SUPPLEMENTARY DATA

Genomic evolution and personalized therapy of an infantile fibrosarcoma harboring an *NTRK* oncogenic fusion

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SUPPLEMENTARY MATERIALS AND METHODS

Molecular pathology analysis

H&E staining and pan-TRK immunohistochemistry were performed as described[1] on formalin-fixed paraffin-embedded tumor tissue. *ETV6* break-apart FISH was performed on formalin-fixed paraffin-embedded tumor tissue using the LSI ETV6 (TEL) (12p13) dual color, break-apart rearrangement probe (Abbott Molecular, Illinois, U.S.A.) followed by counterstaining with 4,5-diamidino-2-phenylindole. Signals from 50 non-overlapping interphase nuclei were counted using the BX63 automated fluorescence microscope (Olympus Corporation, Tokyo, Japan). Computer-based documentation and image analysis were performed using the SoloWeb imaging system (BioView Ltd, Rehovot, Israel).

Tumor sequencing

DNA obtained from the first representative surgical tumor biopsy (T1), the second computed tomography-guided percutaneous tumor biopsy (T2) and from blood leukocytes used as a matched normal control was isolated using the Nucleospin TriPrep Kit according to manufacturer's instructions (Macherey-Nagel, Düren, Germany). Libraries enriched with exonic sequences were prepared using the SureSelect Human All Exon V6 kit (Agilent, Santa Clara, CA, U.S.A.), and further processed using the Illumina TruSeq Exome Kit (Illumina, San Diego, CA, U.S.A.) for sequencing on Illumina HiSeq 2500 and Illumina NextSeq sequencers. The INFORM consortium[2] provided WES and RNA sequencing data from tumor tissue at the time of tumor resection (T3). Data were generated using the Illumina HiSeq 4000 system as described in the INFORM registry (Registry Code: NCT-2013-0220; German Clinical Trial Register ID: DRKS00007623).

SNV analysis

Paired-end sequencing reads were trimmed, filtered with trim galore![3] and aligned against the human genome build hs37d5 with bwa mem[4]. Duplicates were marked using GATK[5]. Bam files

were sorted and indexed with samtools[6]. As some smaller biopsies had very low sequencing coverage, we restricted downstream analysis to samples with at least 40X (average) coverage. Following GATK best practices guidelines[7], all bam files were recalibrated, and mutations were called and filtered with MuTect2[8]. Mutation impact predictions were obtained from VEP[9], and mutations in all samples were filtered against the cancer gene census v91[10]. Mutations with any variant reads in the matched normal sample or a variant allele frequency below 10% were removed from further analysis. To identify mutations that might be below the detection limit of the variant caller, we genotyped the remaining variants across all samples.

Copy number variant analysis

All samples were genotyped against the panel of 1000 genomes SNPs (phase 3)[11] using alleleCount. The coverage at every SNP location was divided by the corresponding coverage in the normal sample and log-normalized to obtain a coverage track. ASCAT v2.5.2[12] was used to infer purity and copy number profiles using the log-normalized coverage and the B-allele frequencies from the 1000 genomes loci.

SUPPLEMENTARY TABLES

Suppl. Table S1. Overview of therapy response evaluation in infantile fibrosarcoma.

Time point (months)	Imaging modality	Response evaluation according to RECIST (version 1.1)[1]	Lesions (mm)			
			Lesion A	Lesion B	Lesion C	Lesion D
0	MRI[2]	baseline at diagnosis	78	-	-	-
2.4	MRI	PD[4]	61	47 (new)	-	-
4.5	MRI	PR[5]	10	30	-	-
6.4	MRI	PD	resolved	25	40 (new)	47 (new)
8.2	MRI	PD	-	23	49	57
10.1	MRI	PR	-	21	25	38
11.8	CT scan[3]	PD	-	21 (calcified)	37	49
14.1	MRI	PR	Lesions A-D resolved, non-measurable lesions remaining (longest diameter <10 mm or lymph nodes with ≥10 to <15 mm short axis). No disease detectable in biopsy of one of those non-measurable lesions.			
17.8	MRI	SD[6]				
27	MRI	SD				

[1] Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009.
[2] Magnetic resonance imaging
[3] Computed tomography scan
[4] Progressive disease
[5] Partial response
[6] Stable disease

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