

Supplementary material for Asklund T, et al., Early and persisting response to vismodegib in a patient with bone metastasizing medulloblastoma, Acta Oncologica, 2013;52:862–866.

Materials and methods

RNA extraction and real-time RT-PCR analysis

Total RNA was extracted from frozen sections from two independent parts of the medulloblastoma, and from Daoy cells, using the RNeasy kit (Qiagen) according to the manufacturer's instructions. A 400 ng aliquot of RNA was reverse transcribed using random hexamer primers and Superscript III polymerase (Life Technologies), and run on an ABI Sequence Detection System using Power SYBR Green (Applied Biosystems). The real-time RT-PCR analysis of biological replicates measured in technical triplicates utilized the housekeeping genes hRPLP0 and h18S as internal controls to normalize the expression levels.

Expression data after normalization were analyzed using the $2^{-\Delta\Delta CT}$ method.

Primer sequences for real-time RT-PCR

PTCH2-fw	5'-TCTTTCTGGGACTGTTGGC CTTTGG
PTCH2-rev	5'-CCTCCCCCAGCTTCTCCTT GGTGT
GLI2-fw	5'-GACATGCGACACCAGGAAGG AAGG
GLI2-rev	5'-GCCGGATCAAGGAGATGTC AGAGA
GLI1-fw	5'-TCTGGACATACCCACCTCC CTCTG
GLI1-rev	5'-ACTGCAGCTCCCCCAATTTT TCTGG
RPLP0-fw	5'-AGGCCTTGGGCATCACCAC AAAGAT
RPLP0-rev	5'-TCACCAGGCCAAAGGAGAA GGGAGA
18S-fw	5'-GCCCCAGCCGCCTGGATACC
18S-rev	5'-TCACCTCTAGCGGCGCAATA CGAA