Massive parallel sequencing unveils homologous recombination deficiency in follicular dendritic cell sarcoma

Luisa Lorenzi,[1] Torsten Haferlach,[2] Luigi Mori,[3] Matteo Simbeni,[1] Wencke Walter,[2] Piera Balzarini,[1] Manja Meggendorfer,[2] Claudia Döring,[4] Silvia Lonardi,[1] Mattia Bugatti,[1] Claudio Agostinelli,[5] Jay Mehta,[6] Anita Borges,[7] Abbas Agaimy,[8] Ingrid Simonitsch-Klupp,[9] José Cabeçadas,[10] Elias Campo,[11] Stefano Aldo Pileri,[12] Fabio Facchetti[1] Martin Leo Hansmann[13] and Sylvia Hartmann[4]

[1]Pathology Unit, ASST Spedali Civili di Brescia, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [2]MLL Munich Leukemia Laboratory, Munich, Germany; [3]Laboratory of Molecular Medicine, Department of Clinical and Experimental Science, University of Brescia, Brescia, Italy; [4]Dr Senckenberg Institute of Pathology, Goethe University, Frankfurt, Germany; [5]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [6]Neuberg Oncopath, Mumbai, India; [7]Histopathology, SRL Diagnostics, Mumbai, India; [8]Institute of Pathology, University Hospital, Erlangen, Germany; [9]Institute of Pathology, Medical University of Vienna, Vienna, Austria; [10]Department of Pathology, Portuguese Institute of Oncology, Lisbon, Portugal; [11]Hematopathology Section, Hospital Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain; [12]Division of Hematopathology, European Institute of Oncology (IEO) IRCCS, Milan, Italy and [13]Institute for General Pharmacology and Toxicology, Goethe University, Frankfurt, Germany

Correspondence: L. Lorenzi
luisa.lorenzi@unibs.it

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Abstract

Standardized treatment options are lacking for patients with unresectable or multifocal follicular dendritic cell sarcoma (FDCS) and disease-related mortality is as high as 20%. Applying whole-genome sequencing (WGS) in one case and whole-exome sequencing (WES) in additional twelve cases, this study adds information on the molecular landscape of FDCS, expanding knowledge on pathobiological mechanisms and identifying novel markers of potential theragnostic significance. Massive parallel sequencing showed high frequency of mutations on oncosuppressor genes, particularly in *RB1*, *CARS* and *BRCA2* and unveiled alterations on homologous recombination DNA damage repair-related genes in 70% (9/13) of cases. This indicates that patients with high-stage FDCS may be eligible for poly ADP ribose polymerase inhibition protocols. Low tumor mutational burden was confirmed in this study despite common PDL1 expression in FDCS arguing on the efficacy of immune checkpoint inhibitors. *CDKN2A* deletion, detected by WGS and confirmed by fluorescence *in situ* hybridization in 41% of cases (9/22) indicates that impairment of cell cycle regulation may sustain oncogenesis in FDCS. Absence of mutations in the RAS/RAF/MAPK pathway and lack of clonal hematopoiesis-related mutations in FDCS sanction its differences from dendritic cell-derived neoplasms of hematopoietic derivation. WGS and WES in FDCS provides additional information on the molecular landscape of this rare tumor, proposing novel candidate genes for innovative therapeutical approaches to improve survival of patients with multifocal disease.

Introduction

Follicular dendritic cell sarcoma (FDCS) is a malignant neoplasm of mesenchymal derivation with morphological and phenotypical features of FDC, dendritic cells of mesenchymal-derivation of the B follicle.[1-4] Included in the group of "histiocytic and dendritic cell neoplasms" in the revised 4th edition of World Health Organization (WHO) classification[4] as well as in the International Consensus Classification,[5] it was moved to a novel chapter of "stroma-derived neoplasms of lymphoid tissues" in the most recent WHO classification.[6] FDCS can occur both at nodal and extranodal sites and can be metastatic and lethal in about a fifth of the cases.[7] Complete tumor resection is the treatment of choice for localized disease while patients with multifocal or unresectable FDCS are

alternatively treated with radio- and/or chemotherapy in accordance with lymphoma, or sarcoma-like regimens with variable outcomes.[7]

Large, massive parallel sequencing studies performed on dendritic- and histiocytic-derived tumors have unveiled important biological pathways commonly driving some of these tumors. MAPK/ERK pathway is activated in Langerhans cell histiocytosis, Erdheim Chester disease as well as in some histiocytic sarcoma and in selected cases of Rosai-Dorfman disease.[8,9] Clinical responses after targeted therapies in these settings are well documented and have changed the natural history for these diseases.[9-11] In contrast, evidence indicates that the molecular mechanisms driving FDCS are different. Mutations affecting genes such as *KRAS*, *NRAS*, *MAP2K1* and *BRAF* are rare in FDCS,[7,12,13] in keeping with its non-hematopoietic, but mesenchymal derivation.[12,14]

Previous studies indicated an oncosuppressor-driven biology in FDCS,[12,15] however, recurrent mutations of diagnostic and prognostic significance are lacking and biomarkers of theragnostic relevance are still missing.[7]

By whole-exome sequencing (WES) and whole-genome sequencing (WGS) approach this study aims to provide novel molecular information on FDCS indicating innovative biomarkers of therapeutical significance.

Methods

Case selection and histopathological review

The discovery cohort consisted of 13 FDCS samples selected by tissue availability, tumor cell content and DNA quality to undergo massive parallel sequencing (MPS). One case (case #13) underwent WGS, 12 cases WES (cases #1-#12). The latter were also included in three tissue micro arrays (TMA) together with 22 additional cases, considered as validation cohort.

The clinical data regarding the cohort undergoing MPS are reported in Table 1. It included seven males and six females; the average age was 66 years (range, 36-84). Diagnosis was classical FDCS in all cases but one (#3), which was diagnosed as Epstein-Barr virus–positive inflammatory follicular dendritic cell sarcoma/tumor (EBV-IFDCS/T).[5,6] All cases underwent pathological revision by a consensus of expert hematopathogists (FF, SAP, MLH, SH); morphology and phenotype of the discovery cohort are detailed in Table

Table 1. Clinical features of 13 follicular dendritic cell-derived tumors/sarcoma undergoing massive parallel sequencing.

Patient ID	Age in years/sex	Site	Diagnosis	Disease at presentation	Therapy	Characteristic at follow-up (months from diagnosis)	Sample for NGS	NGS
#1	71/F	Supraclavicular lymph node	FDCS	Unifocal	Surgery Radiotherapy	NA	FF	WES
#2	57/F	Soft tissue	FDCS	Unifocal	Surgery Radiotherapy Chemotherapy	Dead of disease (156)	FFPE	WES
#3	55/M	Spleen	EBV-IFDCS/T	Unifocal	NA	NA	FF	WES
#4	73/F	Retroperitoneum	FDCS	Unifocal	Surgery	Alive (20)	FF	WES
#5	74/M	Left tonsil	FDCS	Unifocal	Surgery	NA	FFPE	WES
#6	68/F	Lymph node	FDCS	Multifocal	Chemotherapy	Dead of disease (40)	FF	WES
#7	69/M	Lymph node	FDCS	Unifocal	NA	Alive (59)	FF	WES
#8	76/M	Soft tissue	FDCS	NA	NA	NA	FF	WES
#9	53/F	Lymph node	FDCS	Unifocal	Surgery	Dead for other causes (3)	FFPE	WES
#10	84/M	Parotid	FDCS	Multifocal	Chemotherapy	Dead of disease (13)	FF	WES
#11	36/M	Retroperitoneum	FDCS	Unifocal	NA	NA	FFPE	WES
#12	64/M	Axillary lymph node	FDCS	Unifocal	Surgery Radiotheray	Alive (3)	FF	WES
#13	79/M	Stomach	FDCS	NA	Surgery	NA	FF	WGS

FDCS: follicular dendritic cell sarcoma; EBV-IFDCS/T: Epstein-Barr virus–positive inflammatory follicular dendritic cell sarcoma/tumor; FF: fresh frozen; FFPE: formalin-fixed paraffin-embedded; F: female; M: male; WES: whole-exome sequencing; WGS: whole-genome sequencing; NA: not available.

2. Written informed consent was obtained from patients in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee at the University Hospital Frankfurt (157-17).

Immunohistochemistry and fluoresensce *in situ* hybridization studies

Immunohistochemistry was performed for the following antibodies: Serglycin and FDC-SP as previously reported,[16] p16 (clone E6H4, dilution 1:4, CINtec histology kit from Roche); PD-L1 (clone 22C3, dilution 1:40; Agilent).
Structural variants (SV) and copy number variations (CNV) identified by WGS or described in the literature were tested by fluorescence *in situ* hybridization (FISH) with the following probes: CDKN2A/CEP9 (Vysis Abbott Molecular); SS18 Break Apart (Vysis Abbott Molecular); 1p36/1q25 and 19q13/19p13 (Vysis Abbott Molecular); CIC Break Apart (Empire Genomics). Interpretation of *CDKN2A* deletion was supported by counting at least 50 nuclei for each case.

Next-generation sequencing and interpretation of variants

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) or FF samples including at least 20% of tumor cell content without tumor enrichment or microdissection. Next-generation sequencing was performed by amplicon-based massive parallel sequencing technology on an Illumina Nova Seq 6000 (San Diego, CA, US) platform at Munich Leukemia Laboratory, where bioinformatics analysis was carried out using the software Variant Interpreter (Illumina) and open-source databases. Tumor mutational burden and mutational signature were calculated by Base Space Variant Interpreter algorithms exclusively from WGS data.
Single nucleotide variants/insertions deletions (SNV/indel) were extracted and prioritized by the variant filtering strategy detailed in *Online Supplementary Figure S1*.[17-19]
Eligibility of FDCS patients to poly ADP ribose polymerase (PARP) inhibition protocols was performed by manually interrogating the dataset of gene variants for the prevalence of mutations on genes involved in the mechanism of double strand beaks (DSB), as previously described.[20]

Results

Mutations in homologous recombination-related genes mutations are common in follicular dendritic cell sarcoma

SNV analysis lead to the identification of one or more mutations of known oncosuppressor genes in 12 of 13 FDCS (92%) (Table 3). *CARS, RB1* and *WRN* genes were the most recurrently mutated in this cohort (each gene mutated in at least 3 cases). All five cases with a single or no mutation on oncosuppressor genes (#1, #3, #4, #12, #13) were unifocal at presentation, they included both nodal and extranodal cases and the EBV-IFDCS/T sample

Table 2. Cytological and phenotypical features of 13 cases of follicular dendritic cell derived tumors/sarcoma undergoing massive parallel sequencing.

Patient ID	Cytology	CD-LF	Mitosis/ 10 HPF	CD21	CD23	CD35	CXCL13	Clu	Cl4	D2-40	EBV/ EBER	SRGN	FDCS-SP
#1	Spindled	No	10	+	+	+	+	+	+	+	-	+	+
#2	Epithelioid	Yes	4	+	-	+	+	+	-	+	-	+	+
#3	Spindled	No	4	+	+	+	-	-	-	-	+	-	-
#4	Mixed	No	2	-	-	-	-	+	-	+	-	+	-
#5	Epithelioid	No	1	+	+	-	+	+	+	+	-	NA	+
#6	Epithelioid	No	6	+	-	+	+	+	-	+	-	+	+
#7	Mixed	No	6	+	-	+	+	+	+	-	-	+	+
#8	Spindled	N	3	+	+	+	+	+	+	+	-	+	+
#9	Spindled	No	0	+	-	+	+	+	-	-	-	+	-
#10	Mixed	No	2	+	+	-	+	-	-	+	-	-	+
#11	Mixed	Yes	9	+	+	+	+	+	+	+	-	+	+
#12	Epithelioid	Yes	1	-	-	-	+	+	NA	+	-	+	-
#13	Spindled	No	1	+	-	+	+	+	NA	+	-	NA	NA

CD-LF: Castleman disease-like features; Clu: clusterin; Cl4: claudin 4; FDC-SP: follicular dendritic cell-secreted protein; HPF: high power field; NA: not available; SRGN: serglycin; EBV: Epstein-Barr virus; EBER: EBV-encoded small RNA *in situ* hybridization.

(*Online Supplementary Table S1*; Table 1).
Notably, cases #2 and #10 showed the highest number of mutations in this group of genes and both showed an aggressive clinical course leading to patients' death (Table 1). Patient #2 had a previous diagnosis of Hyaline-vascular type Castleman disease (HV-CD) and developed multiple and subsequent nodal and extranodal FDCS during more than 10 years; patient #10 presented with a multifocal extranodal disease with rapid fatal evolution. After surgery, systemic therapy was attempted in both patients, ineffectively.
In 70% of cases (9/13) (Table 4) at least one pathogenic mutation of HRD genes was found. *BRCA2* and *RB1* were the most mutated genes in this group; they occurred in three cases and were simultaneously found in two (#7 and #10). Mutations on *TP53* and *CHEK2* were also identified in two cases. Notably, in the previously mentioned aggressive

Table 3. Pathogenic mutations identified on known oncosuppressor genes in 13 follicular dendritic cell-derived tumors/sarcoma cases.

Gene	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
APC	-	-	-	-	-	L1129S[a]	-	-	-	-	S843G[a]	-	-
ARHGEF12	-	-	-	-	-	-	-	-	-	-	-	D1158V[a]	-
BRCA1	-	-	-	-	-	-	-	-	-	-	L1700*[b]	-	-
BRCA2	-	-	-	-	-	-	L1137F[a]	G1194V[a]	-	I2285V[a]	-	-	-
CARS	-	A241S[a]	-	-	-	-	-	-	G342S[a]	R678*[c]	-	-	-
CBFA2T3	-	R697K[a]	-	-	-	-	-	-	-	-	-	-	-
CDH11	-	-	-	-	-	-	-	-	A475V[a]	-	-	-	-
CREBBP	-	-	-	-	-	-		S1934P[a]	-	-	-	-	-
DDX5	-	-	-	-	G22A[a]	-	-	-	-	-	-	-	-
FLT3	-	-	V194M[a]	-	-	-	-	-	-	-	-	-	-
JAK2	-	-	-	-	-	-	-	-	-	G571S[a]	-	-	-
NUP98	-	-	-	G16S[a]	-	-	-	-	--	-	-	-	-
RB1	-	-	-	-	-	-	D604N[a]	-	-	H483Lfs*[b]	-	-	S360Ksfs*[b]
RUNX1	-	-	-	-	-	-	-	S424P[a]	-	-	-	-	-
SMARCA4	-	-	-	-	-	-	-	-	-	P195L[a]	-	-	-
STK11	-	F354L[a]	-	-	-	-	-	-	-	-	-	-	-
TCF3	-	-	-	-	-	-	-	-	-	P213F[a]	G431S[a]	-	-
TNFAIP3	-	R697K[a]	-	-	-	-	-	-	-	-	-	-	-
TP53	-	-	-	-	-	E286K[a]	-	-	-	R213*[c]	-	-	-
TSC2	-	-	-	-	R1745H[a]	-	-	-	-	S1338L	-	-	-
TSC222D1	-	-	-	-	-	-	-	-	-	-	-	-	-
WRN	-	Y1034*[c]	-	-	-	K635Q[a]	-	-	-	-	A693G[a]	-	-

Mutation consequence: [a]missense mutation; [b]frameshift mutation; [c]stop-gain mutation; *stop codon; #: case.

Table 4. Table of mutations identified in follicular dendritic cell-derived tumors/sarcoma on PARP inhibitor-sensitive genes.

Gene	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
BRCA2	-	-	-	-	-	-	L1137F[a]	G1194V[a]	-	I2285V[a]	-	-	-
RB1	-	-	-	-	-	-	D604N[a]	-	-	H483Lfs*[b]	-	-	S360Kfs*[b]
TP53	-	-	-	-	-	E286K[a]	-	-	-	R213*[c]	-	-	-
CHEK2	-	-	-	-	-	-	-	-	-	Q487*[c]	-	-	-
MLH1	-	-	-	-	-	-	-	-	-	-	-	-	-
ATR	-	M1996T[a]	-	-	-	-	-	-	-	-	-	-	-
BRCA1	-	-	-	-	-	-	-	-	-	-	L1700*[b]	-	-
XPA	-	-	-	-	-	-	R258Yfs*[b]	-	-	-	-	-	-
PARG	-	-	-	-	-	-	-	I574V[a]	-	-	-	-	-

Mutation consequence: [a]missense mutation; [b]frameshift mutation; [c]stop-gain mutation; *stop codon; #: case; PARP: poly ADP-ribose polymerase.

cases (#2 and #10), at least one mutation of HRD was also detected (Table 4).

Structural variants and copy number variations detection in follicular dendritic cell sarcoma

WGS, which was performed on case #13, highlighted a high number of SV, including translocations, inversions, CNV including both gains and losses, affecting mainly chromosomes 3, 5, 9, 13 and chromosome 19. They are outlined in the circos plot in Figure 1 and listed in *Online Supplementary Table S2*. The ploidy level in case #13 was 1.94. In summary, 114 SV (76 translocations, 33 inversions and 5 deletions) and 48 CNV were identified. The karyotype of case #13 was complex, however, by comparison with previously publishes studies we could not identify analogy with previously reported SV.[21-26] In order to evaluate the impact of NGS findings in identifying recurrent translocations of diagnostic significance, variations occurring in regions of interest in cancer biology were explored by FISH probes on tissues slides of case #13.

FISH probe for the 18q11.2 locus did not identify rearrangements reported on the same locus by WGS, t(16;18)(p11.2;q11.2) and t(18;22)(q11.2;q11.23), additionally, the *CIC* and 19q co-deletion probes confirmed the CNV gain detected by WGS but did not identify rearrangements (*data not shown*). The discrepancies between structural variations observed by WGS and the FISH analysis could be explained by the fact that diagnostic FISH probes are designed for specific inquiries and the size of rearrangements detected by WGS may be too small to be identified *in situ*. Still, FISH could indeed confirm the deletion of the *CDKN2A* gene, encoded in chromosome region 9p21 where three inversions were detected by WGS. FISH found homozygous deletion (gene/CEP9 ratio=0.15) and CNV gain of chromosome 9 (average CEP9/nucleus ratio=6.3) (Figure 2). Accordingly, immunostaining for p16 protein was completely negative in tumor cells (Figure 2). In order to calculate the incidence of *CDKN2A* deletion in FDCS, FISH was performed on a total of 22 FDCS and found deletions in 41% of cases (9/22, with gene/CEP9 ratio ranging from 0.14 to 0.53), indicating that deletion of the *CDKN2A* locus is a recurrent event in this tumor. P16 expression evaluated by immunohistochemistry (IHC) could not predict *CDKN2A* deletion: deleted cases (9) were either p16 protein-positive or negative (6/9) or focally positive (3/9); non-deleted cases were either strongly positive (3/13), partially positive (4/13) or negative (6/13).

Whole-genome sequencing in follicular dendritic cell sarcoma shows enrichment in mutational signatures SBS16, SBS8, and SBS9 and low tumor mutational burden

Mutational signatures (MS) are specific patterns of somatic mutation accumulation occurring in cancers, that can predict pathogenetic mechanisms, pathways enrichments, or cancer subtypes, with high accuracy.[27] MS in cancer biology is widely applied and in continuous evolution, with inclusion of novel molecular alterations (e.g., doublet base substitution, insertions and deletions, CNV).[27]

MS evaluation can be reliably performed from WGS data where the incorporation of non-coding DNA allows to investigate the mutational processes involved in cancer genomics.

Therefore, by performing the first MS analysis in a case of FDCS we unveiled novel information. As shown in Figure 3, single base substitution (SBS) signature showed high contribution of SBS1 and SBS5, mutational signatures associated with age and found across many tumor subtypes. The age of the patient (79 years old) and the neoplastic nature of the analyzed tissue confirm the accuracy of this analysis. Other MS identified were SBS16, SBS8 and SBS9. Recent studies have suggested that SBS16 and SBS8 are associated with early and late replication timing, respectively,[28,29] however the meaning of such information remains still uncertain. In contrast, SBS9 is recognized as associated with polymerase-η activity, known to be implicated in the B-cell germinal center reaction and somatic hypermutation. This information suggests that, despite extra-nodal localization of the disease (stomach in this case), FDCS is likely to originate from a tertiary germinal center structure. Lastly, MS of FDCS included SBS28, a signature of unknown

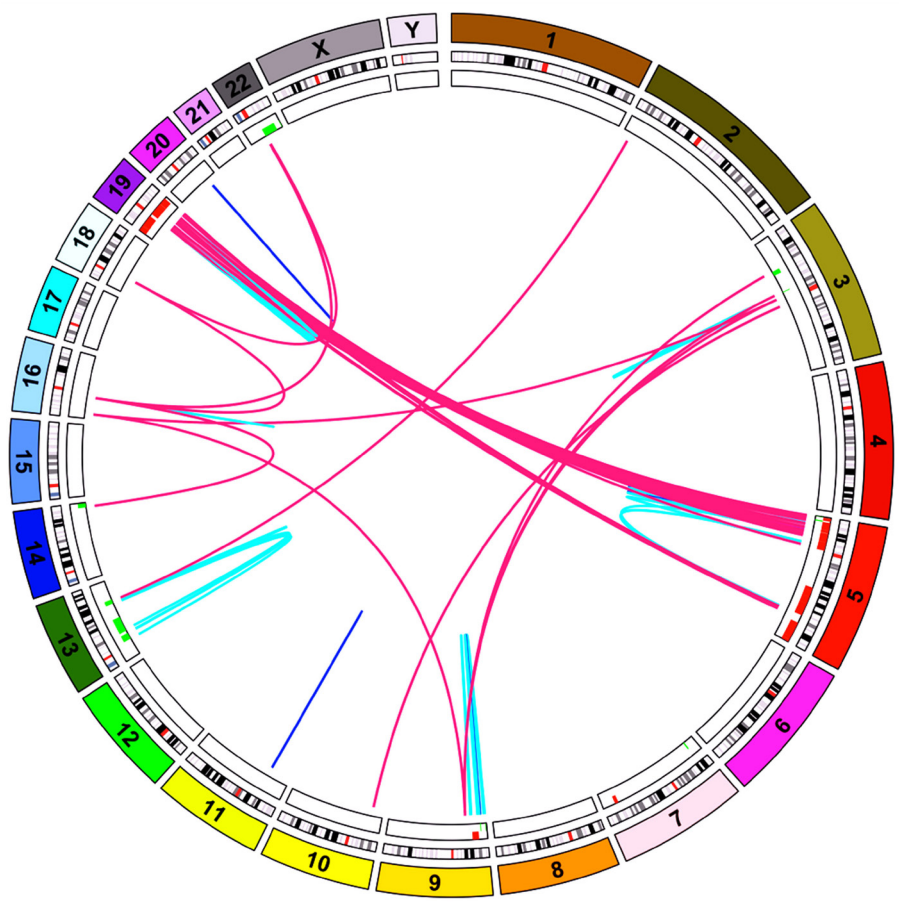


Figure 1. Structural and copy number variations in follicular dendritic cell sarcoma. Circos plot outlines variations identified in case #13; chromosomes are represented in the outer ring, translocations and inversions are depicted by stripes, magenta and light blue, respectively. Small deletions are shown in blue; copy number variations are indicated in the inner cycle: losses in green, gains in red.

significance associated with gastric cancer indicating that the site of disease may contribute to the mutational landscape. Any inference on MS in FDCS requires confirmation on additional cases in following studies.

Tumor mutational burden (TMB) is a novel theragnostic biomarker of response to immune-checkpoint therapies. It can be appropriately evaluated on large panels, such as WGS, while a fully standardized pipeline for its calculation on targeted panels is still missing.[30] TMB, calculated by WGS on FDCS #13, resulted low (1,66 mutations/megabase). Notably, a prevalent low TMB has been reported in most mesenchymal tumors[31,32] and is in keeping with data recently obtained using a different targeted, approach in 44 cases of FDCS.[12] Taken together, these results can have clinical implications since low TMB may indicate low number of neo-antigens and poor or absent response to immunotherapy. However, it is known that tumor cells in FDCS often express PD-L1[7,14] as confirmed also in this co-

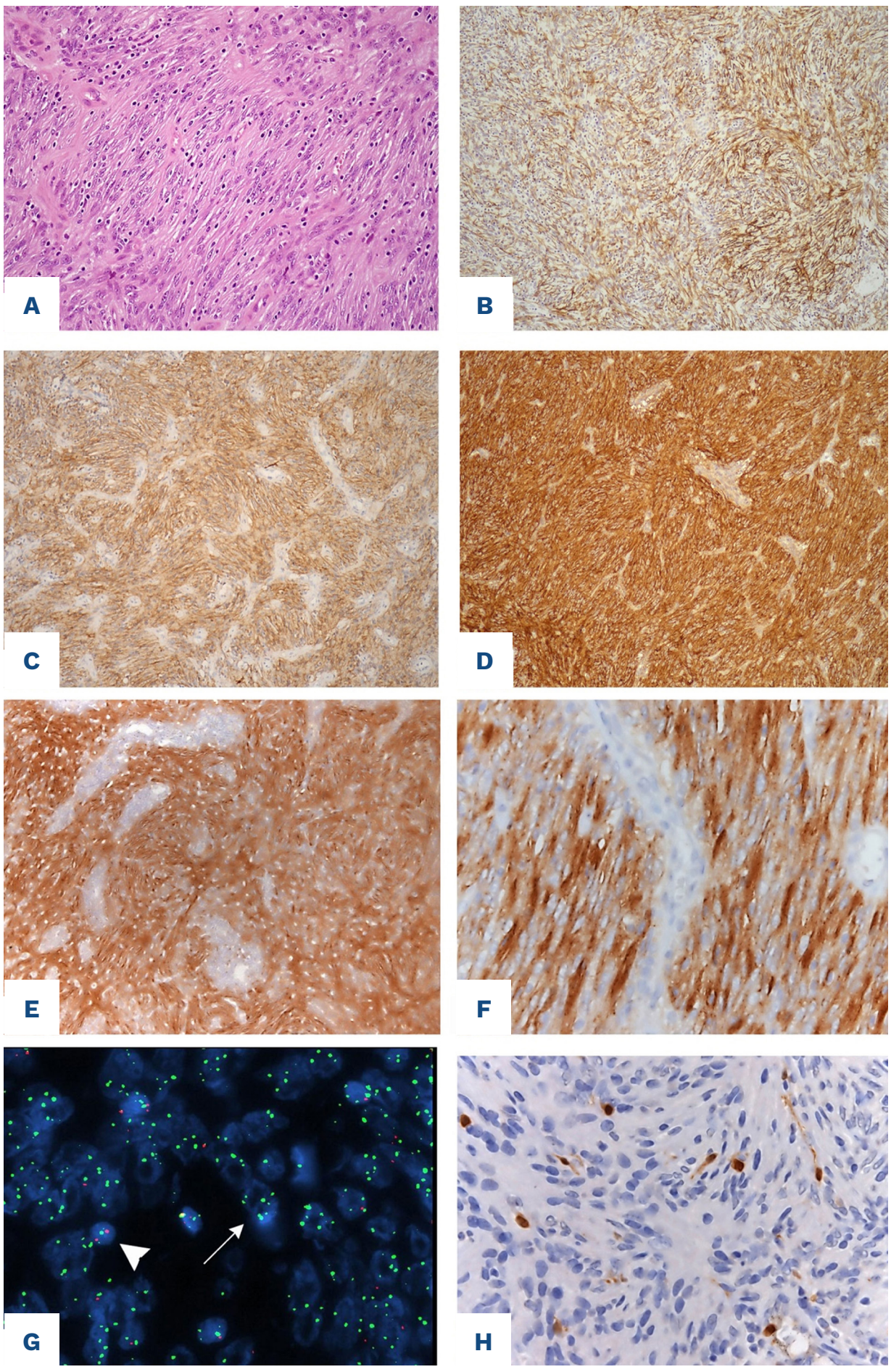


Figure 2. Histological features of follicular dendritic cell sarcoma. On histology, case #13 shows neoplastic follicular dendritic cells (FDC) with spindle cell morphology, associated with some small, intermingled lymphocytes (A) (hematoxylin and eosin staining, 100X magnification) and characterized by unequivocal expression of FDC markers CD21 (B), CXCL13 (C) and clusterin (D) (100X magnification). PD-L1 (22C3) was strongly and diffusely positive on nearly 100% of tumor cells (E, F) (magnification 40X-200X). CDKN2A deletion evaluated by *fluorescenze in situ* hybridization detected large, atypical nuclei with less than 2 red signals (G, arrow) (average ± standard deviation CDKN2A/CEP9 ratio 0,15±0,63). Notably, the centromeric probe showed numerous signals (average ± standard deviation CEP9 6,30±2,85) suggesting polisomy of chromosome 9. Note the normal signal of a reactive lymphocyte (2 red and 2 green signals, arrowhead). Accordingly, p16 protein was not expressed on tumor cells (H).

hort (52%, 16/31). Based on this evidence, immunotherapy approaches have been attempted with variable results.[33,34] In previous gene expression studies, CNV gain of chromosome 9p24 was proposed as mechanism of PD-L1 expression in FDCS.[14,35] By WGS of case #13 we observed lack of 9p24 CNV gain despite intense and diffuse PD-L1 protein expression (Figure 2), indicating that other mechanisms than gene amplification can lead to PD-L1 expression in FDCS.

Mutations on RAS/RAF pathway genes, *PDGFRB* and mutations associated with clonal hematopoiesis are uncommon in follicular dendritic cell sarcoma

WES and WGS on FDCS confirmed some observations previously obtained by targeted approaches. Mutations on genes of the *RAS/RAF* pathway commonly mutated in hematopoietic-derived tumors such as *BRAF, KRAS, MAP2K1, NF1, NRAS* or *PTPN11* were not detected among 13 cases of FDCS[7,12,15] (*Online Supplementary Table S1*).

The *PDGFRB* p.Asn666Ser mutation, previously described in HV-CD and in FDCS[12,36] was not identified in this cohort. Two missense variants of unknown pathogenicity were found on *PDGFRB* in two cases (p.Ser408Cys and p.Ala6Val) (*Online Supplementary Table S1*), one of which showing CD-like features at histology. Functional studies are needed to define the role of these mutations on stromal cells and their significance in FDCS pathogenesis.

Clonal hematopoiesis (CH) is an emerging concept and indicates the clonal expansion of mutated hematopoietic cells occurring with human aging, associated with increased risk of hematological neoplasms[37] and other non-hematological disorders likely due to the involvement of mutated

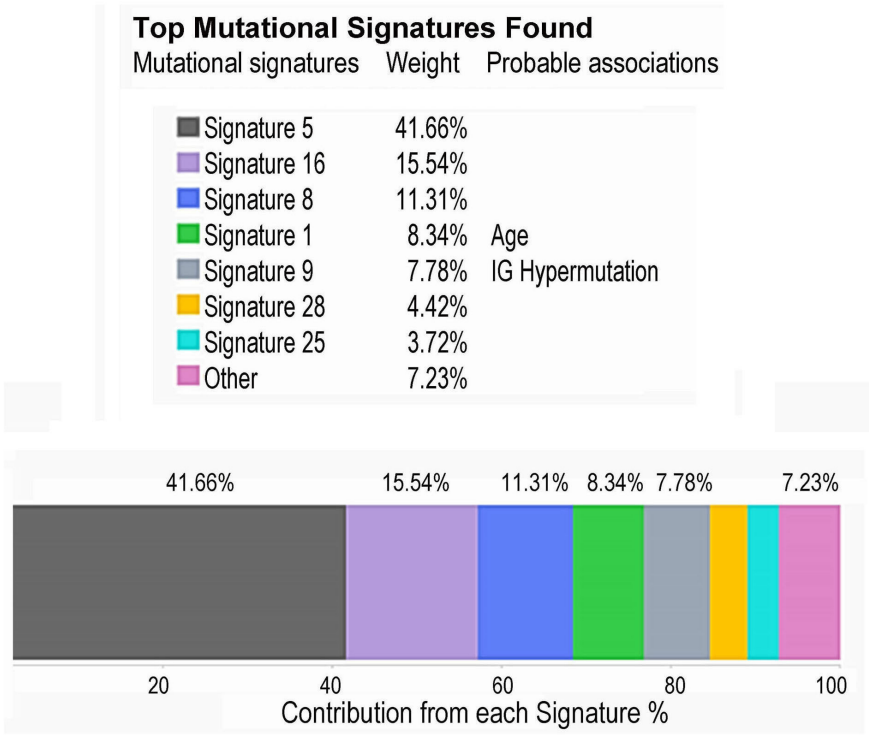
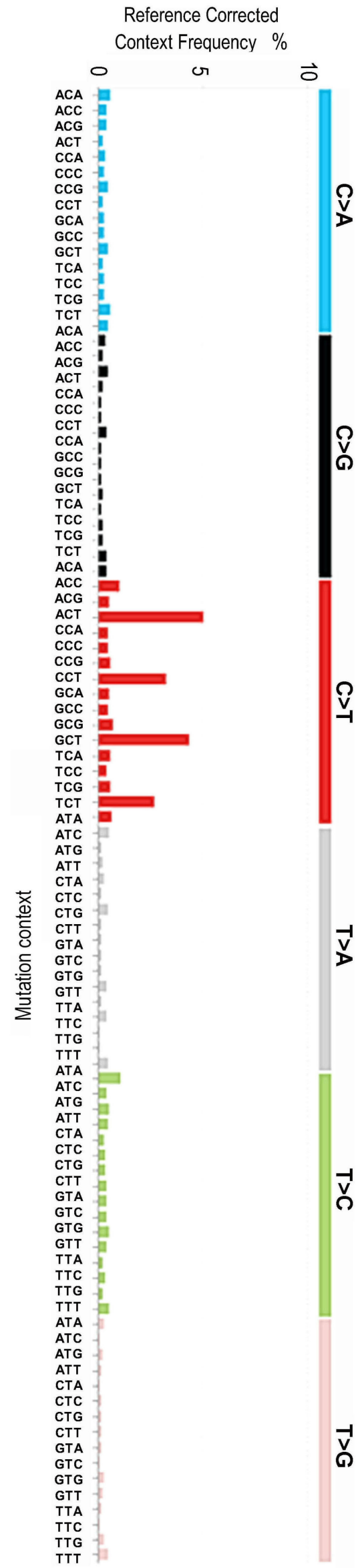


Figure 3. Mutational signatures in follicular dendritic cell sarcoma. Whole-genome sequencing allowed to identify mutational signatures associated with case #13 which include, among others, signature 9, associated with immunoglobulin (IG) hypermutation.



myeloid cells in the inflammatory cascade.[38] Using WES and WGS we interrogated data on the incidence of CH-related mutations in 13 FDCS. No significant mutations on *TET2* or *DNMT3* were detected, pathogenic mutations of *ASXL1* were found in case #4 and of *PPM1D* in case #10, both occurring on elderly patients (73 and 84 years old, respectively).

Association between FDCS and lymphomas is extremely rare[39,40] and immunoglobulin or T-cell receptor gene clonality was only sporadically identified in FDCS.[7] Recently, mutational studies on B- and T-cell lymphomas unveiled a variety of alterations associated with lymphoma pathogenesis and diagnosis in specific settings.[41] By comparing FDCS mutational landscape with lymphoma-related genes we found few pathogenic mutations in genes specifically associated with T- and germinal-center B-cell lymphomas, with the exception for genes associated with epigenetic remodeling. *KMT2D* and *SETD2* mutations with plausible deleterious effect were found in seven of 13 (54%) and two of 13 (15%) FDCS cases, respectively. *NOTCH2* gene, often mutated in marginal zone lymphoma, was mutated in three (23%). It should be noted that *KMT2D* and *NOTCH2* mutations were detected at very low frequency, arguing on the biological significance of such alterations (*Online Supplementary Table S1*).

Discussion

FDCS is an uncommon disease, with unpredictable outcome, occasionally lethal, in need of an effective therapeutical approach. The actual treatment protocols include surgery and chemo- and/or radiotherapy, based on the center experience. The discovery of driving alterations is a priority that could support the identification of effective drugs to cure or control metastatic disease. To this aim, this study, combining WGS and WES in a relatively large cohort of a rare sarcoma, confirms some previous data and adds novel information on its molecular landscape.

In line with previous studies,[12,15] a tumor suppressor driven pathobiology was observed in this cohort of FDCS, with common *CDKN2A* deletion and frequent mutations on *RB1*, *BRCA2, WRN* and *TP53*. Furthermore, accumulation of inactivating mutations on these genes was associated with multifocal disease and poor prognosis.

On the basis of this information, using WES/WGS we investigated the incidence of mutations on genes involved in the repair of double strand breaks (DSB, i.e., homologous recombination DNA damage repair, HRD). Notably, mutations on HRD-related genes were found in 70% of cases, indicating the so called “BRCAness phenotype”. It is known that HRD in cancer promotes genomic instability leading to chromosomal alterations.[42] In FDCS chromosomal alterations are often reported by classical karyotyping[21-26] and were found also by WGS in this study. This suggests that chromosomal “scarring” in FDCS may be a secondary pathogenic event, a consequence of HRD and further studies are warranted to specifically evaluate the correlation between HRD and chromosomal alterations in this setting. Still, these results prospect the opportunity for patients with unresectable disease to be candidate for PARP inhibitor therapy. Approved for breast, ovarian and recently for prostate cancer, this therapeutical approach, induces replication stall, accumulation of cytotoxic substances and formation of DSB in HRD-mutated cells by preventing the activity of PARP enzymes, leading to selected apoptosis of the neoplastic cells. Notably, the literature reports one single case of unresectable FDCS occurring in a patient carrying *BRCA2* germline mutations who reached disease stabilization with PARP inhibitors.[43]

This could be the first targeted approach to be applied in FDCS, since as confirmed in this study, FDCS lack mutations on genes of the *RAS/RAF* pathway, thus excluding it from targeted therapies currently applied in histiocytic and dendritic tumors of hematopoietic derivation.[7,12,15]

Furthermore, the common PDL1 gene and protein expression, detected on FDCS tumor cells in this and in previous studies[7,14] was proposed as a potential marker of immune checkpoint inhibition response. However, the low tumor mutational burden found in FDCS, in this and another studies,[12] questions on the efficacy of this therapeutical approach.

By applying WGS in FDCS, we could investigate, for the first time, mutational signatures in this rare tumor and identified the occurrence of the germinal center (GC)-related signature SBS9. This may suggest that the neoplastic proliferation of FDC can support the mutational activity typically occurring in the B-follicle GC, even after neoplastic transformation. Alternatively, this may suggest that FDC are a target of aberrant somatic hypermutation machinery in the GC leading to their malignant transformation. This is surprising, as aberrant somatic hypermutation has been identified as leading cause of a variety of GC-derived B-cell lymphomas[44,45] but has, to our knowledge, not been demonstrated to occur in FDCS so far. However, expression of activation-induced cytidine deaminase, the enzyme responsible for the somatic hypermutation process, has been well documented in FDC networks.[46]

When comparing the mutational landscape of FDCS with that of B- and T-cell lymphomas of GC derivation, only one gene, the epigenetic modifier *KMT2D*, was altered in a significant number of cases, though at low frequency. Whether this is related to aberrant somatic hypermutation or to an incipient mechanism of tumorigenesis may be further explored; however, the association of FDCS with B-cell lymphoma is an extremely rare event.[7]

Lastly, despite the presence of FDCS with morphological features resembling Castleman disease, including one case developing from a previous HV-CD, the CD-related mutation *PDGFRB* Asn666Ser was not found in this study, while two novel missense *PDGFRB* mutations of unknown

significance were detected in two cases, one with HV-CD features. These findings deserve further evaluation.
In conclusion, this study, applying massive parallel sequencing in FDCS, confirms its molecular difference from hematopoietic-derived neoplasms, the pivotal role of oncosuppressor genes in its pathobiology and indicates a novel therapeutic approach with PARP inhibition that warrants further investigation for patients with non-resectable FDCS.

Disclosure
No conflicts of interest to disclose.

Contributions
LL and SH designed the study, interpreted the data and wrote the manuscript. TH developed the concept of the study, contributed essential material, revised the manuscript. LM, MS, PB, SL and MB performed experiments on tissue and genomic data, revised the manuscript. WW, MM and CD performed genomic data analysis and revised the manuscript. JM, AB, AA, ISK, JC, EC and SAP contributed with essential material and revised the manuscript. FF and MLH developed the concept of the study, contributed essential material, revised the manuscript.

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Data-sharing statement
Original data from sequencing analysis are available in Online Supplementary Tables S1 and S2.

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