Brief Report

Effects of Combined Treatment With Arsenic Trioxide and Itraconazole in Patients With Refractory Metastatic Basal Cell Carcinoma

Mina S. Ally, MD; Katherine Ransohoff, BA; Kavita Sarin, MD, PhD; Scott X. Atwood, PhD; Melika Rezaee, BA; Irene Bailey-Healy, MLS; Jynho Kim, DVM, PhD; Philip A. Beachy, PhD; Anne Lynn S. Chang, MD; Anthony Oro, MD, PhD; Jean Y. Tang, MD, PhD; A. Dimitrios Colevas, MD

IMPORTANCE Tumor resistance is an emerging problem for Smoothened (SMO) inhibitor–treated metastatic basal cell carcinoma (BCC). Arsenic trioxide and itraconazole antagonize the hedgehog (HH) pathway at sites distinct from those treated by SMO inhibitors.

OBJECTIVE To determine whether administration of intravenous arsenic trioxide and oral itraconazole in patients with metastatic BCC is associated with a reduction in *GLI1* messenger RNA expression in tumor and/or normal skin biopsy samples.

DESIGN, SETTING, AND PARTICIPANTS Five men with metastatic BCC who experienced relapse after SMO inhibitor treatment underwent intravenous arsenic trioxide treatment for 5 days, every 28 days, and oral itraconazole treatment on days 6 to 28. Data were collected from April 10 to November 14, 2013. Follow-up was completed on October 3, 2015, and data were analyzed from June 5 to October 6, 2015.

MAIN OUTCOMES AND MEASURES The primary outcome was the change in messenger RNA levels of the GLI family zinc finger 1 (*GLI1*) gene (HH-pathway target gene) in biopsy specimens of normal skin or BCC before and after treatment. Secondary objectives were evaluation of tumor response and tolerability.

RESULTS Of the 5 patients (mean [SD] age, 52 [9] years; age range, 43-62 years), 3 completed 3 cycles of treatment and 2 discontinued treatment early owing to disease progression or adverse events. Adverse effects included grade 2 transaminitis and grade 4 leukopenia with a grade 3 infection. Overall, arsenic trioxide and itraconazole reduced *GLI1* messenger RNA levels by 75% from baseline ($P < .001$). The best overall response after 3 treatment cycles was stable disease in 3 patients.

CONCLUSIONS AND RELEVANCE Targeting the HH pathway with sequential arsenic trioxide and itraconazole treatment is a feasible treatment for metastatic BCC. Although some patients experienced stable disease for 3 months, none had tumor shrinkage, which may be owing to transient *GLI1* suppression with sequential dosing. Continuous dosing may be required to fully inhibit the HH pathway and achieve clinical response.

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Author Affiliations: Department of Dermatology, Stanford University, Stanford, California (Ally, Ransohoff, Sarin, Rezaee, Bailey-Healy, Chang, Oro, Tang); Department of Dermatology, School of Medicine, Stanford University, Stanford, California (Ransohoff, Atwood, Kim, Beachy, Oro); Cancer Center, Stanford University, Stanford, California (Colevas).

Corresponding Author: Jean Y. Tang, MD, PhD, Department of Dermatology, Stanford University, 450 Broadway St, Redwood City, CA 94063 (tangy@stanford.edu).

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Most cutaneous basal cell carcinomas (BCCs) are successfully treated by surgical resection. However, locally advanced (unresectable) or metastatic BCC (mBCC) have a poor prognosis, with a mean survival ranging from 8 months to 3.6 years.[1] Basal cell carcinomas require the hedgehog (HH) pathway for growth. Hedgehog pathway inhibitors, such as vismodegib[2] and sonidegib phosphate, target the G-protein–coupled receptor Smoothened (SMO) and are recommended as first-line treatment for advanced BCC or mBCC by the National Comprehensive Cancer Network.[3]

The emergence of resistance has limited vismodegib's efficacy[2,4] and led the search for therapeutics downstream from SMO. Investigators[5] found that 50% of SMO inhibitor-resistant BCCs had SMO mutations with disruption of ligand responsiveness or release of autoinhibition. Itraconazole, a widely used oral antifungal agent, is a potent HH pathway antagonist that suppresses BCC carcinogenesis and reduces messenger RNA (mRNA) expression of the GLI family zinc finger 1 (*GLI1* [OMIM 165220]) gene in murine BCCs[6] and in patients with nonadvanced cutaneous BCCs.[7]

Arsenic trioxide, which is approved by the US Food and Drug Administration for treatment of acute promyelocytic leukemia, inhibits the HH pathway downstream from SMO by preventing ciliary trafficking and destabilizing *GLI2*. Arsenic trioxide can bypass acquired mutations in SMO found in resistant tumors.[8] Combined treatment with arsenic trioxide and itraconazole (ATO-ITRA) has an additive HH inhibitory effect shown to be effective in murine BCCs.[9]

To assess the HH inhibitory effect and clinical efficacy of ATO-ITRA in human BCC, we performed an open-label proof-of-concept, phase 2 biomarker trial in patients with mBCC whose tumors were clinically resistant to SMO inhibitors.[10] The primary goal of our study was to determine whether administration of intravenous (IV) arsenic trioxide and oral itraconazole in patients with mBCC is associated with a reduction in *GLI1* mRNA expression in tumor and/or normal skin biopsy samples. Secondary end points included the evaluation of tumor response, safety profile, and presence of HH pathway mutations in SMO inhibitor–resistant BCCs before treatment.

Methods

Patients and Treatments

This study was conducted from April 10 to November 14, 2013. We enrolled and treated patients with biopsy-confirmed mBCC that progressed after treatment with SMO inhibitors (**Table 1**). The Stanford University institutional review board reviewed and approved the study protocol. All patients were required to have results of laboratory evaluations (liver function tests and levels of potassium, magnesium, calcium, and creatinine) within reference ranges and an electrocardiogram with a QTc of less than 450 milliseconds at baseline, a tumor evaluable by RECIST (Response Evaluation Criteria in Solid Tumors) criteria, version 1.1[11] (computed tomography was performed before and after treatment), and eligibility for pretreatment and posttreatment biopsy of mBCC or normal skin. Patients with major comorbidities, Eastern Cooperative Oncology Group performance status[12] greater than 2, and cardiac arrhythmias were excluded. All patients provided written informed consent.

Based on prior dosing schedules in solid tumors,[13] patients were treated with IV arsenic trioxide, 0.3 mg/kg daily, for 5 days every 28 days for a total of 3 cycles or until disease progression or unacceptable toxic effects occurred. Oral itraconazole, 400 mg/d, was given between arsenic trioxide infusion days. One treatment cycle was defined as complete IV arsenic trioxide treatment on days 1 to 5 and oral itraconazole treatment on days 6 to 28 (**Figure**, A). We did not administer the drugs at the same time to limit adverse events, which were graded according to the Common Terminology for Adverse Events (version 3.0; http://ctep.cancer.gov/). Patients were followed up for no more than 2 years after study completion, with follow-up completed on October 3, 2015.

Table 1. Patient Demographics and Clinical Characteristics

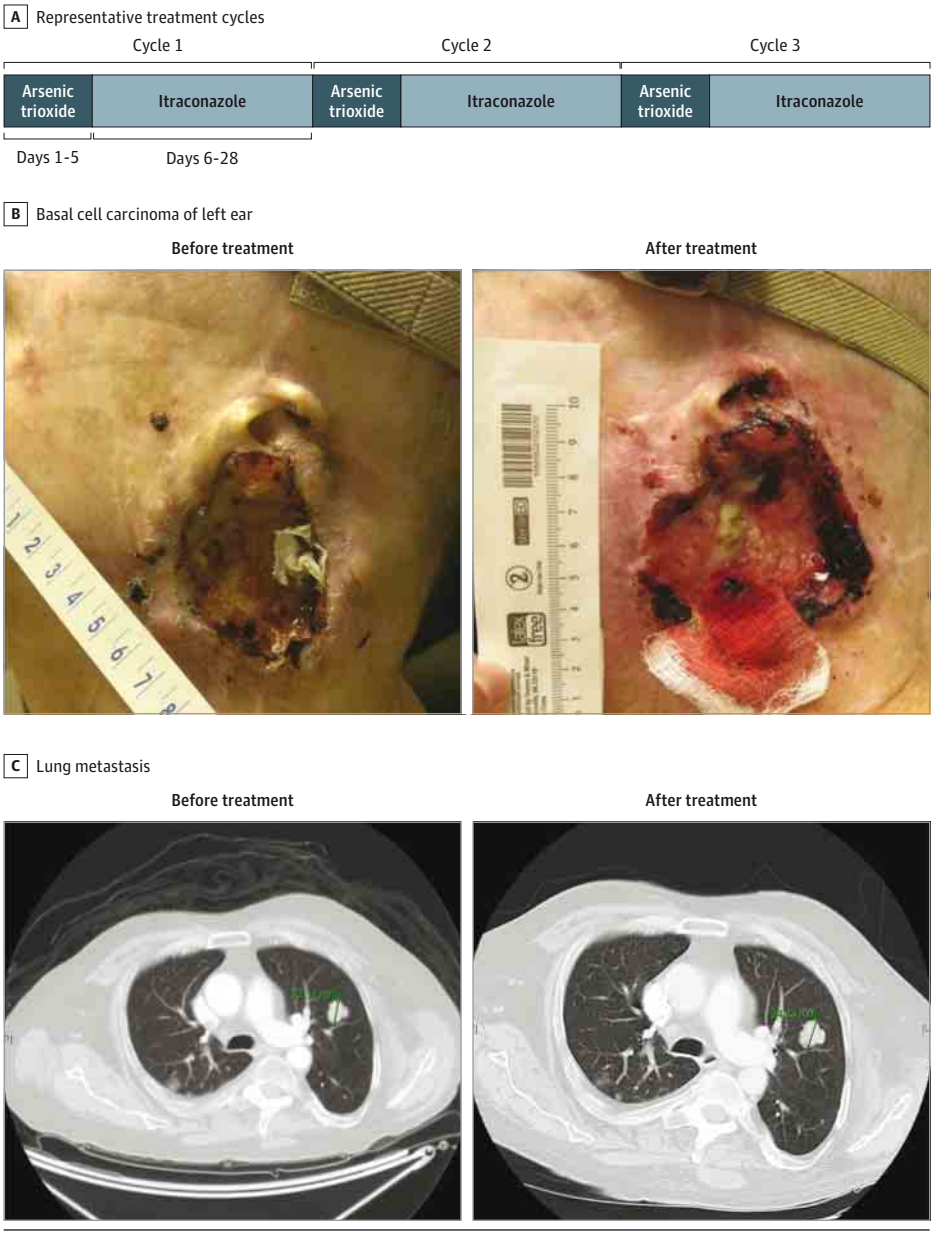
Patient No./ Sex/Age, Decade	Gorlin Syndrome Present	No. of Cutaneous BCCs at Baseline	Site of Metastasis	Prior Therapy (Duration, mo)	Interval From Prior to Study Therapy, mo	No. of Treatment Cycles Completed During Trial[a]
1/M/50s	No	0	Bone marrow and lymph nodes	Vismodegib (10) Sonidegib phosphate (3)	1	3
2/M/40s	Yes *PTCH1* mutation p.P1164L	2	Lungs and lymph nodes	Vismodegib (42) LEQ506 (12)	1	3
3/M/50s	Yes *PTCH1* mutation p.P662Q	13	Lung	Radiotherapy Vismodegib (18) IPI926 (16) Sonidegib (3)	1	3
4/M/60s	No	4	Pleura and lymph nodes	Radiotherapy Vismodegib (8)	0.5	2
5/M/40s	No[b]	11	Lung	Vismodegib (36) Sonidegib phosphate (14)	1	1

Abbreviations: BCCs, basal cell carcinomas; *PTCH1*, *Drosophila* patched gene.

[a] Patient 1 had 3 additional treatment cycles and patient 2 had 1 additional treatment cycle from their primary oncologist after completion of 3 cycles during the study. Patients with responding or stable disease and toxic effects of less than grade 2 could be treated with additional courses of therapy after completion of 3 cycles.

[b] The patient had a chromosome Y translocation to chromosome 7, resulting in high levels of sonic hedgehog protein and predisposing to multiple BCCs.[14]

Figure. Effect of Combined Arsenic Trioxide and Itraconazole Treatment



Photographs demonstrate a basal cell carcinoma of the left ear before and after 3 cycles of arsenic trioxide–itraconazole treatment, which shows no clinical improvement. Scales are in centimeters. Computed tomography of lung metastasis before and after treatment also shows no reduction in size.

Tumor Biopsy and Biomarker Evaluations

Target BCC lesions underwent biopsy at enrollment and study end. Biopsy specimens were stained with hematoxylin-eosin to confirm the presence of BCC. To assess HH pathway activity, fresh biopsy samples were stored in RNA stabilization reagent (RNAlater; Qiagen) (described in the eMethods in the Supplement).

Statistical Analysis

Data were analyzed from June 5 to October 6, 2015. This pilot study enrolled 5 patients to estimate the variance and effect size of ATO-ITRA treatment on the HH pathway and tumor size. Based on 2 prior clinical trials (with itraconazole[7] and vismodegib[2]), we hypothesized that ATO-ITRA would need to decrease *GLI* mRNA levels by 50% to have a clinically meaningful anti-BCC effect. With our sample size, we can detect a 54% (SD, 30%) decrease in *GLI* mRNA levels ($\beta = 0.80$; 2-sided $\alpha = .05$), assuming no change in *GLI* mRNA levels in untreated samples (effect size = 1.0, Wilcoxon signed rank test).

Results

Patient Demographics

We enrolled 5 men with a mean (SD) age of 52 (9) years. Patients experienced disease progression after a mean (SD) of 32 (21) months of treatment with SMO inhibitors and an initial partial response (Table 1). Three of 5 patients completed all 3 treat-

Table 2. Response to Study Treatment by Cycle 3

Patient No.	Site of mBCC Target Lesion	Mutations From Sequencing of Resistant Tumor		Change, %		Best Overall Response	Treatment After Disease Progression	Current Status (Duration of Follow-up, mo)
		SMO Gene	*PTCH1* Gene	*GLI1* mRNA Level	Sum of Longest Diameters of Measurable Target Lesions			
1[a,b]	Bone[a] and lymph nodes	*SMO* p.R168H	*PTCH1* p.W900X	−65 (skin)	4	SD	Palliative radiotherapy	AWD (11)
2[a,c,d]	Lymph nodes and lung	None	None	−75 (skin)	10	SD	Carboplatin	Deceased (19)
3[a,d]	Lung	*SMO* D473G	None	−90 (BCC)	11	SD	Carboplatin	AWD (8)
4[a,d,e]	Lymph nodes and pleura	None	*PTCH1* p.K80E	−90 (BCC)	13	PD	Cisplatin and paclitaxel	AWD (19)
5[f]	Lung	SMO p.G529C, p.P739S, p.Q123H	p.G1239E p.Q1149X p.F17L	−75 (BCC)	NE	NE	LY2940680[g]	AWD (6)

Abbreviations: AWD, alive with disease; mBCC, metastatic basal cell carcinoma; mRNA, messenger RNA; NE, not evaluable; PD, progressive disease; *PTCH1*, *Drosophila* patched gene; SD, stable disease; SMO, Smoothened.

[a] Indicates disease was evaluable by RECIST (Response Evaluation Criteria in Solid Tumors, version 1.1) criteria.

[b] The patient had stable disease after cycle 3 and developed progressive disease after cycle 6 owing to the development of a new tumor in his cervical spine; he continued off-study treatment with his primary oncologist.

[c] Underwent only posttreatment imaging at the end of cycle 4 (not available for imaging after cycle 3) after an additional treatment cycle off-study with his primary oncologist, which showed stable disease. After cycle 4, a nonmalignant grade 2 pleural effusion was found and treatment was discontinued.

[d] Had at least 2 measurable target lesions.

[e] Results refer to imaging after cycle 2; the patient discontinued treatment at this point because a new pleural lesion was noted.

[f] Disease was unevaluable owing to a lack of timely posttreatment imaging secondary to an adverse event.

[g] The patient enrolled in a phase 1 clinical trial for a new SMO inhibitor.

ment cycles. The remaining 2 patients discontinued the trial early because of disease progression and an arsenic trioxide-attributed adverse event.[14]

Drug-Related Adverse Events

Combination therapy with ATO-ITRA was associated with documented grade 1 and 2 adverse events,[13] including leukopenia, increased serum urea nitrogen and creatinine levels, transaminitis, and dyspnea. Patient 3 had grade 4 leukopenia and a grade 3 infection after the cycle 3 arsenic trioxide infusion. He recovered after hospital admission and IV antibiotic treatment. Patient 5 discontinued treatment after the first cycle owing to grade 1 asymptomatic atrial flutter. He underwent repeated electrocardiography during a 1-week period, and the arrhythmia spontaneously resolved.

Biomarker Analysis

All patients underwent pretreatment and posttreatment biopsy. Posttreatment biopsy was performed after 1 week of IV arsenic trioxide treatment during the second cycle. Paired biopsy specimens were taken from cutaneous BCCs in 3 patients and normal skin in 2 patients owing to a lack of mBCCs available for biopsy. In a paired analysis, ATO-ITRA reduced *GLI1* mRNA expression by 75% from baseline ($P < .001$) (**Table 2**).

Tumor Response

The best overall response after 3 treatment cycles for the 4 patients with evaluable data was stable disease in 3 patients and progressive disease in 1 patient (Table 2 and Figure). Four patients survived with disease and 1 patient died after a mean follow-up of 12.6 (range, 6-19) months from the date of trial enrollment.

Sequencing Results

All patients provided BCC tissue that was resistant to previous SMO inhibitor therapy, which allowed assessment for HH pathway mutations (Table 2). Of these, 2 patients had functional mutations in SMO at site D473, a mutation known to cause HH inhibitor resistance by altering the drug-binding pocket of SMO,[15] and 1 patient had a mutation at site R168H, a well-known germline polymorphism.[16] No mutations were detected in the downstream HH pathway gene suppressor of fused homolog (*SUFU*; OMIM 607035) or *GLI1*.

Discussion

We have demonstrated the first off-label use of sequential ATO-ITRA for mBCC treatment. Combined ATO-ITRA can reduce HH pathway activity in humans by 75% compared with baseline, which is consistent with findings in murine models.[9] However, the degree to which this reduction in HH activity can result in clinical response is unknown. Although some patients experienced arrest of tumor growth during treatment (stable disease for 3 months), none experienced tumor shrinkage.

Our dosing regimen of ATO-ITRA reduces HH pathway activity by 75% after 1 month compared with a 90% reduction with vismodegib.[4] This HH pathway reduction leads to clini-

cally stable disease at best compared with a response rate of 30% with vismodegib treatment in mBCC.[2]

In a prior study, an itraconazole dosage of 400 mg/d for nonmetastatic BCC reduced HH activity by 65% after 1 month.[7] However, tumor size reduction was noted in vismodegib-naive patients but not those with prior vismodegib treatment.[7] All patients in our trial had received previous vismodegib treatment. These results suggest that only vismodegib-naive patients may benefit from itraconazole treatment and may explain our lack of response in patients with mBCC already treated with vismodegib. The lack of clinical response in our patients despite pathway inhibition may also be the result of suboptimal dosing or transient pathway suppression because our IV arsenic trioxide schedule included 5 days per month. Arsenic trioxide dosing schedules for induction treatment of acute promyelocytic leukemia use continuous daily IV[17] or oral[18] administration, which may lead to better pathway suppression and clinical outcomes.

To our knowledge, no effective therapy exists for patients with mBCC whose tumors have progressed after vismodegib treatment. A recently completed clinical trial using sonidegib phosphate (800 mg/d) for patients with advanced BCC and secondary acquired resistance to nonsonidegib SMO inhibitors also demonstrated no clinical tumor response to treatment.[19] Whether disease progression after HH inhibitor therapy results in a poor response to subsequent therapy remains unknown. These tumors might be growing in an HH pathway–independent manner.

This study demonstrates the feasibility of targeting the HH pathway with the use of an ATO-ITRA regimen, but our findings suggest that this dose and schedule do not have significant clinical efficacy for SMO inhibitor–resistant mBCC in patients receiving second-line therapy. Our next study will test higher doses or continuous dosing schedules with ATO-ITRA to more fully inhibit the HH pathway, which may yield a better clinical response.

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Study concept and design: Tang, Colevas.
Acquisition, analysis, or interpretation of data: Ally, Ransohoff, Sarin, Atwood, Rezaee, Bailey-Healy, Kim, Beachy, Chang, Oro, Tang, Colevas.
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REFERENCES

1. Wysong A, Aasi SZ, Tang JY. Update on metastatic basal cell carcinoma: a summary of published cases from 1981 through 2011. *JAMA Dermatol*. 2013;149(5):615-616.

2. Sekulic A, Migden MR, Oro AE, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med*. 2012;366(23):2171-2179.

3. National Comprehensive Cancer Network. Basal cell skin cancer. 2015; https://www.nccn.org/store/login/login.aspx?ReturnURL=http://www.nccn.org/professionals/physician_gls/pdf/nmsc.pdf. Accessed June 14, 2015.

4. Tang JY, Mackay-Wiggan JM, Aszterbaum M, et al. Inhibiting the hedgehog pathway in patients with the basal-cell nevus syndrome. *N Engl J Med*. 2012;366(23):2180-2188.

5. Atwood SX, Sarin KY, Whitson RJ, et al. Smoothened variants explain the majority of drug resistance in basal cell carcinoma. *Cancer Cell*. 2015;27(3):342-353.

6. Kim J, Tang JY, Gong R, et al. Itraconazole, a commonly used antifungal that inhibits hedgehog pathway activity and cancer growth. *Cancer Cell*. 2010;17(4):388-399.

7. Kim DJ, Kim J, Spaunhurst K, et al. Open-label, exploratory phase II trial of oral itraconazole for the treatment of basal cell carcinoma. *J Clin Oncol*. 2014;32(8):745-751.

8. Kim J, Lee JJ, Kim J, Gardner D, Beachy PA. Arsenic antagonizes the hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. *Proc Natl Acad Sci U S A*. 2010;107(30):13432-13437.

9. Kim J, Aftab BT, Tang JY, et al. Itraconazole and arsenic trioxide inhibit hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists. *Cancer Cell*. 2013;23(1):23-34.

10. ClincalTrials.gov. Arsenic Trioxide in Treating Patients With Basal Cell Carcinoma. NCT01791894. https://clinicaltrials.gov/ct2/show/NCT01791894?term=NCT01791894&rank=1. Accessed September 5, 2014.

11. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.

12. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5(6):649-655.

13. Kindler HL, Aklilu M, Nattam S, Vokes EE. Arsenic trioxide in patients with adenocarcinoma of the pancreas refractory to gemcitabine: a phase II trial of the University of Chicago Phase II Consortium. *Am J Clin Oncol*. 2008;31(6):553-556.

14. Gomez-Ospina N, Chang AL, Qu K, Oro AE. Translocation affecting sonic hedgehog genes in basal-cell carcinoma. *N Engl J Med*. 2012;366(23):2233-2234.

15. Metcalfe C, de Sauvage FJ. Hedgehog fights back: mechanisms of acquired resistance against Smoothened antagonists. *Cancer Res*. 2011;71(15):5057-5061.

16. Wang XD, Inzunza H, Chang H, et al. Mutations in the hedgehog pathway genes *SMO* and *PTCH1* in human gastric tumors. *PLoS One*. 2013;8(1):e54415.

17. George B, Mathews V, Poonkuzhali B, Shaji RV, Srivastava A, Chandy M. Treatment of children with newly diagnosed acute promyelocytic leukemia with arsenic trioxide: a single center experience. *Leukemia*. 2004;18(10):1587-1590.

18. Zhu HH, Wu DP, Jin J, et al. Oral tetra-arsenic tetra-sulfide formula versus intravenous arsenic trioxide as first-line treatment of acute promyelocytic leukemia: a multicenter randomized controlled trial. *J Clin Oncol*. 2013;31(33):4215-4221.

19. Chang AS. Open-label pilot study of LDE225 in advanced basal cell carcinoma patients who have been treated previously with a non-LDE225 Smoothened inhibitor. *J Invest Dermatol*. 2014;134(suppl 1):S101.