A Phase III, Double-Blind, Placebo-Controlled Clinical Trial to Assess Cyclopamine as a Chemopreventive Agent Inhibiting Recurrence of Basal Cell Carcinoma following Surgical Resection

Sara Herman

A. Study Purpose and Rationale

Basal cell carcinoma (BCC) has been found to be one of the most commonly occurring tumors in humans. The incidence of BCC continues to increase 5% annually likely as a result of the average individual's annual increase in sun exposure, the environmental factor most closely associated with BCC development.[1,2] Additionally, patients presenting with a history of at least one BCC are at a significant risk of developing additional tumors. The likelihood of developing a second cutaneous BCC reaches approximately 40 percent over five years, with the recurrence rate being the highest in the initial first three years.[1]

Despite their high incidence, BCCs rarely metastasize, and therefore BCC's mortality rate is quite low. Although the mortality is low, BCC can cause considerable morbidity through local spread and destruction of surrounding structures, leading to scarring and disfigurement. Ultimately, a safe and effective means of preventing BCC would be beneficial to reduce the significant morbidity associated with BCC.

Progress has been achieved in understanding the pathogenesis of BCCs through studies examining both sporadic BCCs and BCCs that develop in the context of the basal cell nevus syndrome (BCNS). BCNS is a rare, autosomal dominant disorder that is due to germ line mutations of a tumor suppressor gene, *patched* (PTCH), on chromosome 9.[3] Affected individuals are especially likely to develop BCCs in large numbers, in addition to rare CNS tumors, secondary to the fact that they are born with only one functional PTCH allele. Histologically, the appearance of the tumors in both the sporadic and familial forms of BCC is identical. Furthermore, studies suggest that the development of BCC follows the 'two-hit hypothesis' of tumorigenesis; two acquired mutations in PTCH may be sufficient for the development of sporadic BCC while only one somatic mutation, in addition to the inheritance of a defective PTCH allele, is required in familial cases of BCC.[4]

The PTCH protein is a transmembrane receptor for an important component of the sonic hedgehog (Shh) signaling pathway, the hedgehog (Hh) protein. The family of Shh proteins mediate the embryonic development of a variety of organs to such an extent that loss of Shh signaling can result in severe developmental abnormalities, including cyclopia. Furthermore, inappropriate activation of the Shh pathway has been associated with the development of multiple human tumors, including BCC, medulloblastoma, and rhabdomyosarcoma.[5] Understanding the interaction between the Hh protein, the PTCH receptor, and a third protein, *smoothened* (SMO) is essential for appreciating both the pathogenesis of BCC and the mechanisms underlying potential therapies. In the absence of Shh signaling, PTCH binds to SMO, suppressing SMO's activity. However, when Hh binds to PTCH, SMO suppression is silenced, resulting in the induction of transcription factors downstream which have been shown to have profound effects on cell behavior and function. They have also been associated with different aspects of tumor development.[4] The deregulation in Hh signaling, via either loss of function mutations in the PTCH gene or activating mutations in SMO, leads to constitutive overexpression of the Shh signal. Ultimately, Shh overexpression has been connected to the development of BCC and other tumors.[4]

Cyclopamine, a plant-derived steroidal alkaloid, has been shown to inhibit the cellular response to Hh signaling by antagonizing the proto-oncogene SMO.[6] It therefore blocks abnormal cell growth by

inhibiting cell signaling downstream associated with both PTCH and SMO mutations. Chronic oral cyclopamine administration at 10μg/day dramatically inhibits BCC growth in *Ptch1*$^{+/-}$ heterozygous knock-out mice by causing differentiation of the tumor cells and efficient apoptotic death through elevation of pro-apoptotic protein expression.[7] Cyclopamine's modulation of the hedgehog/smoothened signal transduction pathway makes its use highly advantageous in the treatment of basal cell carcinomas and other tumors that use this pathway for proliferation and prevention of apoptosis.

Patients presenting with at least one prior BCC are at high risk for developing subsequent BCCs and are therefore an ideal target population for chemoprevention. The purpose of this hypothetical study will be to determine the efficacy of cyclopamine as a chemopreventive agent for the secondary development of basal cell carcinoma.

B. Study Design and Statistical Analysis

This study will be a hypothetical five-year randomized, double-blinded, placebo-controlled clinical trial investigating cyclopamine as a chemopreventive agent for the development of subsequent BCC in patients with their first occurrence of BCC. The study sample will consist of consecutive patients diagnosed with BCC and presenting for surgical resection of their lesion. At the time of resection, patients will be invited to participate in this clinical trial. Participation in the trial will begin one week after excision of the presenting BCC.

Patients will be randomly assigned to the study group or the control group via block randomization. The Research Pharmacy at Columbia University will generate randomization codes. All study personnel will be blinded to the study drug administration. Patients assigned to the control group will receive a placebo topical SPF 30 cream to be applied once daily over all sun-exposed areas, including, but not limited to, face, neck, and arms, for the duration of the study. Patients assigned to the study group will receive topical SPF 30 cream that appears and smells identical to the placebo, but which contains 18mM concentration of cyclopamine, also to be applied once daily to sun exposed areas for the duration of the study. Compliance will be assessed every 3 months using patient surveys.

The study population will consist of 423 patients in each group. This sample size allows detection of a 10% reduction in the recurrence of BCC, i.e. from a projected 5-year occurrence rate of 40%. This sample size also allows for up to a 10% dropout rate. Patients will not be crossed over between groups, and patients will remain in the study for the duration of the study period, regardless of development of new BCC. The primary endpoint will be occurrence of BCC during the 5-year study period. An additional endpoint will be mean number of BCC over the 5 year time period. Finally, time to occurrences of BCC during the 5 year study period will be assessed.

A chi-square test with 0.05 alpha level will be used to determine if there are significant differences in the incidence of BCC over the five year study period. Additionally, the non-parametric Wilcoxon rank sum test will be used to compare mean number of occurrences of BCC in the two groups during the five year study period. Time to occurrence of BCC will be compared using Kaplan-Meier survival curves. The Cox proportional hazards regression analysis will be used to adjust for predisposing variables between the two groups.

C. Study Procedure

Initial evaluation, which will include a β-HCG urine analysis for all women of childbearing age, and enrollment will be performed in the Columbia University dermatology attending physicians' offices. Participation in the study will begin 1 week after excision of the presenting BCC. Thereafter, the patient will receive topical administration of either cyclopamine or placebo, and will return for a dermatologic exam every 3 months. At each visit, any adverse effects will be noted, and patients will complete a survey to assess their compliance. Additionally, all female patients of childbearing age will receive a β-HCG urine analysis to assess for pregnancy. All patients will remain in the study protocol for the duration of the study period, regardless of incidence of BCC. Only significant adverse effects, non-

compliance, or pregnancy will be grounds for dropout from the study.

D. Study Drugs

A. Cyclopamine, approximately .5mL of 18mM concentration in base SPF 30 cream, applied topically to sun exposed areas, including, but not limited to, face, neck, and arms, once daily for approximately 5 years.

B. Placebo, approximately .5mL of base SPF 30 cream topical administration to sun exposed areas once daily for approximately 5 years.

E. Medical Device

Not applicable.

F. Study Questionnaires

See Figure 1 and Figure 2.

G. Study Subjects

Patients aged 20-65 years with Fitzpatrick Skin Types I, II, III, and IV presenting with a first occurrence of BCC for surgical resection will be eligible for inclusion in this study. The Fitzpatrick skin typing system is used to categorize individuals based on their potential risk for sunburn. Patients with skin types V and VI, or those patients who rarely or never sunburn, are less likely to develop BCCs, as they are less likely to experience UVB-induced DNA damage, and will therefore be excluded from the study in order to avoid confounding by other predisposing factors to the development of BCC.

Patients must be in good health, likely to remain in the area and able to complete the 5 year study. Patients must be willing to abstain from use of topical medications, including corticosteroids, vitamin A derivatives, and/or alpha-hydroxy acids. Female patients that are of childbearing age must be willing to utilize a reliable method of birth control throughout the duration of the study.

Exclusion criteria:

- Patients with Fitzpatrick Skin Types V and VI
- Known condition predisposing the patient to a cutaneous neoplasia, i.e. Basal Cell Nevus Syndrome (BCNS) or Xeroderma Pigmentosum (XP)
- Known exposure to arsenic
- Known skin condition which is likely to require use of topical medications
- Pregnancy or planned pregnancy during the duration of the 5-year study

H. Recruitment of Subjects

Consecutive patients who are diagnosed with a first occurrence of BCC by a Columbia University dermatologist, and have treated the lesion with surgical resection, will be invited to participate in this study.

I. Confidentiality of Study Data

Any information obtained from this study and identified with a particular patient will remain confidential.

J. Potential Conflict of Interest

None.

K. Location of Study

Columbia Presbyterian Medical Center: Irving Pavilion 12th floor Dermatology office suites.

L. Potential Risks

As neither Phase I nor Phase II clinical trials have been conducted to assess the potential adverse effects of cyclopamine in human subjects, potential risks cannot be specifically outlined. However, as this is a hypothetical Phase III trial, prior studies in mice have shown the survival of a cohort of mice receiving placebo and a second cohort receiving 10μL cyclopamine po, per drinking water, daily is similar, indicating that cyclopamine does not affect the overall survival of these mice.[7] Additionally, in a preliminary proof of concept study conducted by S. Tas and O. Avci, the maximal topical administration of cyclopamine of 120μL every four hours produced no short-term or long-term adverse effects.[8] Cyclopamine has been shown to have teratogenic effects, including cyclopia, cebocephaly, anophthalmia, or microphthalmia, in lab animals.[9] Therefore, it should not be utilized in pregnant females in order to avoid these detrimental congenital effects.

M. Potential Benefits

The potential benefit for the patient is the possible chemopreventive effect of cyclopamine. This benefit will only be applicable to those enrolled in the study group. In addition, each participant will receive comprehensive dermatologic exams every 3 months to monitor for recurrence of BCC. These exams will be provided free of charge for the duration of the 5 year study.

N. Alternative Therapies

Patients may choose not to participate in this study.

O. Compensation to Subjects

None.

P. Costs to Subjects

Patients will not be billed for participation in this study.

Q. Minors as Research Subjects

Not applicable.

R. Radiation or Radioactive Substances

Not applicable.

S. References

1. Marcil, I. and Stern, RS. "Risk of developing a subsequent nonmelanoma skin cancer in patients with a history of nonmelanoma skin cancer: a critical review of the literature and meta-analysis." *Arch Dermatol* 2000;136(12):1524-30.

2. Zak-Prelich M et al. "Environmental risk factors predisposing to the development of basal cell carcinoma." *Dermatol Surg* 2004;30(2 Pt 2):248-52.

3. High, A. and Zedan, W. "Basal Cell Nevus Syndrome." *Curr Opin Oncol* 2005; 17:160-166.

4. Dicker, T. et al. "Molecular and Cellular Biology of Basal Cell Carcinoma." *Australian Journal of Dermatology* 2002; 43:241-246.

5. Taipale, J et al. "Effects of Oncogenic Mutations in *Smoothened* and *Patched* Can Be Reversed by Cyclopamine." *Nature* 2000; 406:1005-1009.

6. Miller, SJ. and Yu, TC. "Cyclopamine as a Potential Therapeutic Agent for Treatment of Tumors Related to Hedgehog Pathway Mutations." *Dermatol Surg* 2002; 28:187.

7. Athar et al. "Inhibition of *Smoothened* Signaling Prevents Ultraviolet B-Induced Basal Cell Carcinomas through Regulation of Fas Expression and Apoptosis." *Cancer Research* 2004;64:7545-7552.

8. Tas, S. and Avci, O. "Rapid Clearance of Psoriatic Skin Lesions Induced by Topical Cyclopamine." *Dermatology* 2004; 209(2):126-131.

9. Keeler RF. "Teratogenic effects of cyclopamine and jervine in rats, mice, and hamsters." *Proc Soc Exp Biol Med* 1975; 149:302-306.

10. American Cancer Society. Cancer facts and figures 2000. Available at: www.cancer.org.2001. Accessed August 2005.

Figure 1: Study Questionnaire: Initial Evaluation

Cyclopamine as Chemopreventive Agent: Initial Evaluation

Name: MRN:
Age:
Sex:

Presenting Diagnosis:
Date of Diagnosis:
Location:
Size:
Description:
Date of Excision:

Exclusion Criteria: (Circle one)

Fitzpatrick Skin Type V or VI		Y	N
Known condition predisposing to BCC		Y	N
Known exposure to arsenic		Y	N
Known skin condition likely to require use of topical medications	Y	N	
β-HCG urine analysis negative		Y	N
Planned pregnancy during the duration of the 5 year study		Y	N

Other Medical Conditions:

Current Medications:

Method of Contraception (If Applicable):

Figure 2: Study Questionnaire: Follow-up Evaluation

Cyclopamine as Chemopreventive Agent: Follow-up Evaluation

Name: MRN:
Date:

History:
Estimated number of missed doses:
Adverse effects from therapy:

Dermatologic exam:
New BCC (Circle one): BCC None

If new lesion is present:
Date of Diagnosis:
Location:
Size:
Description:

Other comments:

(If Applicable)
β-HCG urine analysis negative Y N
Method of Contraception: