
eMethods. RNA Analysis and Genetic Sequencing

This supplementary material has been provided by the authors to give readers additional information about their work.
**eMethods.** RNA Analysis and Genetic Sequencing

Total RNA was purified from stored samples with the RNeasy Mini Kit (Qiagen). cDNA was generated and quantitative real-time polymerase chain reaction was performed using the Brilliant II SYBR Green QRT-PCR Master Mix Kit (Agilent Technologies) on a Mx3000P qPCR System (Agilent Technologies). Fold change in $GLI1$ mRNA expression was measured using $\Delta\Delta C_t$ analysis, with $GAPDH$ as the internal control gene. Polymerase chain reaction primer sequences were:

$GLI1$ forward: 5’ GCCGTGCTAAAGCTCCAGTGAACAC 3’

$GLI1$ reverse: 5’ AGAAGTCGAGGTGGTGCTGCTGCCC 3’

$GAPDH$ forward: 5’ AATCCCATCACCATCTTCCA 3’

$GAPDH$ reverse: 5’ TGGACTCCACGACGTACTCA 3’

In cases where the metastatic BCC was inaccessible for biopsy, we obtained biopsies from other cutaneous BCCs or normal skin as a surrogate, as has been done in prior studies assessing the effect of HH-inhibitors on HH signaling in BCC 4.

**Exome and SMO sequencing**

We also assessed the presence or absence of HH pathway mutations in SMO inhibitor-resistant BCC prior to initiation of ATO/itraconazole using whole exome sequencing and targeted SMO sequencing.
For exome sequencing, we purified genomic DNA from BCC samples stored in RNAlater using the DNeasy Blood and Tissue Kit (Qiagen). Capture libraries were constructed from 2 μg of DNA using the SureSelect Human All Exon V4 Kit (Agilent Technologies). Enriched exome libraries were multiplexed and sequenced on the Illumina HiSeq 2500 platform to generate 100-bp paired-end reads.

For targeted sequencing of SMO, the coding regions of SMO were amplified using the Access Array platform (Fluidigm). The samples were amplified in a multiplex format with genomic DNA (100 ng) according to the manufacturer's recommendation (Ambry Genetics). Subsequently, the multiplexed library pools were subjected to deep sequencing using the Illumina MiSeq platform to generate 150-bp paired-end reads.

Sequencing reads were aligned to the human reference genome sequence (hg19) using Burrows-Wheeler Aligner (BWA) software. SAM to BAM conversion and marking of PCR duplicates were performed using Picard tools (version 1.86), followed by local realignment around In/Dels and base quality score recalibration using the Genome Analysis Toolkit (GATK) (v2.3.9; Broad Institute). Somatic SNVs and In/Dels were called using GATK. Variants were selected if they resulted in a predicted change to the protein coding sequence and if they occurred in genes listed in the human HH signaling pathway in the KEGG database (v2.3.9). SNPs and In/Dels were further analyzed for functional annotations using ANNOVAR.