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ORIGINAL REPORT

Open-Label, Exploratory Phase II Trial of Oral Itraconazole for the Treatment of Basal Cell Carcinoma

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ABSTRACT

Purpose
Itraconazole, a US Food and Drug Administration–approved antifungal drug, inhibits the Hedgehog (HH) signaling pathway, a crucial driver of basal cell carcinoma (BCC) tumorigenesis, and reduces BCC growth in mice. We assessed the effect of itraconazole on the HH pathway and on tumor size in human BCC tumors.

Patients and Methods
Patients with ≥ one BCC tumor > 4 mm in diameter were enrolled onto two cohorts to receive oral itraconazole 200 mg twice per day for 1 month (cohort A) or 100 mg twice per day for an average of 2.3 months (cohort B). The primary end point was change in biomarkers: Ki67 tumor proliferation and HH activity (*GLI1* mRNA). Secondary end points included change in tumor size in a subset of patients with multiple tumors.

Results
A total of 29 patients were enrolled, of whom 19 were treated with itraconazole. Itraconazole treatment was associated with two adverse events (grade 2 fatigue and grade 4 congestive heart failure). Itraconazole reduced cell proliferation by 45% ($P = .04$), HH pathway activity by 65% ($P = .03$), and reduced tumor area by 24% (95% CI, 18.2% to 30.0%). Of eight patients with multiple nonbiopsied tumors, four achieved partial response, and four had stable disease. Tumors from untreated control patients and from those previously treated with vismodegib showed no significant changes in proliferation or tumor size.

Conclusion
Itraconazole has anti-BCC activity in humans. These results provide the basis for larger trials of longer duration to measure the clinical efficacy of itraconazole, especially relative to other HH pathway inhibitors.

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INTRODUCTION

Basal cell carcinoma (BCC) is the most commonly diagnosed human cancer, with approximately 2 million new cases in the United States every year.[1] Patients affected by the rare heritable basal cell nevus (Gorlin) syndrome (Online Mendelian Inheritance in Man No. 109400) may develop hundreds to thousands of BCCs.[2] Patients with basal cell nevus syndrome inherit one defective copy of the tumor suppressor gene *PTCH1*, which acts as a primary inhibitor of the Hedgehog (HH) signaling pathway.[3-5] *PTCH1* gene mutations and loss of the remaining wild-type allele also occur in sporadic BCC.[3-5] Essentially all BCCs have malignant activation of the HH signaling pathway, which is commonly measured by the expression of a target gene encoding the *GLI1* transcription factor.[2-4] The recent US Food and Drug Administration (FDA) approval of vismodegib, an antagonist of the essential HH pathway component Smoothened (SMO), validates the effectiveness of HH pathway inhibition in the treatment of BCC.[6] Vismodegib is approved for locally advanced (inoperable) or metastatic BCC, which comprises only 2% of all BCCs. A majority of BCCs can be treated by surgical excision, although surgery can lead to significant scarring and morbidity.[5] Given the rising incidence of BCC and its costly treatment—one of the highest among Medicare recipients[7]—there is a large unmet clinical need for nonsurgical forms of treatment.[8] Although some chemotherapeutic creams such as imiquimod and fluouracil are FDA approved, these topical options are effective only against the superficial subtype of

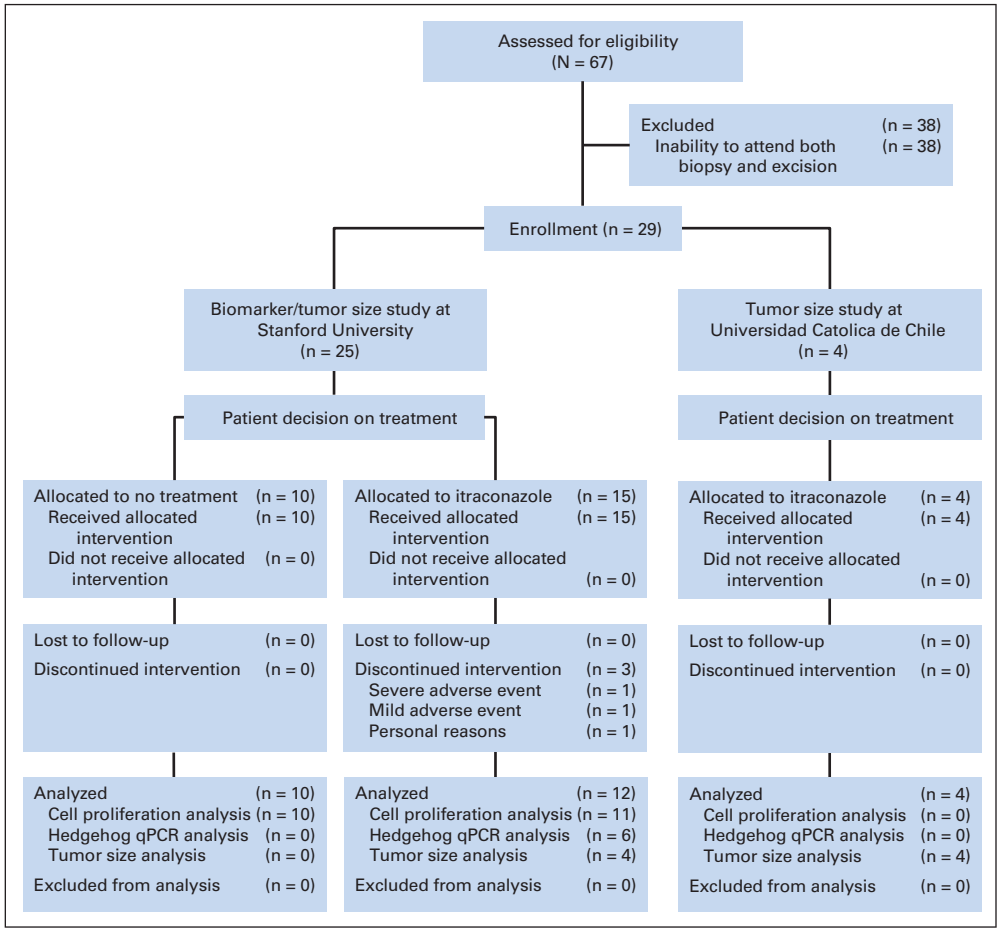

Fig 1. CONSORT diagram. qPCR, quantitative polymerase chain reaction.

BCC, which comprises only 30% of all BCCs.[9,10] The frequent adverse effects (hair loss, weight loss, muscle cramps) of vismodegib preclude it as a viable treatment option for a majority of nonadvanced BCCs.[6]

We performed a screen of FDA-approved drugs and identified itraconazole, a widely used oral antifungal agent, as a potent HH pathway antagonist.[11] Previously, we demonstrated that itraconazole suppresses autochthonous murine BCC carcinogenesis, induces tumor necrosis, and reduces *GLI1* mRNA expression in mice.[11] To assess the mechanism of action and clinical efficacy of itraconazole in human BCC, we performed an open-label, proof-of-concept phase II trial in two cohorts of patients with sporadic BCC. The primary goals of our two cohorts were to evaluate whether itraconazole-induced inhibition of HH pathway activity (*GLI1* mRNA) could reduce tumor proliferation (Ki67) and tumor size in human BCC and to evaluate whether significant reductions in tumor size could be achieved with lower doses of itraconazole administered over a longer duration.

PATIENTS AND METHODS

Patients and Treatments

We recruited patients with ≥ one BCC tumor > 4 mm (longest diameter), with no comorbidities and normal liver function tests within 1 year before enrollment. Those taking medications that might affect BCC tumors or the metabolism of itraconazole (eg, anticonvulsants, corticosteroids) and those unable to attend both initial biopsy and surgical excision at the same institution were excluded from the study. All patients had AST/ALT measured after 1 month of itraconazole.

We screened 67 patients for eligibility and enrolled 29 at two sites; 37 patients were excluded from enrollment because of their inability to have both biopsy and excision at the same site. Cohort A (Stanford University, Stanford, CA) enrolled 25 patients from April 2010 to December 2010, and cohort B (Universidad Católica de Chile, Santiago, Chile) enrolled four patients from July 2010 to September 2011 (CONSORT diagram; Fig 1). Only one patient met clinical diagnosis for basal cell nevus syndrome.

In cohort A, patients received itraconazole in 200-mg tablets orally twice per day between the time of biopsy and time of definitive excision (routinely 4 weeks). The primary goal of cohort A was to assess tumor biomarkers (Ki67 proliferation and HH pathway activity [*GLI1* mRNA]) and tumor size change after 1 month of treatment. In cohort B, patients received 100-mg tablets orally twice per day. The primary goal of cohort B was to determine whether a lower itraconazole dose administered over a longer duration (mean, 2.3 months; range, 1 to 4 months) could still achieve clinically significant effects. Patients otherwise eligible but unwilling to take itraconazole were enrolled onto the control arm of the study and received no treatment. The parallel studies were approved by the respective institutional review boards.

Three patients from cohort A were previously treated with vismodegib and experienced progression during therapy, likely as a result of secondary resistance to vismodegib.[12] Because itraconazole also antagonizes SMO, and little is known about how resistance to one drug may affect the efficacy of another SMO inhibitor, those previously treated with vismodegib were analyzed separately from vismodegib-naive patients.

Therefore, we ultimately analyzed three discrete groups: one, patients who received no itraconazole; two, patients who received itraconazole without prior vismodegib treatment; and three, patients who received itraconazole with prior vismodegib treatment.

Tumor Biopsies and Biomarker Evaluations (cohort A)

At the time of enrollment, patients in cohort A had target BCC lesions (one or two of the most representative tumors) selected for biopsy. Biopsied tumors were paraffin embedded and stained for Ki67 to assess cell proliferation. Cells with positive nuclear staining (indicating actively proliferating cells) were counted by Flagship Biosciences (Flagstaff, AZ) and normalized to the total number of nuclei present in the BCC. Cell proliferation data presented here are expressed as percentage of cells with a positive signal. To assess HH pathway activity, fresh biopsy samples were flash frozen, placed in Trizol (Invitrogen, Carlsbad, CA), and homogenized using MP FastPrep 24 (MP Biomedicals, Solon, OH). Total RNA was purified with phase Lock Gels tubes (Eppendorf, Hamburg, Germany) and an RNA PureLink Micro Kit (Invitrogen). cDNA was synthesized with SuperScript III Reverse Transcriptase (Invitrogen). Real-time polymerase chain reaction was performed on a Bio-Rad iCycler using iQ SyberGreen Supermix (Bio-Rad, Hercules, CA). Fold change in *GLI1* mRNA expression was measured using $\Delta\Delta C_t$ analysis, with HPRT1 as the internal control gene and normal skin collected from patients with BCC as control samples for normalization of the BCC samples. Polymerase chain reaction primer sequences were:

- *GLI1* forward: 5′ GAAGTCATACTCACGCCTCGAA 3′
- *GLI1* reverse: 5′ CAGCCAGGGAGCTTACATACAT 3′
- *HPRT1* forward: 5′ GGTCAGGCAGTATAATCCAAAG 3′
- *HPRT1* reverse: 5′GGACTCCAGATGTTTCCAAAC 3′

In cases where the biopsy/excision had limited tumor volume, samples were prioritized for cell-proliferation analysis. No tumors were biopsied for biomarker analysis in cohort B, because its primary goal was to evaluate the effect of low-dose itraconazole on tumor size.

Tumor Size Measurements

In cohort A, 11 of 15 patients had only one BCC tumor. This target lesion was biopsied and excised for biomarker analysis. Target lesions were not assessed for tumor size change, because they were biopsied at baseline. Only four of 15 patients had ≥ one BCC tumor, and nontarget lesions (n = 42) were observed for tumor size change because these were not biopsied at baseline. Tumor area was assessed with calipers measuring longest perpendicular diameters before and after 1 month of itraconazole 200 mg twice per day. In contrast, all tumors from the four patients (n = 14 BCCs) in cohort B were assessed for tumor size change using calipers before and after treatment with itraconazole 200 mg per day (average duration, 2.3 months). Percent change in tumor area from both cohorts (eight patients total with 57 tumors) was calculated from date of enrollment to date of last clinical assessment.

For each patient measured for tumor area, validated BCC clinical response parameters were used per Skvara et al[13] and defined as follows: complete response, no longer any visible evidence of a lesion consistent with BCC; partial response, although a BCC still remains, there is a visible decrease in size; and no response, the BCC has not visibly decreased in size. A dermatologist investigator who was blinded to treatment groups performed all clinical evaluations using photographs taken before and after treatment. We did not use RECIST criteria for solid tumors, because a majority of BCC lesions were < 10 mm in size, and tumor lesions were primarily assessed for biomarker changes.

Statistical Analysis

As an exploratory trial, we calculated that a minimum of 27 patients needed to be enrolled to detect a two-fold difference between the change in Ki67 in patients not treated versus treated with itraconazole (β, 0.80; two-sided α, 0.05). Nonparametric Mann-Whitney rank sum test was used for unpaired comparisons, and Wilcoxon signed rank test was used for paired comparisons. All *P* values reported are two sided.

RESULTS

Patient Demographics

We enrolled 29 patients with a total of 101 BCC tumors; 19 patients with a total of 90 BCCs consented to treatment with itraconazole (cohort A, n = 15; cohort B, n = 4). At baseline, itraconazole-treated patients and control patients were similar in age, sex, BCC type, and location of BCC tumors (Table 1). Tumors appeared on classically sun-exposed regions of the body, including the face, neck, and trunk. Patients were predominantly older men (mean age, 60 years) with nodular BCC subtypes. Patients receiving itraconazole had, on average, almost 5× as many tumors at baseline compared with those not treated with itraconazole (P = .13). Average duration of observation/treatment was approximately 1 month among control patients and itraconazole-treated patients in cohort A (n = 76 tumors) and 2.3 months among itraconazole-treated patients in cohort B (n = 14 tumors). Three patients in cohort A were previously treated with vismodegib and experienced disease progression and had stopped vismodegib for ≥ 6 months before starting itraconazole.

Drug-Related Adverse Events

Two of 19 patients discontinued itraconazole because of previously described adverse effects associated with itraconazole. One grade

Table 1. Patient Demographic and Clinical Characteristics by Treatment Group (N = 29)

Characteristic	Control (n = 10) No.	Control (n = 10) %	Itraconazole (n = 19) No.	Itraconazole (n = 19) %
Age at initial biopsy, years				
Average	68.5		62.2	
Range	53 to 81		44 to 85	
Sex				
Male	8	80	15	79
Female	2	20	4	21
Total No. of BCC tumors present	11		90	
Average No. of BCC tumors per patient	1.1		4.77	
BCC tumor type*				
Nodular	6	43	21	50
Superficial	4	29	14	33
Infiltrative	2	14	5	12
Micronodular	2	14	2	5
BCC tumor location				
Face	6	55	35	39
Back	2	18	17	19
Shoulder	0	0	13	14
Neck	0	0	8	9
Chest	0	0	6	7
Leg	1	9	6	7
Arm	2	18	2	2
Scalp	0	0	2	2
Ear	0	0	1	3
History of vismodegib treatment	0	0	3	
Average length of treatment, months				
Stanford patients (200 mg twice per day)	1.1		1.0	
Chile patients (100 mg twice per day)	NA		2.3	

Abbreviations: BCC, basal cell carcinoma; NA, not applicable.
*Includes only tumors that were biopsied.

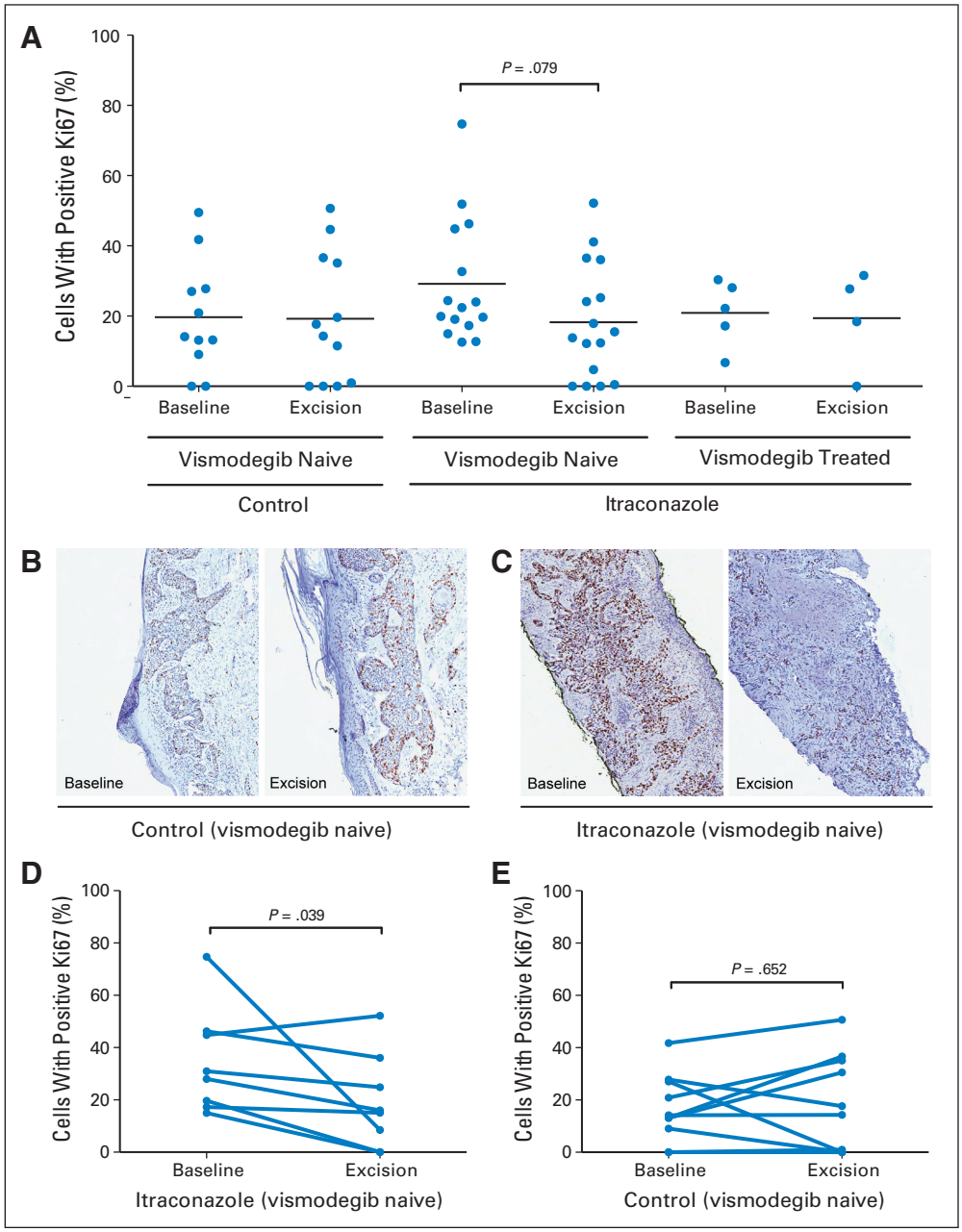

Fig 2. Ki67 quantification and analysis of basal cell carcinoma (BCC) tumors. (A) Unpaired analysis of individual tumors from control patients and itraconazole-treated patients. Ki67 staining of BCC tumors at baseline and excision from (B) control patients and (C) vismodegib-naive patients treated with itraconazole. Positive (brown) staining indicates proliferating nuclei; negative (blue) staining indicates no proliferative activity. Paired analysis of patient Ki67 averages among (D) vismodegib-naive patients and (E) control patients treated with itraconazole.

4 congestive heart failure occurred in a patient who had undiagnosed heart disease from prior adriamycin treatment for Hodgkin lymphoma 20 years ago (Fig 1). A second patient discontinued because of grade 2 fatigue. Other adverse effects were mild, and all adverse effects were reversible on drug discontinuation. No elevations in liver function testing (AST/ALT) were detected in any patient during treatment.

Biomarker Analysis (cohort A only)

A total of 63 biopsies from 22 of 25 enrolled patients in cohort A were available for biomarker analysis (Fig 1). We compared the change in Ki67 proliferation in biopsies taken at baseline and at excision 1 month later in itraconazole-treated patientss (20 BCCs) and untreated/control patients (12 BCCs). Itraconazole decreased cell proliferation by 35% in vismodegib-naive patients in unpaired analysis ($P = .079$; Figs 2A through 2C). Within patient comparisons of Ki67 activity from baseline to excision, itraconazole decreased cell proliferation by 45% in vismodegib-naive patients (n = 8; pairwise $P = .04$; Fig 2D). Control patients and those previously treated with vismodegib, by contrast, showed no significant changes in cell proliferation (Fig 2E).

For analysis of HH signaling pathway, a total of 24 biopsies were collected from itraconazole-treated patients and analyzed for *GLI1* mRNA levels. In an unpaired analysis, itraconazole decreased *GLI1* mRNA by 65% in tumors from vismodegib-naive patients compared with baseline ($P = .028$; Fig 3A). Examining only paired tumors from four patients (vismodegib naive), itraconazole decreased *GLI1* mRNA by 45% in tumors after 1 month compared with baseline (Fig 3B). Tumors from patients with prior vismodegib treatment did not

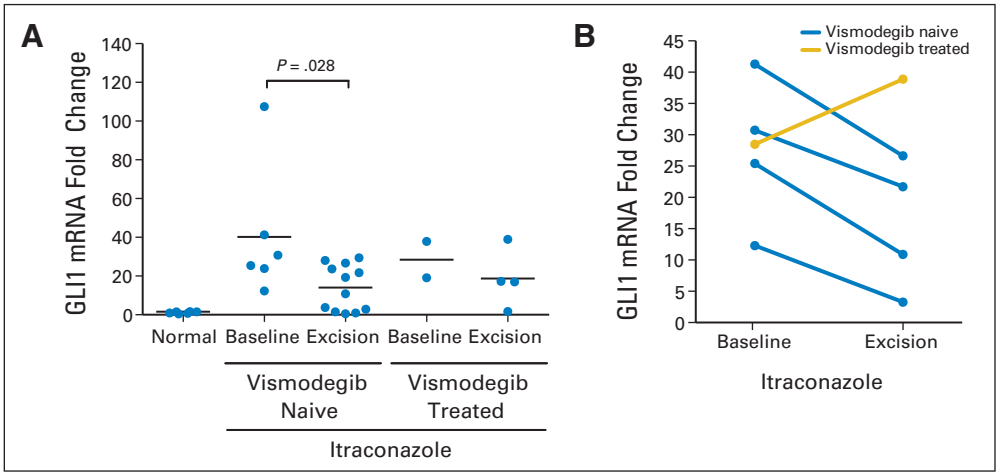

Fig 3. Levels of GLI1 mRNA expression in basal cell carcinoma tumors from patients treated with itraconazole for 1 month. Some patients had previously received treatment with vismodegib and were no longer responsive to vismodegib. (A) Unpaired analysis of GLI1 mRNA levels from tumors at baseline and excised tumors after itraconazole treatment. As control, GLI1 mRNA was assessed in normal nontumor skin. GLI1 mRNA decreased by 65% in vismodegib-naive patients treated with itraconazole from baseline to excision (P = .028). (B) In paired analysis of tumors from same patients, itraconazole decreased GLI1 expression by 45% after 1 month in vismodegib-naive patients only.

demonstrate a significant decrease in *GLI1* mRNA expression. Other BCC clinical trials have also demonstrated no change in *GLI1* mRNA expression in placebo-treated or control patients.[14,15]

Tumor Size Changes and Clinical Response (cohorts A and B)

Itraconazole reduced tumor size and promoted re-epithelialization in BCC tumors of eight of 29 vismodegib-naive patients (Fig 4). In cohort A, four patients had > one BCC tumor, and these nontarget lesions were observed for tumor size change, because these were not biopsied at baseline (n = 43). Tumor area was assessed with calipers measuring longest perpendicular diameters before and after 1 month of itraconazole 200 mg twice per day. In the four patients in cohort B, all 14 BCC tumors were assessed for tumor size change using calipers before and after treatment with itraconazole 100 mg twice per day (average duration, 2.3 months). None of cohort B tumors were biopsied for biomarker analysis, because the goal of cohort B was to assess a lower dose of itraconazole for a longer duration. Percent change in tumor area from both cohorts (eight patients total with 57 tumors) is shown in Figure 5. On average, tumors from vismodegib-naive patients decreased by 24% (95% CI, 18.2% to 30.0%) after treatment with itraconazole (Fig 5). Furthermore, average tumor reductions in patients from cohort B were comparable to those of cohort A (P = .435). This finding suggests that the alternate administration of itraconazole (lower doses over longer duration) can still achieve clinically significant reductions.

Despite the general reduction in size, none of the BCC tumors disappeared completely with itraconazole treatment after an average of 1.1 months (cohort A, n = 43 tumors) and 2.3 months (cohort B, n = 14 tumors). Four patients experienced partial response, and four had stable disease using previously described guidelines.[16] In clinical assessments, neither control patients nor patients with a history of vismodegib treatment showed any changes in tumor size.

DISCUSSION

In this proof-of-concept study, we have demonstrated the first off-label use, to our knowledge, of an FDA-approved drug for BCC treatment that targets HH pathway activity. Itraconazole can reduce BCC tumor size via inhibition of the HH signaling pathway after 1 month of treatment. These results provide the basis for larger trials of longer duration to measure the clinical efficacy of itraconazole.

As an exploratory phase II trial, the primary end point of this study was to determine whether itraconazole at commonly prescribed doses of 200 to 400 mg daily could reduce tumor proliferation and HH pathway in human BCCs after 1 month. Only four patients in cohort B were treated for > 1 month, and thus, we do not have results on the clinical anti-BCC efficacy of itraconazole over a longer treatment period. Furthermore, we cannot directly compare the short-term efficacy of itraconazole with that of vismodegib, the first FDA-approved HH inhibitor that showed a 40% response rate in patients with locally advanced BCC treated for > 10 months.[6] Itraconazole seems to have less clinical utility compared with vismodegib as first-line treatment, because the half maximal inhibitory concentration of itraconazole is 100× less potent in vitro compared with vismodegib, and itraconazole reduces HH pathway by 65% after 1 month in contrast to a 90% reduction by vismodegib.[15] Itraconazole may be effective as second-line therapy, because itraconazole acts on SMO at a site distinct from cyclopamine and vismodegib.[11] As demonstrated by in vitro signaling assays,[17] the efficacy of itraconazole is dose dependent, and future studies should examine whether higher doses of itraconazole administered over longer treatment periods can approach the efficacy seen with vismodegib and other SMO antagonists. However, chronic administration of itraconazole could also have additional adverse effects and toxicity not seen in this exploratory trial, although long-term treatment with 600 to 900 mg per day ranging from 3 to 16 months with manageable toxicities have been reported.[18,19]

Our study has several limitations. First, it was limited by sample size, because we enrolled only 29 patients; however, many patients had multiple tumors, and this study evaluated 101 BCCs. Second, all but one of the patients in this study had nonadvanced BCC, and none had metastatic disease; thus, our results cannot be compared with the 40% overall response rate seen with vismodegib in patients with advanced or metastatic BCC.[6] Third, we only had three patients who experienced progression with vismodegib and did not perform sequencing analyses to determine the mechanism of vismodegib drug resistance. Thus, we could not directly test whether itraconazole could be a viable option as second-line treatment after failure of vismodegib. Fourth,

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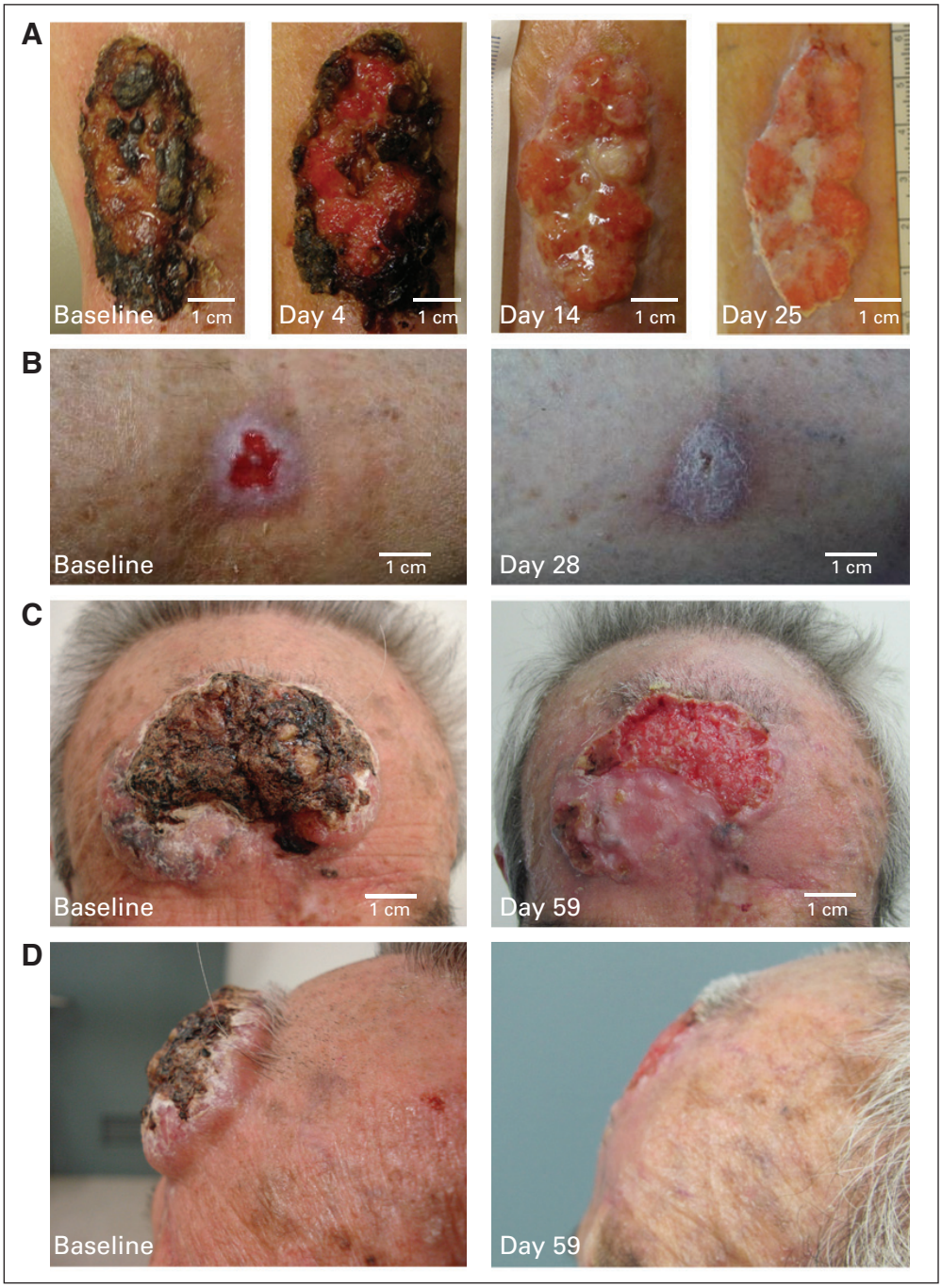

Fig 4. Reduction or re-epithelialization of basal cell carcinoma (BCC) tumors by itraconazole in vismodegib-naive patients. (A) Nodular BCC tumor on leg from enrollment to day 25. (B) Infiltrative BCC tumor on shoulder from enrollment to day 28. (C) Frontal and (D) side view of nodular BCC tumor on forehead from enrollment to day 59.

we only assessed the short-term efficacy of itraconazole, and chronic administration is needed to compare the efficacy and toxicity of itraconazole relative to vismodegib. Future randomized controlled trials are needed to determine the efficacy of itraconazole in a broader range of patients with varying burdens of disease, a potential differential efficacy in vismodegib-resistant versus vismdoegib-naive tumors, and the possibility of whether a double anti-SMO attack would yield superior results. Our preclinical results suggest that a double anti-SMO inhibition reduces tumor growth more effectively than itraconazole or cyclopamine alone.[11]

Recently, combinatorial therapy with arsenic trioxide (ATO), FDA approved for acute promyelocytic leukemia, has been shown to be efficacious in murine BCC and vismodegib-resistant medulloblastoma.[20] ATO acts as an HH antagonist by preventing ciliary trafficking and destabilizing GLI2, a transcription factor downstream of SMO.[17] Combined treatment with itraconazole and ATO might prove

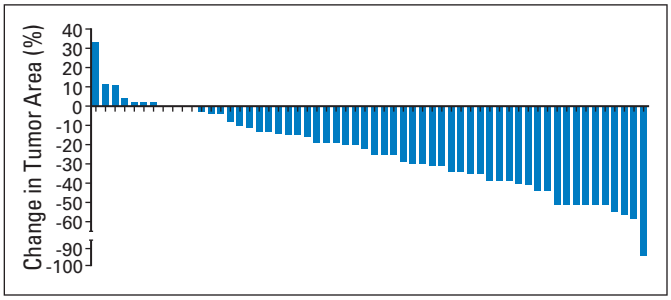

Fig 5. Percentage change in tumor area for 57 basal cell carcinoma (BCC) tumors from eight patients treated with itraconazole. During itraconazole treatment, tumor area decreased by average of 24% (95% CI, 18.2% to 30.0%). In cohort A, four patients had multiple BCC tumors, and nontarget lesions were available for tumor size measurement. Tumor area was approximated by multiplying longest perpendicular diameters of tumors, measured by calipers. Nonbiopsied tumors were assessed for tumor area change at baseline and after 4 weeks. In cohort B, BCC tumors were not assessed for biomarkers; they were assessed only for clinical size change at baseline (after initial biopsy confirming BCC diagnosis) and after treatment with itraconazole 200 mg for average of 2.3 months. Percent change in area was calculated from date of enrollment to date of last clinical assessment.

particularly beneficial in BCC tumors resistant[21] to SMO antagonists, such as vismodegib, that mimic and compete for binding with cyclopamine.[20] Assuming vismodegib-resistant BCCs behave similarly to medulloblastoma,[22,23] combined itraconazole-ATO therapy may overcome resistance resulting from *SMO* mutations and *GLI2* amplifications, because itraconazole inhibits SMO through a mechanism distinct from cyclopamine,[11] and ATO inhibits the *GLI* transcription factors. To this end, we have initiated a trial of combination itraconazole and ATO in patients with metastatic BCCs for whom vismodegib has failed (clinicaltrials.gov NCT01791894).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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