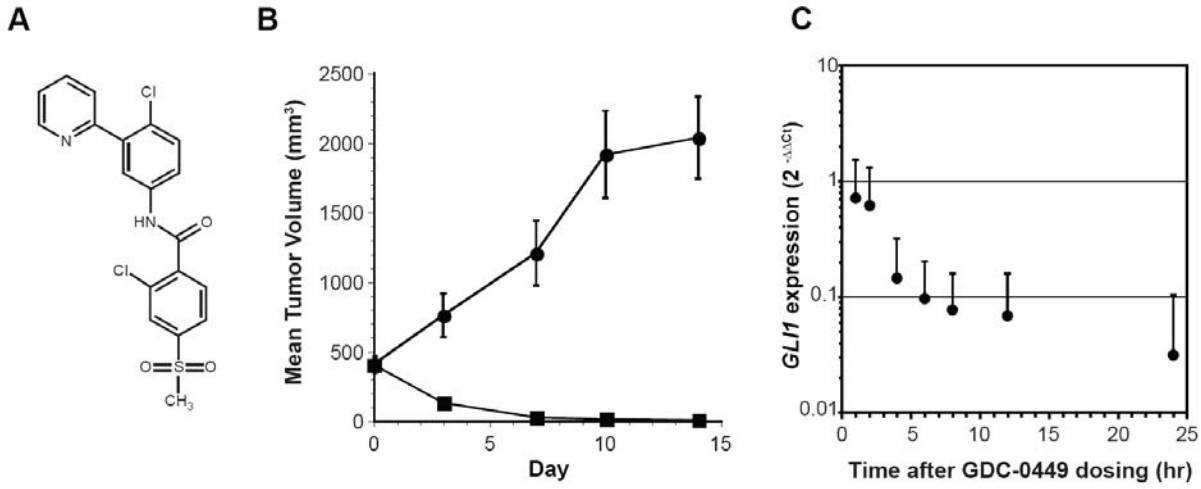
Supplementary Appendix

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

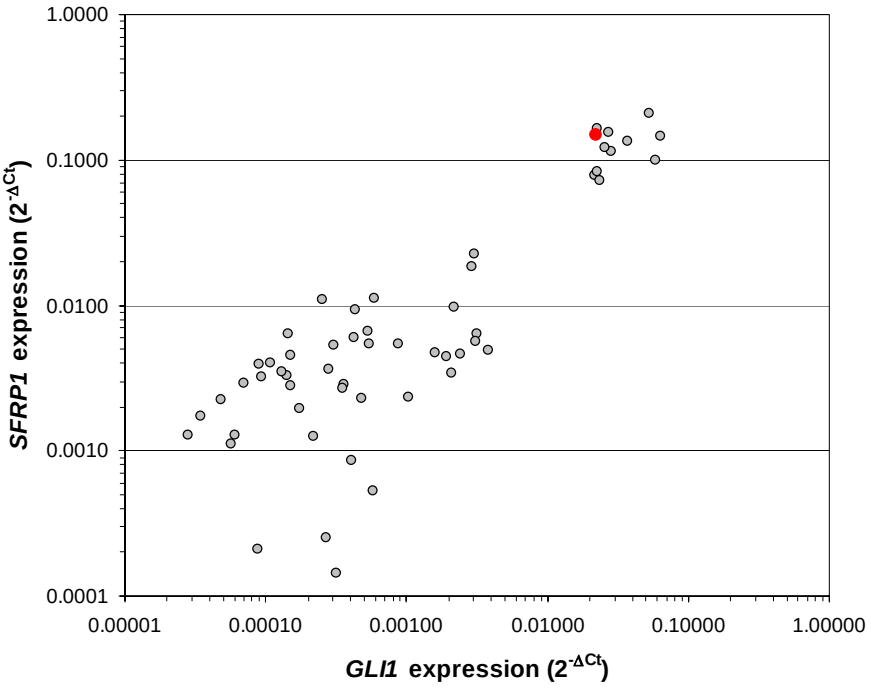
Supplement to: Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med 2009;361:1173-8. DOI: 10.1056/NEJMoa0902903.

Supplementary Figure 1: GDC-0449 causes complete regression in a mouse model of medulloblastoma. A. Chemical structure of GDC-0449, a 2-pyridyl amide Hedgehog pathway inhibitor (HPI). **B.** *Ptc*$^{+/-}$ medulloblastoma allograft tumors (n=5 per group) were treated orally with 100 mg/kg GDC-0449 (squares) or vehicle control (circles) once daily for 14 days. Following 7 days of dosing all tumors treated with GDC-0449 had regressed to a volume of < 50 mm^3. **C.** In a parallel study 100 mg/kg GDC0449 was administered in a single dose. Medulloblastoma allograft tumors collected from 1 to 24 hours following drug administration were analyzed for expression of *Gli1* mRNA by quantitative PCR. GDC-0449 treatment led to significant suppression of *Gli1* expression by 4 hours, maximal by 8 hours, with continued suppression at 24 hours.

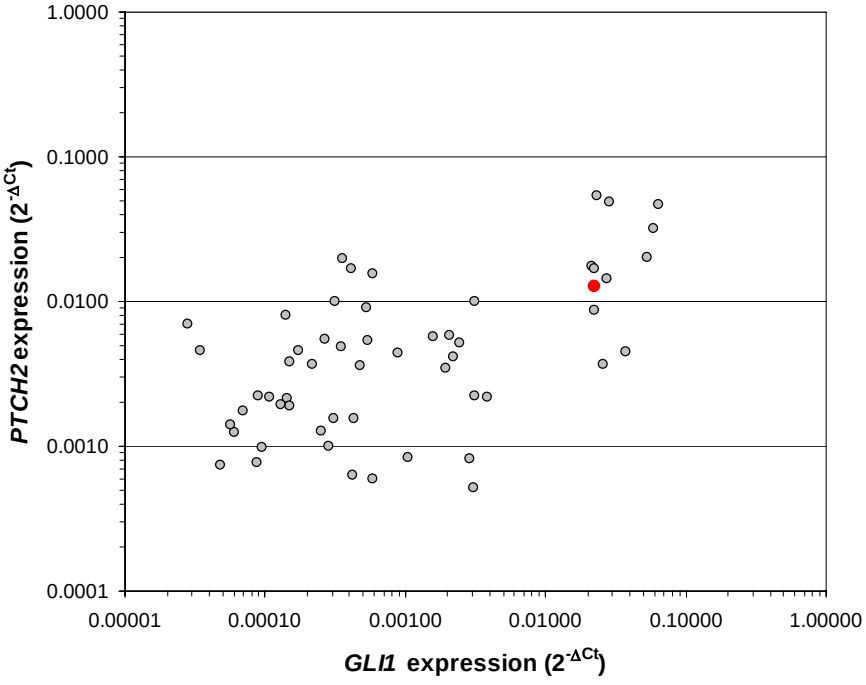


Supplementary Figure 2: Tumor-specific Hedgehog pathway activation. Relative mRNA expression levels of Hedgehog pathway regulated genes in patient tumor (red dot) relative to expression in a panel of banked medulloblastoma samples (grey dots; n=55). **A.** *GLI1* vs. *SFRP1*. **B.** *GLI1* vs. *PTCH2*.

A.

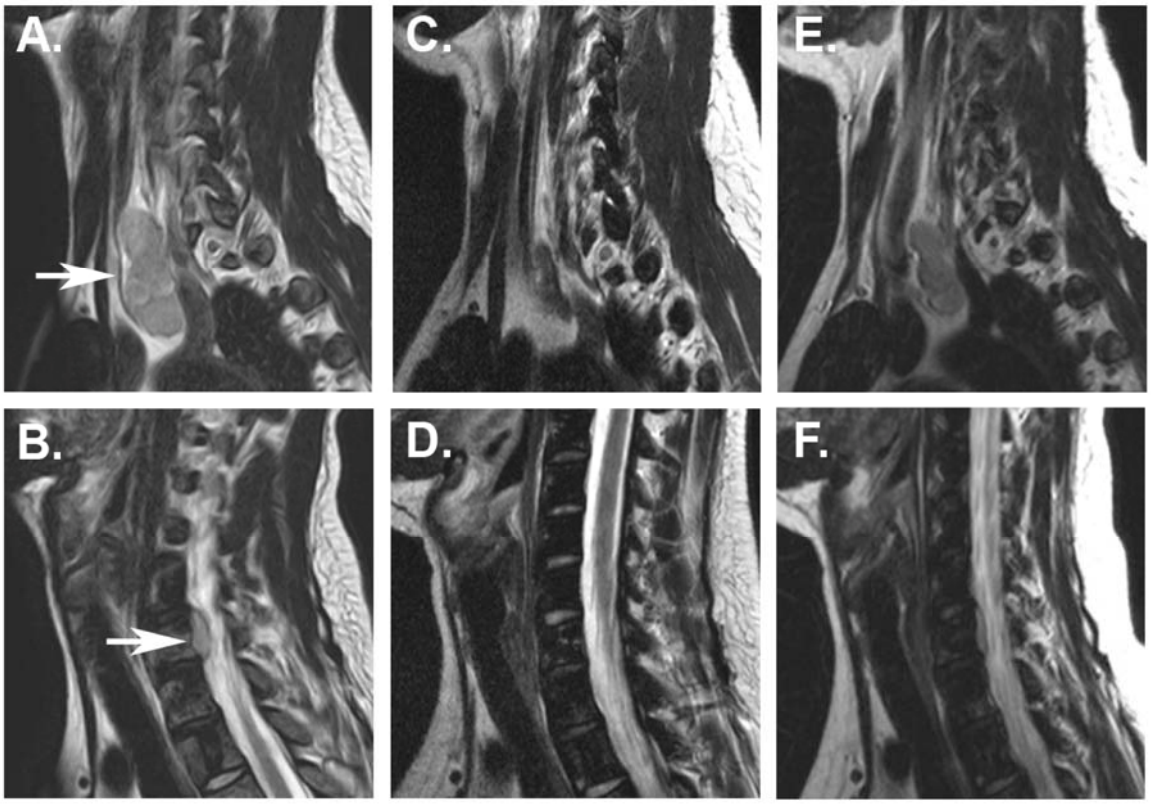


B.



Supplementary Figure 3

Tumor response by cervical/thoracic MRI. A & B. Scan performed day 3 after a single pharmacokinetic dose of GDC-0449 and 4 days before initiating daily GDC-0449, with arrows indicating matted right supraclavicular lymphadenopathy (**A**) and an epidural mass compressing the thecal sac (**B**). **C & D.** Repeat scan after 2 months on GDC-0449, demonstrating resolution of lymphadenopathy and of the epidural mass. **E & F.** Repeat scan images after 3 months of therapy.



Supplementary material: Methods

Gene expression profiling.

Hh pathway mRNA expression levels were assessed by quantitative RT-PCR using standard Taqman techniques. Assays were tested for amplification efficiency on RNA isolated from formalin-fixed, paraffin-embedded specimens and for lack of genomic DNA amplification. Hh target gene levels were normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and results are expressed as normalized expression values ($=2^{-\Delta Ct}$). The primer/probe sets for the transcripts analyzed were as follows; *GAPDH*: forward, 5'-GGGTGTGAACCATGAGAAGTATGA-3'; reverse, 5'- GGTGCAGGAGGCATTGCT-3'; and probe, 5'(VIC)-ACAGCCTCAAGATCAT-(MGBNFQ)-3'; *GLI1*: 5'-CAGGGAGGAAAGCAGACTGACT-3'; reverse, 5'- GGCTTGGCTGTGGCTTCA-3'; and probe, 5-(FAM)- TGCCAGAGGGTGCC-(MGBNFQ)-3'; *PTCH1*: 5'- CCACGACAAAGCCGACTACA-3'; reverse, 5'- GCTCTGCTGCCGGGATT-3'; and probe, 5-(FAM)- CCTGAAACAAGGCTG-(MGBNFQ)-3'; *SFRP1*: 5'-CAAGCCCCAAGGCACAAC-3'; reverse, 5'-ATGATGGCCTCAGATTTCAACTC-3'; and probe, 5-(FAM)- TGTGTCCTCCCTGTGAC-(MGBNFQ)-3'; *PTCH2*: 5'- CGCCCGCTATCAGTTTGC-3'; reverse, 5'-CACGATGGCCTTAGCATGTG-3'; and probe, 5-(FAM)-CCGTTGCTGCTCCAG-(MGBNFQ)-3'.

Gene sequencing.

PTCH1 exons were PCR-amplified from genomic DNA using a pair of nested primers. The internal pair of primers used in the amplification contained m13 forward or m13 reverse primer sequences. The sequence of *PTCH1* exon 15 primers were as follows: 5'outer primer (AATCAGTTTAAGTGTGGTGGTG); 3' outer (GCTCTCATAATCATGACAAAGGAAC); 5' inner

(TGTAAAACGACGGCCAGTCAAGGTATTAACTAGACAGCTTCTC); 3’ inner (CAGGAAACAGCTATGACCCATGACAAAGGAACCTGTTGAA). After PCR, free nucleotides and excess primer were removed using ExoSAP-IT kit (USB); PCR products were sequenced in both directions using a m13 sequencing primers. PCR products were cycle-sequenced using BigTerminator Kit (Applied Biosystems). All sequencing products were resolved on a 3730xl sequencing machine (Applied Biosystems). Sequence trace files were analyzed using Sequencher (GeneCodes) and/or Mutation Surveyor (SoftGenetics LLC).