

Supplementary Appendix

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

Supplement to: Van Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. *N Engl J Med* 2009;361:1164-72. DOI: 10.1056/NEJMoa0905360.

Inhibition of the hedgehog pathway in advanced basal-cell carcinoma

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SUPPLEMENTARY MATERIAL:

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Supplementary Figure 1

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Supplementary Table 1. Probe and Primer Sequences for Taqman qRT-PCR and PCR Amplification of *PTCH1* and *SMO* exon 9

Taqman Primer Probes			
Gene	Primer 1	Primer 2	Probe
<i>GLI1</i>	CAGGGAGGAAAGCAGACTGACT	GGCTTGGCTGTGGCTTCA	FAM-TGCCAGAGGGTGCC-MGB
<i>SMO</i>	TGAGTGGGAGCGCAGCTT	GCCCGATGGTCACATTGG	FAM-CGGGACTATGTGCTATGT-MGB

Gene Sequencing Primers	
Exon	Primer Sequence
SMO.e9.5i	TGTAAAACGACGGCCAGTACCCAGTAGACTCAGAAAACCC
SMO.e9.3i	CAGGAAACAGCTATGACCAAACCTAAAGATGGGGTCCCA
SMO.e9.5o	TGATTGTCTGAGTCTACCCAGTAG
SMO.e9.3o	CCGACAAAACCTAAAGATGGGG
PTCH.e2.5i	TGT AAA ACG ACG GCC AGT AGT CTC GAG GGC GAG TCC GTG T
PTCH.e2.3i	CAG GAA ACA GCT ATG ACC GCT GGC GAA TAT CTC TAT CAA CC
PTCH.e2.5o	AGC CCC CCA TGA CGC TCA GAT
PTCH.e2.3o	GTG CGC TGG CGA ATA TCT CTA T
PTCH.e3.5i	TGT AAA ACG ACG GCC AGT GGC AGG TAG TCA GAT AAC AGA TAA A
PTCH.e3.3i	CAG GAA ACA GCT ATG ACC GGC CTA AAC CAG CAG CCT TCT
PTCH.e3.5o	TGT ATC CTA TGG CAG GTA GTC AGA T
PTCH.e3.3o	ATA ACG GGG CCT AAA CCA GCA GC
PTCH.e4.5i	TGT AAA ACG ACG GCC AGT GAC AGA TGC TTA AAA GTG ATC G
PTCH.e4.3i	CAG GAA ACA GCT ATG ACC GCC ATG CGT TAG GTT AAG GCA
PTCH.e4.5o	TGC TCG TTT TGA CAG ATG CTT
PTCH.e4.3o	GAA GAG GCC ATG CGT TAG GTT A
PTCH.e5.5i	TGT AAA ACG ACG GCC AGT GGC AAA AAT TTC TCA GGA ACA CC
PTCH.e5.3i	CAG GAA ACA GCT ATG ACC CCG ACT ATT CAC TCA AAA AAT GCA C
PTCH.e5.5o	ACA GGT TAC ATG GAT CAG GTA T
PTCH.e5.3o	CCC CCG ACT ATT CAC TCA AAA AA
PTCH.e6.5i	TGT AAA ACG ACG GCC AGT TGG GCG CAG CCG TGT TAC TTT A
PTCH.e6.3i	CAG GAA ACA GCT ATG ACC AAG TGA ACG ATG AAT GGA CAC A
PTCH.e6.5o	ACA AGG TGG ATG CAG TGG GCG
PTCH.e6.3o	AGA CAA AGA CGA TCA TGG AGA ATG
PTCH.e7.5i	TGT AAA ACG ACG GCC AGT GCC CTT AAT GCA CTG GAT TTT AAC
PTCH.e7.3i	CAG GAA ACA GCT ATG ACC TCA GGA GGG AAG TGG CTT TTG
PTCH.e7.5o	CAC ACA AGC CCT TAA TGC ACT G
PTCH.e7.3o	CGA TGT CAG GAG GGA AGT GGC
PTCH.e8.5i	TGT AAA ACG ACG GCC AGT GGC TGG GGA TTT GAG GCA GTG
PTCH.e8.3i	CAG GAA ACA GCT ATG ACC CAA GTT CCC AGA ATT GCA ATG T
PTCH.e8.5o	AGC CAG TGA GTT GGG GGA GCT
PTCH.e8.3o	CCA TCA AGT TCC CAG AAT TGC
PTCH.e9.5i	TGT AAA ACG ACG GCC AGT CCT GAG ATC TGT GCT GTC GAG
PTCH.e9.3i	CAG GAA ACA GCT ATG ACC GAG CAG TCA TGG AAA AGT AAA GAC T
PTCH.e9.5o	TGT GTC ACC TGA GAT CTG TGC T
PTCH.e9.3o	GCA GGA GCA GTC ATG GAA AAG TAA
PTCH.e10.5i	TGT AAA ACG ACG GCC AGT TGG AGG GAA ACA TTA GAA TCA CAA C
PTCH.e10.3i	CAG GAA ACA GCT ATG ACC CCG GTG AGA AGG ACA CAC AGC
PTCH.e10.5o	AAG TTC TGC CTT TTG ATT TGG G
PTCH.e10.3o	CCC TTC CGG TGA GAA GGA CAC
PTCH.e11.5i	TGT AAA ACG ACG GCC AGT GCA GAG TCC TAA CTA GCT TTA GAA T
PTCH.e11.3i	CAG GAA ACA GCT ATG ACC TGG GTC AGA CTG AGG AAA ATT AAA G
PTCH.e11.5o	TGC TGG TGG CAG AGT CCT AAC
PTCH.e11.3o	TAC TGG GTC AGA CTG AGG AAA AT
PTCH.e12.5i	TGT AAA ACG ACG GCC AGT ATC TGC TCA CAC AGT CTG TGC TC
PTCH.e12.3i	CAG GAA ACA GCT ATG ACC ACC GCA GAC ATG GGA TGC TGG
PTCH.e12.5o	GCC ACG TAT CTG CTC ACA CAG T
PTCH.e12.3o	CAG AGA CCG CAG ACA TGG GAT
PTCH.e13.5i	TGT AAA ACG ACG GCC AGT CAT CTT TAT TTT CGA AGC AGT CCT C
PTCH.e13.3i	CAG GAA ACA GCT ATG ACC CAC AGG CTG AAA GAG TTC TCT CA
PTCH.e13.5o	CAA ATG CTT CAA GAG GAA AGG G
PTCH.e13.3o	ATA AGT CCA CAG GCT GAA AGA GTT C
PTCH.e14.5i	TGT AAA ACG ACG GCC AGT CAC AGT GAA AAA TGG CAG AAT G
PTCH.e14.5o	TCT CCG TAC ACA GTG AAA AAT GG
PTCH.e14.3i	CAG GAA ACA GCT ATG ACC AAG CAA TCT GAT GAA CTC CAA AGG
PTCH.e14.3o	AAG TAG AAG CAA TCT GAT GAA CTC C
PTCH.e15.5i	TGT AAA ACG ACG GCC AGT CAA GGT ATT AAC TAG ACA GCT TCT C
PTCH.e15.3i	CAG GAA ACA GCT ATG ACC CAT GAC AAA GGA ACC TGT TGA A
PTCH.e15.5o	AAT CAG TTT AAG TGT GGT GGT G
PTCH.e15.3o	GCT CTC ATA ATC ATG ACA AAG GAA C
PTCH.e16.5i	TGT AAA ACG ACG GCC AGT ACG TCT AAG AGC CCC CTG CCC T
PTCH.e16.3i	CAG GAA ACA GCT ATG ACC TGT CAA GCA GCC TCC ACC AGG
PTCH.e16.5o	TGA TGG ACG TCT AAG AGC CCC
PTCH.e16.3o	CAC GCT GTC AAG CAG CCT CCA
PTCH.e17.5i	TGT AAA ACG ACG GCC AGT TCT GTG CTC TCA AGG CAG AAG T
PTCH.e17.3i	CAG GAA ACA GCT ATG ACC TGT TGC TGA GTT TGG AGA ACC A
PTCH.e17.5o	TCA GCT TCT GTG CTC TCA AGG

Supplementary Table 2. Listing of Grade 2 Adverse Events by MedDRA Preferred Terms

Adverse Event	Grade 2
Muscle Spasms	4
Anorexia	3
Dyspnea	3
Chest Pain	2
Cough	2
Dysgeusia	2
Erythema	2
Musculoskeletal Chest Pain	2
Upper Respiratory Tract Infection	2
Weight Decreased	2
Anemia	1
Anxiety	1
Asthenia	1
Back Pain	1
Bowen's Disease	1
Bronchitis	1
Candidiasis	1
Cheilitis	1
Constipation	1
Cranial Nerve Pareses	1
Cystitis	1
Decubitis Ulcer	1
Diarrhea	1
Duodenal Ulcer	1
Dyspepsia	1
Dysphagia	1
Dysuria	1
Eye Infection	1
Facial Palsy	1
Fatigue	1
Flank Pain	1
Gout	1
Headache	1

Hemorrhage	1
Humerus Fracture	1
Hydrothorax	1
Hyperglycemia	1
Hyperhidrosis	1
Hypertension	1
Hypocalcemia	1
Influenza	1
Insomnia	1
Jaw Pain	1
Limb Abscess	1
Lobar Pneumonia	1
Localized Infection	1
Lymphopenia	1
Nausea	1
Neck Pain	1
Neuralgia	1
Oesophageal Stenosis	1
Oral Pain	1
Pain in Extremity	1
Photophobia	1
Pleural Effusion	1
Pleurisy	1
Pneumonia	1
Respiratory Tract Congestion	1
Sinusitis	1
Skin Infection	1
Supraventricular Tachycardia	1
Upper Abdominal Pain	1
Vomiting	1
Wound Infection	1

Supplementary Table 3. *PTCH1* and/or *SMO* mutations identified in tumor tissue from 9 out of 10 patients with BCC.^a

Patient ID	GDC-0449 Phase I Disease Group	Mutation		Mutation present in patient-matched normal tissue?
		nucleic acid	amino acid	
1	LA	<i>PTCH1</i> _901G>A	<i>PTCH1</i> _238G>E	No
		<i>PTCH1</i> _2118C>T	<i>PTCH1</i> _644P>S	No
		<i>PTCH1</i> _2236C>T	<i>PTCH1</i> _683S>F	No
		<i>PTCH1</i> _3780C>T	<i>PTCH1</i> _1198P>S	No
2	MET	<i>PTCH1</i> _2749-1G>A	intronic_splice site	na
4	LA	<i>PTCH1</i> _3465G>A	<i>PTCH1</i> _1093G>R	Yes
		<i>PTCH1</i> _3771A>T	<i>PTCH1</i> _1195T>S	Yes
5	MET	<i>PTCH1</i> _465T>TA	<i>PTCH1</i> _Y93>stop	na
9	MET	<i>PTCH1</i> _450-462_del	<i>PTCH1</i> _fs>112stop	na
11	LA	nd	nd	
14	MET	<i>SMO</i> _1884G>T	<i>SMO</i> _W535L	No
		<i>PTCH1</i> _1891C>T	<i>PTCH1</i> _568P>L	No
		<i>PTCH1</i> _2254C>T	<i>PTCH1</i> _689P>L	No
17	MET	<i>PTCH1</i> _895G>A	<i>PTCH1</i> _236W>stop	na
23	MET	<i>PTCH1</i> _2776G>A	<i>PTCH1</i> _863W>stop	na
27	MET	<i>PTCH1</i> _3000C>T	<i>PTCH1</i> _938Q>stop	na

LA = locally-advanced, MET = metastatic, na = not available, nd = not detected

^a When available, patient-matched normal skin was also sequenced to determine if the identified mutation was somatic or germline.

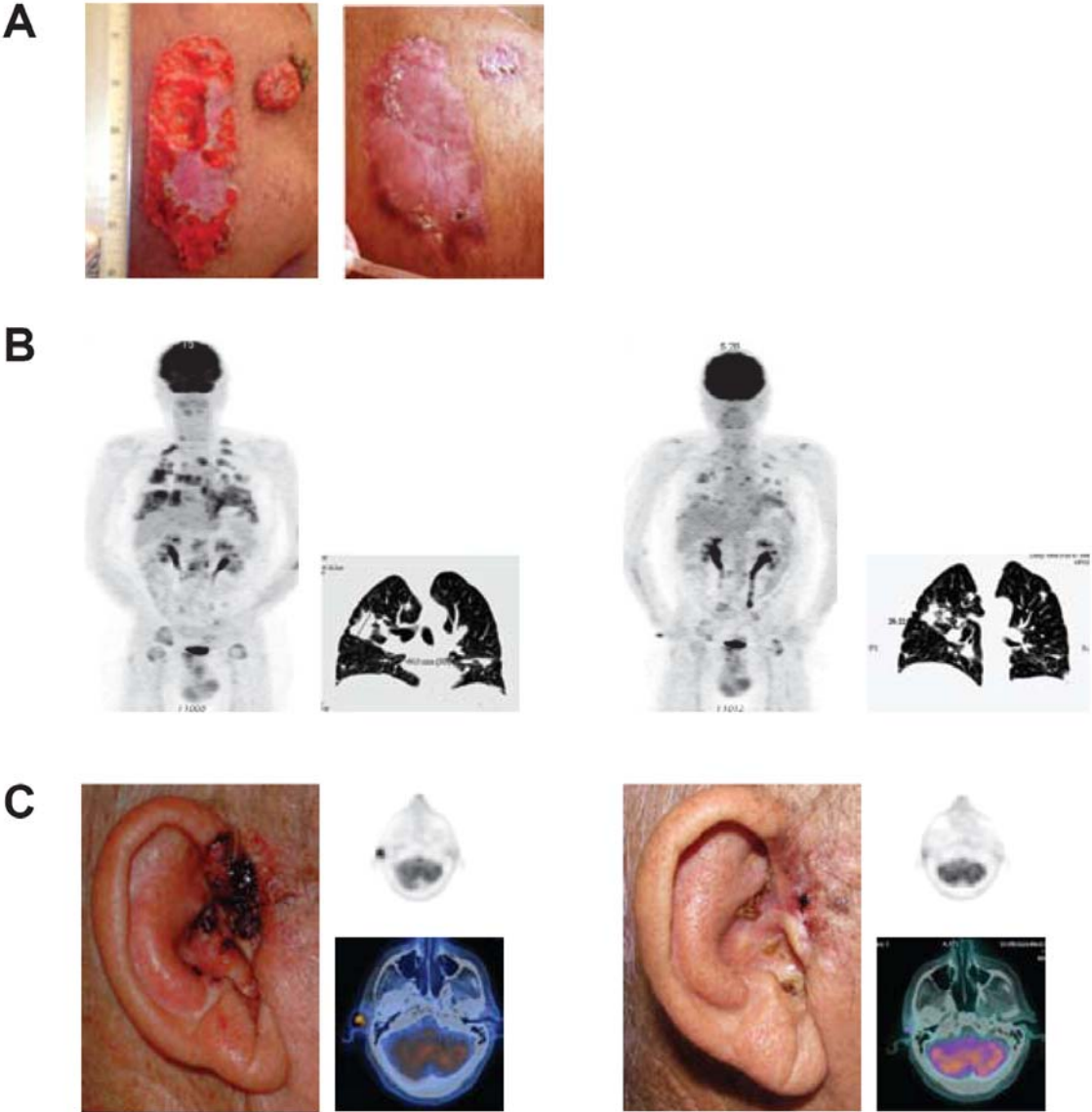
Supplementary Figure 1. GDC-0449 Activity in Other Patients with Advanced BCC

A. 49 year old male with locally advanced BCC shown with multiple large lesions on upper right back at baseline and at the onset of a clinical partial response after 3 months of GDC-0449 administration (Patient 7)

B. 67 year old male with BCC metastatic to lung, soft tissue and bone, with PET and CT (coronal view) scans at baseline and at 8 months, at time of confirmed radiologic partial response (Patient 2)

C. 83 year old male with BCC in the right external ear and invasion into the parotid gland, shown with PET/CT scans at baseline and at the onset of a clinical partial response after 2 months of GDC-0449 administration (Patient 11)

Supplementary Figure 1. GDC-0449 Activity in Other Patients with Advanced BCC



Supplementary Figure 2. Identification of a Homozygous, Intronic *PTCH1* Mutation in a Lung Biopsy from Patient 2

A. Nucleotide sequence tracing shows a homozygous intronic mutation (-1G>A) in a dinucleotide splice site consensus sequence of *PTCH1* in an archival lung tumor specimen obtained from Patient 2. This mutation maps to the splice site junction of an exon mapping to the 2nd extracellular loop of PTCH1.

B. MassARRAY assays of gDNA isolated from either whole tissue (top) or laser capture micro-dissected (bottom) tumor epithelium are shown. Note the absence of the wild-type G allele in the lower panel.

Supplementary Figure 2. Identification of a PTCH1 Homozygous Intronic Mutation in an Archival Patient Lung Tumor Specimen

