on DNA and DNA repair mechanisms.[18,19] There was a loss of heterozygosity (LOH) in the BRCA1 gene, but without any alteration in the second allele. There was no variation in other HRD genes except for several alterations in the Fanconi anemia complementation group M (*FANCM*) gene: (1) a mutation of uncertain significance (ENST00000267430.9:c.1667A>G) with a variant allele frequency (VAF) of 72%. The variation was also present at the germline level at heterozygous state and confirmed using Sanger sequencing (Fig 2B). The somatic VAF at 72% thus implied an allelic imbalance of FANCM with a probable LOH in all tumor cells, since tumor cellularity reached 53%; (2) this variant was associated with four single-nucleotide polymorphisms (rs1367580, rs11845507, rs4900664, and rs7141145) that have been linked to a 2-fold increased risk of osteosarcoma.[20]

The association of the SBS3 mutational signature with a high HRD scar score combined with a biallelic inactivation of *FANCM* (pathogenic germline mutation with somatic LOH) can be predictive of sensitivity to DNA-damaging chemotherapeutic agents and poly (ADP-ribose) polymerase (PARP) inhibitors.[21] The molecular biology tumor board proposed maintenance treatment using olaparib, a PARP inhibitor, at a dose of 300 mg twice daily. With a trough concentration of 1,065 ng/mL, our patient was in the expected range for adequate exposure to olaparib. After 3 months of treatment, she had complete metabolic response, maintained after 14 months of treatment (Figs 1A and 1B).

Discussion

The c.1667A>G variant found in *FANCM* is considered to be of unknown significance according to the American College of Medical Genetics and Genomics (ACMG) criteria,[22] the recommendations of the French NGS-DIAG network (19/12/2017), and the recommendations of the French Group of Oncology Cytogenomics (CGFCO). It corresponds to a p.D556G protein modification in an evolutionary conserved aspartic acid (D) in the C-terminal helicase domain supporting its pathogenicity (Fig 2C). Associated with LOH at the other locus, it probably led to the biallelic inactivation of FANCM. This early carcinogenic event, present in all cancer cells if we consider the VAF of 72% and the tumor cellularity of 53%, contributed to impaired homologous recombination and to the SBS3 signature.[23] The *FANCM* gene is also reported to be a breast cancer susceptibility gene with a possible tumor suppressor role,[20,24] implicated in regulating repair pathways with BRCA1/2.[25] Moreover, in a large cohort of patients with sarcomas, 10% of the patients harbored a germline variation (variant of unknown significance or a pathogenic variant) in genes associated with Fanconi anemia, suggesting a major role in sarcoma genesis[26] and possible therapeutic implications for PARP inhibitors. However, to date, one should keep in mind that a HRD signature may not be systematically associated with functional HRD.[27,28] In addition, data from evidence-based medicine arw lacking to demonstrate a link between HRD signature and response to PARP inhibitors.

Our well-structured multidisciplinary approach contributed to overcoming some of the limitations of precision medicine trials. Temporal heterogeneity was reduced, as frozen tumor samples were obtained at the time of disease progression. Indeed, metastatic cells can derive from a minority clone within a primary tumor or a metastatic localisation.[29] When tumor samples are obtained several months or even years before tumor progression, this can challenge the reliability of the treatments proposed.[30]

To overcome spatial heterogeneity (intratumor heterogeneity but also heterogeneity between different metastases in a single patient), optimally, multiple sampling should be carried out. An alternative method to multiple sampling, the Req-Seq method, which pools samples from the same tumor in a single analysis, seems a promising approach.[31]

Large genomic trials in precision medicine can also be affected by technological limitations for detecting molecular alterations. Formalin fixation leads to DNA changes, which makes variant detection more difficult.[32] Frozen conservation thus remains the standard method for whole-genome analysis. In addition, sequencing technologies are rapidly changing, and molecular biology platforms need to upgrade their machines accordingly, since earlier technologies are limited in detecting genomic abnormalities of low prevalence.[33] Our SeqOIA platform uses the available state-of-the-art technology, and is supported by a multidisciplinary molecular tumor board, which is essential for interpreting results. Indeed, the functional value of an identified abnormality is still a challenge for therapeutic decisions, and polygenic interactions remain an unexplored domain for artificial intelligence.

Finally, adequate treatments may not yet be available for each target identified, and physicians should rethink ways of assessing treatment response, with each patient being his or her own control for response duration to previous treatments.[30]

Radiation-associated sarcomas after breast cancer treatment are rare cancers with a poor prognosis.[34,35] Osteosarcomas are very heterogeneous with a complex genomic profile and no recurrent genomic alteration.[36] For our patient, the exemplary situation of a simple genomic signature enabled us to obtain a complete response to a targeted treatment, thus highlighting the benefits of precision medicine in oncology.

This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All treatment decisions are taken in a multidisciplinary tumor board. The patient provided her written informed consent to participate in the 2025 French Genomic Program (SeqOIA platform) and for the publication of this work.

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EQUAL CONTRIBUTION

P.L.-P. and G.B. contributed equally as cosenior authors to this work.

DATA SHARING STATEMENT

The data sets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

The authors would like to thank Sarah Leyshon and Angela Swaine for the revision of the English language.

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APPENDIX 1. SUPPLEMENTARY METHODS

Genome Analysis

Genome analysis was carried out using the patient's immediately snap-frozen tumor and germline blood samples. In compliance with French law on bioethics (2004-800, 06/08/2004), she had been informed of the research use of what remained of her samples after establishing the diagnosis. She did not oppose it, and her informed consent was obtained.

DNA purification was performed using QIAmp DNA Mini Kit (Qiagen), its quantification using Nanodrop, and finally qualification by electrophoresis. Then genome analysis was conducted using next-generation sequencing and Sanger sequencing.

Next-Generation Sequencing

Validation and reporting policy. Variant classification is based on the American College of Medical Genetics and Genomics-ACMG criteria (Richards S et al. Genetics in Medicine 2015), the French NGS-DIAG network recommendations (19/12/2017), and the French Group of Oncology Cytogenomics—GFCO recommendations. Gene and variant nomenclature follows the Human Gene Nomenclature Committee—HGNC (https://www.genenames.org/) and Human Genome Variation Society—HGVS (https://varnomen.hgvs.org/) recommendations, respectively. Only pathogenic (class 5) and probably pathogenic (class 4) variants are included in the medical biology report. Variants of uncertain significance (class 3) are only detailed in the report if they are of interest in the downstream PCR. Regions not covered by the technical validation threshold are not reanalyzed using a complementary method. Reported variants meet internal quality criteria, but are not confirmed either by a complementary method or on a second sample.

Test performance. The SeqOIA molecular biology laboratory implements a validation method on the basis of the French National Institute of Cancer recommendations (March 2016), those of the French NGS-DIAG network (NGSDiag_002_V1, NGSDiag_003_V1, NGSDiag_004_V1), as well as the literature (Jennings LJ *et al.* The Journal of Molecular Diagnostics 2017).

Quality metrics. Standard thresholds are indicated in parentheses. A test or a variant that does not pass one or more of the thresholds can be used or reported, subject to the reservations mentioned in the technical report (Appendix Table A1).

Production of WGS sequences. Nucleic acid quantification and qualification were obtained on Spark, TECAN, and Fragment Analyzer, Agilent, respectively. The fragments were generated by sonication (LE220plus; Covaris). Size selection and subsequent purification steps were performed on magnetic beads (Sera-Mag magnetic beads; GE Healthcare). The preparation of the library was performed without amplification (NEBNext Ultra II End repair/A-tailing module & Ligation module; New England Biolabs). The library was qualified by capillary migration (Fragment Analyzer; Agilent) and quantified by qPCR (NEBNext Custom 2X Library Quant Kit Master Mix; New England Biolabs; QuantStudio six Flex Real-Time PCR System, Life Technologies). Libraries were sequenced in 'pair-end' mode (2 series of 150 cycles) by SBS technology (Flow Cell S4, NovaSeq 6000; Illumina).

Production of WES sequences. Quantification and qualification of the nucleic acids were, respectively, obtained on Spark, TECAN, and Fragment Analyzer, Agilent. The fragments were generated by sonication (LE220plus; Covaris). Size selection and subsequent purification steps were performed on magnetic beads (Sera-Mag magnetic beads; GE Healthcare). The preparation of the precapture library (NEBNext Ultra II End repair/A-tailing module & Ligation module; New England Biolabs) was amplified by PCR (KAPA Hifi HotStart ReadyMix; Roche). Exome capture was performed by single-plex hybridization (Twist Human Core Exome Kit + IntegraGen Custom v1; Twist BioScience) and the captured regions were amplified by PCR (KAPA Hifi HotStart ReadyMix; Roche). The final library was qualified by capillary migration (Fragment Analyzer, Agilent) and quantified by qPCR (NEBNext Custom 2X Library Quant Kit Master Mix, New England Biolabs; QuantStudio six Flex Real-Time PCR System, Life Technologies). Libraries were sequenced in 'pair-end' mode (2 series of 100 cycles) by SBS technology (Flow Cell S2; NovaSeq 6000; Illumina).

Bioinformatics analysis. Versions: pipeline_cancer_wgs v2.1.0; snakefile_analysis_2.1; pipeline_config, v.2.1.0; cluster_config,v.2.1.0.

Method: Raw sequencing files (.BCL) of the germline genome (WGS-G), tumor exome (WES-T), tumor genome (WGS-T), and tumor transcriptome (WTS-T), if available, were generated in a demultiplexing step (bcl2fastq, v2.20.0.422, Illumina). The sequences (FASTQ format) were aligned with the reference human genome (GRCh38.92.fa; GRCh38, release-92, July 2, 2018).[15] This alignment step used a Burrows-Wheeler transformation (BWA-MEM, 0.7.15). A cleaning-up process for the alignment files was then performed; this included the marking of PCR duplicates (Picard MarkDuplicates [Picard Tools, 2.8.1]) and a recalibration of base quality scores (BaseRecalibrator, GATK4 [v4.1.0.0]). Variant calling (SNPs and indels) on WGS-G and WTS-T was performed by Haplotype Caller (GATK4, v4.1.0.0). Variant calling (SNPs and indels) on the WES-T was performed by Mutect 2 (GATK4, v4.1.4.1). Variants were annotated using SNPeff (4.3t) and SnpSift (4.3t); the databases queried were SNPEff (v4.3t), 1000Genomes (phase3, v2013-05-02), gnomAD exomes (v2.1. 1), gnomAD genomes (v3), ClinVar (v20190722), COSMIC (coding, v89), COSMIC (non-coding, v89), dbscSNV (v1.1), dbSNP (v20180418), dbNSFP (v4.0), and phastCons (v08-May-2015). Somatic variants were used to calculate mutational load (pyTMB [v1.3.0dev]) and to extract mutational signature (COSMIC [v3] databases integrated with SigProfiler [v1.0.9]). WGS-G and WGS-T alignment files were used to assess microsatellite instability (MSIsensor2 [v20191,121]). Copy-number variations were detected by Facet (v0.5.14) and WisecondorX (v1.1.5), then annotated by AnnotSV (v2.3.2) to which the following databases had been added: Cytoband (December 2013, USCS) and COSMIC (v90). Merge calling was performed independently by Arriba (v1.2.0), STAR-Fusion (v1.9.0), and FusionCatcher (v1.10); merges were validated by FusionInspector (STAR-Fusion v1.9.0) and then annotated by FusionAnnotator (STAR-Fusion, v1.9.0).

Sanger Sequencing

Sequencing of germline DNA was done using the Sanger method using the forward primer 5′-ACAGTTTCGTGACGGTGGTT-3′ and reverse primer 5′-AACTGGCCGTAA-3′ to identify FANCM (reference mutation). 20 μL of PCR products were purified using ExoSAP-IT product cleanup (USB Corporation, Cleveland). BigDye-Terminator-v1.1-Sequencing-Kit (Applied Biosystems) was used for labeling in both forward and reverse directions. An initial denaturing step at 94°C for 3 minutes was performed, followed by 25 cycles at 94°C for 10 seconds, and finally an annealing temperature at 60°C for 20 seconds was applied. BDX-terminator purified products by Sephadex G-50 (Sigma Aldrich, St Louis, MO) were run on a 16-capillary automated sequencer (ABI-PRISM-3130xl-Genetic-Analyzer; Applied-Biosystems, Foster-City, CA). SeqScape-Software v 2.5(Applied Biosystems, Foster-City, CA) enabled nucleotide change determination.

TABLE A1. Quality Metrics of Genomic Analysis

	WGS Germline	WGS Tumor	WES Tumor	WTS Tumor
Mean depth	50.1X (30X threshold)	96.2X (60X threshold)	271.7X (150X threshold)	
No. of bases (≥Q30)	152 GB (85 GB threshold)	287 GB (170 GB threshold)		—
Coverage (mapq > 20)	96.6% (threshold 85% ≥ 15X)	96.5% (threshold 85% ≥ 30X)		

Abbreviations: GB, giga base; mapq, mapping quality; Q, quality score (≥Q30: probability of a wrong base call of one in 1.000 and accuracy of a base call of 99.9%); WES, whole-exome sequencing; WTS, whole-transcriptome sequencing; WGS, whole-genome sequencing.