## ***Next-generation sequencing***

Tumor DNA was extracted at the central lab after macro-dissection.

A total of 20 ng of DNA was used to build The Oncomine Focus DNA Assay panel libraries (Thermo Fisher Scientific, Inc.), using the Ion AmpliSeq™ Library kit 2.0 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. This panel comprises hotspot mutations/small indels and CNVs for 35 and 19 genes, respectively, over 269 amplicons. Ion Xpress Barcode 1–32 and Ion P1 Adapter (Thermo Fisher Scientific, Inc.) were added during the library preparation to allow a maximum pooling strategy of 32 samples/chip. The final amplicon libraries were quantified using the Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific, Inc.) and diluted to 30 pM for the subsequent sequencing.

A total of 30 uniquely barcoded library samples were pooled for sequencing per run on an Ion 530™ chip (Thermo Fisher Scientific, Inc.). Template generation and chip loading were performed using The Ion Chef™ System (Thermo Fisher Scientific, Inc.) using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef. The obtained libraries were finally sequenced using the Ion GeneStudioTM S5 Plus System.

BAM files derived from processed raw data were generated by the Ion Torrent platform-specific pipeline software. Briefly, polyclonal and poor signal profile reads were a priori excluded by the raw sequences, 3′ trimming of reads was performed using Torrent Suite Assay Development Mode v5.0 (Thermo Fisher Scientific, Inc.) and the filtered reads were aligned to the human genome hg19 ([https://www.ncbi.nlm.nih.gov/assembly/GCF\_0000](https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/)

[01405.13/](https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/)). All the BAM files were transferred on the Ion Reporter Software (v. 5.10.5.0) (Thermo Fisher Scientific, Inc.) and analysed by the Oncomine Focus w2.4 - DNA - Single Sample (v. 5.10). The Ion Reporter workflow was applied to identify SNVs, indels and CNVs using the presetted parameters. A cut off of 300× read depth was applied to all analyses in the present study, allowing a uniform evaluation of 10% VAF. Samples with a MAPD 0.5 and therefore lower coverage uniformity were excluded from downstream analysis as suggested by Ion Reporter Software QC guidelines. Finally, a custom filter chain was applied to report only likely somatic mutations with a VAF 0.1 and a minor allele frequency or global allele frequency in ExAC or 5000 exomes databases 1.0E-6. Mutations must also be nonsynonymous and occur in exonic or splice-site regions.