Supplementary Online Content

Bahrami A, Lee S, Wu G, et al. Pigment-synthesizing melanocytic neoplasm with Protein Kinase C Alpha (*PRKCA*) fusion. Published online December 16, 2015. *JAMA Dernatology*. doi:10.1001/jamadermatol.2015.2524.

eMethods.

eFigure. The CGH genome view

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

eMETHODS

RNA Sequencing (RNA-seq) Data Analysis. Paired-end reads from RNA-seq were aligned to the following 4 database files by using BWA (0.5.10) aligner: (1) the human GRCh37-lite reference sequence, (2) RefSeq, (3) a sequence file representing all possible combinations of nonsequential pairs in RefSeq exons, and (4) AceView database flat file downloaded from UCSC representing transcripts constructed from human ESTs. The mapping results from (2) to (4) were aligned to human reference genome coordinates. They were also aligned by using STAR 2.3.0 to the human GRCh37-lite reference sequence without annotations. The final BAM file was constructed by selecting the best alignment among the 5 mappings. The coverage was calculated using an in-house pipeline. RNA-seq was performed with at least 20% of exonic bases covered with a depth of coverage of at least 20×. Structural variations were detected by using CICERO, a novel algorithm that uses de novo assembly to identify structural variation in RNA-seq.

Array Comparative Genomic Hybridization (aCGH) Analysis. aCGH analysis was performed on the FFPE tissue. Briefly, DNA was extracted and purified from the tissue blocks and quantified with a Nanodrop 8000 spectrophotometer (Thermo Scientific). DNA was labeled by using the Agilent Universal Linkage System (ULS) Labeling Kit (Agilent Technologies). Gender-matched genomic DNA from Promega was used as a control. Samples were labeled with the fluorescent dye Cy-5 and cohybridized with the appropriate control DNA labeled with the fluorescent dye Cy-3 onto the microarray from the Agilent SurePrint G3 ISCA CGH + SNP Microarray Kit, 4x180K (Agilent

Technologies). The microarray was left to hybridize for 40 h and stringently washed to remove unhybridized targets. Then, the microarray was scanned at 2 different laser wavelengths, and the fluorescent signal at each probe was compiled by using the Agilent analysis software (Agilent Cytogenomics Edition).

Immunohistochemical Analysis

FFPE tumor sections (4 μm) were processed for immunohistochemical analysis for PKA [R1α] (20/PKA R1α; BD Biosciences) with heat-induced epitope retrieval with ER2 for 20 min on the Leica BOND automated staining system (Leica Biosystems), followed by antibody incubation at a 1:400 dilution for 15 min and staining with the Bond Polymer Refine Red Detection Kit.

eFigure 1: The CGH genome view