Supplementary Online Content

Robertson SJ, Orme L, Teixeira R, et al. Evaluation of crizotinib treatment in a patient with unresectable *GOPC-ROS1* fusion agminated Spitz nevi. *JAMA Dermatol*. Published online June 2, 2021. doi:10.1001/jamadermatol.2021.0025

eMethods. Histologic and Genetic Analysis

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Histologic and Genetic Analysis

Consent: Consent for genetic sequencing was obtained via the Melbourne Melanoma Project (Peter MacCallum Cancer Centre Ethics Approval number 07/38).

Histologic analysis: Surgical samples were processed, formalin-fixed and paraffin-embedded as per standard laboratory protocols. Sections were stained with hematoxylin and eosin via routine diagnostic procedures. Protein expression was detected with antibodies targeting Trk family proteins (TrkA, TrkB, and TrkC; clone C17F1), ROS1 (D4D6), and/or ALK (D5F3), all obtained from Cell Signaling (Danvers, MA),

DNA extractions: Tumour DNA was extracted using an AllPrepDNA/RNA/miRNA Universal kit (80224, Qiagen), according to the manufacturer's instructions. Blood DNA was extracted from whole blood using a Flexigene DNA Kit (51206, Qiagen). All samples were quantified using the NanoDrop (ND1000, Thermoscientific) and Qubit dsDNA HS Assay (Q32851, Life Technologies) and DNA size and quality were tested by gel electrophoresis. Samples with a concentration of less than 50 ng μl^{-1}, or with absence of a high molecular mass band in electrophoresis gels, were excluded from further analyses.

WGS: Whole genome sequencing (WGS) was performed on an Illumina HiSeq X Ten instrument (Illumina, San Diego, California, USA) at Macrogen (South Korea). Library construction was performed using TruSeq DNA Sample Preparation kits (Illumina) according to the manufacturer's instructions. Subsequent 150 base pair (bp) paired-end libraries were sequenced to depths of 72x in the tumour sample and 38x in the matched normal.

WGS processing and quality control: Sequence data were aligned to the GRCh37 assembly using BWA-MEM, resulting in sorted lane level files in sequence alignment/mapping format which were compressed and converted to binary file (BAM) created by samtools 0.1.19.

Sample-level merged BAMs were produced by in-house tools and duplicate reads marked with Picard MarkDuplicates 1.97 (http://picard.sourceforge.net). Quality assessment and coverage estimation were performed by qProfiler and qCoverage respectively (http://sourceforge.net/projects/adamajava).

Somatic mutation detection, UVR signature and telomere length: Somatic mutations and germline variants were detected with a dual calling strategy using qSNP[1] and GATK HaplotypeCaller[2]. Indels of 1–50 bp in length were called with GATK. The predicted consequence of mutations was annotated using Ensembl gene annotation with SnpEff. To determine the proportion of mutations with an ultraviolet radiation (UVR) signature, we used SignatureEstimation[3] to assign mutations to COSMIC version 1 signatures[4]. Somatic copy number was determined using ascatNGS[5]. Structural variants were identified using the qSV tool[6]. Telomere length was determined using qMotif (http://sourceforge.net/projects/adamajava). The number of somatic single nucleotide variants (SNVs), small indels, copy number variations, and structural variants, the proportions of UVR signature mutations, and the relative telomere lengths were compared with previously published data from 140 cutaneous melanomas[7].

Gene fusions were validated orthogonally using a next-generation sequencing-based method that detects *NTRK1/2/3*, *ALK* and *ROS1* rearrangements (Ignyta). RNA was extracted from FFPE sections and its fragmentation assessed by 138 bp qPCR of the *VCP* housekeeping gene. After reverse transcription, sequencing target enrichment was accomplished by anchored multiplex PCR[8,9]. Libraries were sequenced on a MiSeq or MiSeqDx sequencer (Illumina, San Diego, CA). Analysis of sequencing data was performed using Archer Analysis software (v3.2.1; ArcherDX Inc.). Fusion parameters were set to a minimum of 5 valid fusion reads with a minimum of 3 unique start sites within each valid read.

eReferences

1. Kassahn KS, Holmes O, Nones K, et al. Somatic point mutation calling in low cellularity tumors. *PLoS One.* 2013;8(11):e74380.
2. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20(9):1297-1303.
3. Huang X, Wojtowicz D, Przytycka TM. Detecting presence of mutational signatures in cancer with confidence. *Bioinformatics.* 2018;34(2):330-337.
4. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature.* 2013;500(7463):415-421.
5. Raine KM, Van Loo P, Wedge DC, et al. ascatNgs: Identifying Somatically Acquired Copy-Number Alterations from Whole-Genome Sequencing Data. *Curr Protoc Bioinformatics.* 2016;56:15.19.11-15.19.17.
6. Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. *Nature.* 2015;521(7553):489-494.
7. Hayward NK, Wilmott JS, Waddell N, et al. Whole-genome landscapes of major melanoma subtypes. *Nature.* 2017;545(7653):175-180.
8. Zheng Z, Liebers M, Zhelyazkova B, et al. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Med.* 2014;20(12):1479-1484.
9. Murphy DA, Ely HA, Shoemaker R, et al. Detecting Gene Rearrangements in Patient Populations Through a 2-Step Diagnostic Test Comprised of Rapid IHC Enrichment Followed by Sensitive Next-Generation Sequencing. *Appl Immunohistochem Mol Morphol.* 2017;25(7):513-523.