Supplemental Materials and Methods

Supplementary Table 1: Signal intensity of BCC tumor epithelium following human *GLI1* isotopic in situ hybridization analysis on patient-matched specimens

Tx. Group (Pt.#)	Superficial Pre-Tx Biopsy	Deep Pre-Tx Biopsy	Superficial Post-Tx Excision	Deep Post-Tx Excision
Placebo (Pt. 1)	2	2	0	0
CUR61414 (Pt. 2)	2	2	3	3
CUR61414 (Pt. 3)	2	2	3	3
CUR61414 (Pt. 4)	3	3	3	3

*The intensity of hybridization with anti-sense probes was scored in a blinded fashion on a scale of 0 (no labeling over background) to 3 (high hybridization signal) for both superficial (top) and deep (bottom) halves of specimens.
**Pt, patient. Tx, treatment.

Supplementary Table 2: Signal intensity of BCC tumor epithelium following human *PTCH1* isotopic in situ hybridization analysis on patient-matched specimens

Tx. Group (Pt.#)	Superficial Pre-Tx Biopsy	Deep Pre-Tx Biopsy	Superficial Post-Tx Excision	Deep Post-Tx Excision
Placebo (Pt. 1)	2	2	1	1
CUR61414 (Pt. 2)	3	3	3	3
CUR61414 (Pt. 3)	3	3	2	2
CUR61414 (Pt. 4)	3	3	3	3

*The intensity of hybridization with anti-sense probes was scored in a blinded fashion on a scale of 0 (no labeling over background) to 3 (high hybridization signal) for both superficial (top) and deep (bottom) halves of specimens.
**Pt, patient. Tx, treatment.

Supplementary Table 3: Percentage of BCC Epithelial Cells Staining with the Proliferation Marker Ki-67

Tx. Group (Pt.#)	Superficial Pre-Tx Biopsy	Deep Pre-Tx Biopsy	Superficial Post-Tx Excision	Deep Post-Tx Excision
Placebo (Pt. 1)	69.9	79.6	92.5	96.5
CUR61414 (Pt. 2)	57.7	60.4	88.9	90.2
CUR61414 (Pt. 3)	74.8	76.7	88	84.9
CUR61414 (Pt. 4)	55.6	44.4	73.6	55
CUR61414 (Ave.)	62.7	60.5	83.5	76.7
CUR61414 (SEM)	6.1	9.4	5	11

Note: Superficial = top half of biopsy tissue; deep = bottom half of biopsy tissue. Pt, patient. Tx, treatment. SEM, standard error of mean.

Supplementary Table 4: Percentage of BCC Epithelial Cell Tumor Area Stained with the Apoptosis Marker Cleaved Caspase-3

Tx. Group (Pt.#)	Superficial Pre-Tx Biopsy	Deep Pre-Tx Biopsy	Superficial Post-Tx Excision	Deep Post-Tx Excision
Placebo (Pt. 1)	0.021	0.017	0.012	0
CUR61414 (Pt. 2)	0.006	0.002	0.004	0.001
CUR61414 (Pt. 3)	0.081	0.101	0.074	0.0625
CUR61414 (Pt. 4)	0.034	0.044	0.02	0.012
CUR61414 (Ave.)	0.04	0.049	0.033	0.025
CUR61414 (SEM)	0.022	0.029	0.021	0.019

Note: Superficial = top half of biopsy tissue; deep = bottom half of biopsy tissue. Pt, patient. Tx, treatment. SEM, standard error of mean.

Human Studies

Inclusion Criteria:

• Adults at least 18 years old

• Women who were postmenopausal (defined as ≥ 12 months amenorrhea) or had had a hysterectomy. A negative serum pregnancy test was required for all women at screening for study entry.

• Single BCC or multiple BCCs, with only one target lesion to be treated.

• Primary (not recurrent or previously treated) target lesion

• Histopathologic confirmation of superficial or nodular BCC lesions. BCCs with both superficial and nodular components were considered nodular. Only patients with nodular BCCs were enrolled in the PD marker segment.

• BCC diameter of ≥ 5 mm and ≤ 20 mm (≥ 10 mm and ≤ 20 mm for the PD marker segment) prior to biopsy for histopathologic confirmation. The diameter of the BCC was defined as the longest length of the lesion.

• BCC in an easily accessible location, i.e., the limbs, trunk, neck, or head, including the face (but excluding BCC on or behind the ears, the scalp margin, and in the T-zone [defined as the central portion of the face, including the area within 1 cm above the eyebrows, 2 cm lateral and below the eyes and mouth, and 2 cm lateral to the nose])

Exclusion Criteria:

• History of cancer with exception of non-melanoma skin cancer

• Basal cell nevus syndrome (or Gorlin syndrome)

• Evidence of aggressive growth patterns, e.g., severe squamous metaplasia or an infiltrative or desmoplastic or micronodular growth pattern

• Sclerotic BCC

• Concomitant dermatologic disease in the target treatment site

• Previous radiation at target treatment site

• Use of any other investigational drug or therapy within the past month

• Allergy to any of the study drug ingredients

• Clinically significant or unstable or life-threatening medical condition(s)

Dose-Escalation Segment

The first 7 subjects were randomized to receive either 0.09% active drug or placebo (Dose Level 1) in a 6:1 ratio (see Figure 3a). Once preliminary safety data were assessed and all 7 subjects in the treatment group had completed the 4-week treatment period without having more than one dose-limiting toxicity (DLT) in that treatment group, the next 7 subjects were randomized to receive either 0.35% active drug or placebo (Dose Level 2) in a 6:1 ratio. Dose escalation continued for each subsequent dose level in the manner outlined above until the maximum dose

of 3.1% was reached. Treatment of the target BCC (Day 1 to Day 28) consisted of topical application of a continuous thin film of study drug over the entire surface of the target BCC and margins within an area (6.5 cm2) defined by a laminated dosing card. The dosing card was used to indicate the volume of study drug to be applied, and this volume was standard for every subject. Subjects applied one dose of study drug every 12 hours (± 1 hour) for 27.5 days, with the last dose of study drug applied the morning of Day 28, for a total of 55 doses. A separate transparent plastic template detailing at least four anatomical markers was also obtained on Day 1 and used to indicate the location of the BCC lesion for excision at the end of the observation period. Subjects were monitored for safety starting on Day 1, continuing through the observation and follow-up periods. Of particular interest were targeted local skin reactions (LSRs) that included erythema, edema, induration, scaling, vesicles, erosion, ulceration, and crusting; LSRs were assessed at all study visits after Day 1. The treatment area was assessed for clinical clearance and scarring by the evaluating physician prior to complete excision of the target treatment site at the end of the observation period (i.e., Day 42 + 3-day window). If LSRs interfered with the overall assessment of clinical clearance and scarring, the evaluating physician could delay excision of the treatment site until it could be safely excised. In addition, if more than 2 actively treated subjects within a dose group in the dose-escalation segment had LSRs at the end of the observation period, the observation period was to be extended to a 3-week period (instead of a 2-week period) for all subsequent subjects treated at that dose level. The excised tissue was to undergo histopathologic evaluation by the evaluating physician or a dermatopathologist who was blinded to treatment group assignment. The MTD was determined at the end of the dose-escalation segment of the study and was defined as the highest dose level at which fewer than 2 actively treated subjects experienced a DLT. During the dose-escalation segment, if 2 or more subjects receiving active treatment experienced a dose-limiting toxicity (DLT) within a particular dose level, dosing at that level would be stopped and no further dose escalation would occur. Any subject experiencing a DLT would be discontinued from treatment. Three DLTs, all moderate skin ulceration, were observed in the study, one in each segment of the trial. One, in a subject in Dose Level 3 (1.1%), led to treatment discontinuation; another in a subject in the MTD expansion segment (3.1%), occurred 2 weeks after last application of study drug, and a third, in a subject in Segment 3, occurred on Day 4, the last day of treatment. All had mild skin ulceration pre-treatment.

MTD Expansion Segment

Once the MTD was determined, an additional 14 subjects were randomized in a 6:1 ratio (CUR61414 to placebo) to receive the MTD of study drug topically applied twice daily for 27.5 days, with the last (55th) dose applied the morning of Day 28. At least 10 of the 14 subjects enrolled in the MTD expansion segment must have had a biopsy-confirmed nodular BCC.

Subjects were monitored for safety starting on Day 1, continuing through the observation and follow-up periods. The target treatment site was assessed for clinical clearance and scarring by the evaluating physician prior to complete excision of the target treatment site at the end of the observation period (i.e., Day 42 + 3-day window). In the event that LSRs interfered with the overall assessment at that time, the evaluating physician could delay excision of the treatment site until it could be safely excised. The excised tissue underwent histopathological evaluation. Subjects were monitored for safety over a subsequent 2-week period following excision of the treatment site.

PD Marker Segment

Once the MTD was determined at the end of the dose-escalation segment and the MTD expansion segment had commenced, enrollment began for the PD marker segment. A total of 8 subjects with a biopsy-confirmed nodular BCC were randomized in a 3:1 ratio (CUR61414 to placebo) to receive a single dose level of study drug topically applied twice daily at the MTD to the target BCC for 3.5 days, with the final (seventh) dose of study drug to be applied the morning of Day 4. In addition to histopathologic confirmation of the BCC during the screening period, the biopsy sample was also used to determine the pre-treatment level of *GLI1* expression. Treatment procedure was the same as described for the dose escalation segment except that subjects applied one dose of study drug every 12 hours (± 1 hour) for 3.5 days, with the final dose applied the morning of Day 4, for a total of seven doses. Subjects were monitored for safety starting on Day 1, continuing through the follow-up period (Day 5 to Day 18). The target treatment site was completely excised 5 hours (± 1 hour) after application of the last dose (Day 4) for histopathologic evaluation (performed by the evaluating physician or a dermatopathologist who was blinded to treatment assignment) and for evaluation of change in *GLI1* expression (performed by the Sponsor). Subjects were monitored for safety over a subsequent 2-week period following excision of the target treatment site. Subjects were also given the option to give informed consent, allowing their excised tissue samples to be used for additional research related to the understanding of BCC and/or how CUR61414 works.

To examine whether topical application of CUR61414 affected BCC epithelial cell proliferation or apoptosis, pre- and post-treatment skin specimens from these four patients were stained for the proliferation marker Ki-67 and the apoptotic marker cleaved caspase-3. In a blinded manner, digital images of all stained tissue were captured and analyzed using the Ariol SL50 (Applied Biosystems). More specifically, the superficial and deep halves of each tumor were assessed for the percentage of tumor epithelial cells staining (Ki-67) or the percentage of epithelial tumor area staining (cleaved caspase-3).

While the sample size of each dose level was chosen primarily to evaluate the safety and tolerability of CUR61414, expansion at the maximum tolerated dose allowed for evaluation of a minimum of 18 subjects to determine the 95% exact confidence interval (Blyth-Still-Casella) to exclude a 50% response rate if a minimum of 13 out of 18 subjects had a clinical response. In addition, with 18 evaluable subjects, a one-sided paired t-test would have more than 90% power for detecting a one standard deviation decrease in the change in *GLI1* levels with alpha less than 0.05.

Plasma sampling for PK

In the first two segments, an intensive plasma sampling design was utilized following the first dose on Day 1, Day 15, and Day 28 and sparse trough concentration sampling designs were used weekly during the 4-week treatment period. In Segment 3, where the treatment period was only 3.5 days, plasma samples were collected pre-dose on Day 1 and approximately 5 hours after the last dose prior to excision on Day 4. All samples were analyzed for CUR61414 by the LC/MS method.

Clinical and histopathologic clearance assessment

A clinical assessment of the lesion was made on Day 42 prior to excision of the target BCC. The choices for the clinical clearance assessment were: ”no visible lesion,“ “decrease in lesion size,“ “no change in lesion size,” or ”increase in lesion size.“ Histopathologic clearance was assessed by the evaluating physician or dermatopathologist. The histopathologic findings were classified as either ”no residual tumor found” or ”residual tumor found.” In the latter case, physicians were asked to further specify whether margins were clear or involved.