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This supplementary material has been provided by the authors to give readers additional information about their work.
Appendix 1. Key personnel of the VAKCC Trial

Study Chairman’s Office, Providence, RI: Martin A. Weinstock, MD, PhD (Chair), Kimberly Marcolivio, MEd (National Coordinator)

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Cooperative Studies Program Research Pharmacy Coordinating Center, Albuquerque, NM: Mike Sather PhD; Carol Fye MS; Robert J. Ringer, PharmD, BCNP; David Hunt, MS

Dermatopathologists, Providence, RI: Leslie Robinson-Bostom, MD; Gladys Telang, MD; Caroline Wilkel, MD

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Clinical Centers: 1Bay Pines, FL, 2Hines, IL, 3Palo Alto, CA, 4Atlanta, GA, 5San Diego, CA, 6Minneapolis, MN, 7Nashville, TN, 8Denver, CO, 9Boston, MA, 10Philadelphia, PA, 11Durham, NC, 12Miami, FL
Appendix 2. Detailed methods of the VAKCC Trial

Study population
Participants with a history of at least two keratinocyte carcinomas (KCs) in the five years prior to enrollment, at least one of which was on the face or ears, were recruited from 12 VA medical centers (in Denver, Colorado; Philadelphia, Pennsylvania; Palo Alto and San Diego, California; Durham, North Carolina; Minneapolis, Minnesota; Atlanta, Georgia; Miami and St. Petersburg (Bay Pines), Florida; Chicago (Hines), Illinois; Nashville, Tennessee; and Boston, Massachusetts). KCs were not counted towards inclusion criteria if they were located on the vermilion, genitalia, perianal skin, or area of previous radiation therapy. They were also not counted if they had arisen in a scar, area of chronic infection, inflammation, or ulcer; or if they were a recurrence of a previously treated lesion. In addition, participants were required to be English-speaking, able to give consent, able to comply with protocol requirements, and KC-free on the face and ears at enrollment. Patients were excluded if in the last three years they had used systemic 5-fluorouracil (5-FU), oral capecitabine, or field therapy for actinic keratoses (AKs) on the face/ears (e.g. 5-FU cream, imiquimod, diclofenac gel, chemical peel, or photodynamic therapy). We also excluded solid organ transplant recipients; patients with genetic disorders associated with extremely high skin cancer risk (i.e., basal cell nevus syndrome, erythrodysplasia verruciformis, xeroderma pigmentosum); patients with a history of psoralen plus UV-A treatment, arsenic exposure, cutaneous T-cell lymphoma, or radiation therapy to the face and/or ears; patients with allergies to sunscreen, triamcinolone, or 5-FU; patients with high mortality risk due to comorbid illness (less than 50% chance of surviving five years); pregnant or breastfeeding women, women of childbearing potential unwilling to use birth control; patients with a known dihydropyrimidine dehydrogenase enzyme deficiency, and patients currently using methotrexate.

Study design and interventions
The Veterans Affairs Keratinocyte Carcinoma Chemoprevention (VAKCC) trial (Cooperative Studies Program [CSP] 562) was a randomized, double-blind, vehicle-controlled, parallel group, two-arm trial (ClinicalTrials.gov Identifier: NCT00847912) for chemoprevention of KCs in veterans at high risk for these cancers. The intervention was a standard course of topical 5% 5-FU or vehicle control cream, consisting of the same inactive ingredients as the 5-FU cream: white petrolatum, stearyl alcohol, propylene glycol, polysorbate 60, and parabens.

Participants were instructed to apply the study medication twice daily to the face and ears for four weeks—a total of 56 doses. If twice daily application was intolerable due to adverse effects, the medication was stopped and triamcinolone cream, 0.1% was applied twice daily to the face and ears for five days. If this occurred prior to completion of 28 doses, the study medication was resumed three weeks after stopping and applied once daily to complete 56 doses. If still intolerable, the study medication was discontinued for the duration of the study. We considered 28 doses to be the minimum adequate dose to evaluate this treatment for the purposes of this study. We note that a standard course of topical 5-FU for actinic keratosis treatment is two to four weeks twice daily. All participants had completed treatment within 11 weeks from onset of study medication.

Cumulative dose of study medication was calculated in two different ways: 1) primarily using cumulative dose logs kept by study coordinators and documented contemporaneously in medical records; and 2) primarily using case report forms of participant-reported study medication application frequency.

After the active treatment phase, participants were evaluated face-to-face semi-annually starting with the first six-month visit up to the study end date of June 30, 2013. In-person visits, telephone interviews, medical record reviews, and full body skin exams were conducted to collect information on demographic characteristics, medical history, medication use, adverse events, and study endpoints. The primary outcome for this study was the first occurrence of a primary KC on the face or ears that was surgically removed. This includes primary basal cell carcinoma (BCC), primary invasive squamous cell carcinoma (SCC), and primary SCC in situ.

Throughout the study, we provided SPF 30 sunscreen to all participants and educated them about skin cancer, sun safety, and sunscreen use. We also showed participants photographs of moderate to severe reactions to topical 5-FU. Participants were interviewed about medication reactions and photographed to document those reactions at multiple time points.

Randomization and blinding
Randomization was conducted centrally based on a random treatment allocation sequence generated by the computer algorithm written by the study statistician and integrated into the electronic data capture (EDC) system used at the sites to enter study data. The allocation sequence used randomly permuted blocks of variable size to ensure approximate balance over time. To account for potential practice differences, randomization was stratified by site so that a balanced allocation of treatments was achieved within each site. For drug assignment, site personnel would fill out the assignment form in EDC, which would transmit therapy number, treatment designation, and randomization date to a web service software hosted at the Pharmacy Coordinating Center (PCC). The software would verify the match with the randomization treatment allocation sequence and return a box number and tube number which would appear on the EDC form for the site to print and take to their research pharmacy at the site, which would dispense the study drug.

Participants were blinded to treatment assignment and the success of this blinding was evaluated at both six months and the final study visit. Investigators who performed assessments were also blinded to treatment assignment. During the active treatment period, adverse effects were monitored by a designated unblinded investigator at each of the study sites. Blinded investigators first examined participants before they initiated study medication and again starting at six months after enrollment, months after the expected resolution of 5-FU side effects.

Study assessments
Medical records were used by study dermatologists to verify skin cancer history and confirm participant eligibility. Detailed methods are also described elsewhere. The study chairman’s office reviewed all pathology reports of skin cancers diagnosed in the five years prior to enrollment. Before randomization, study dermatologists performed a full body skin exam on each participant and recorded baseline AKs on the face and ears by predefined anatomical region. Those with KCs on the face or ears had them removed and were required to wait 30 days before randomization and starting intervention. Demographic and health related information was also collected at the baseline visit.

During the active treatment period, study medication use was assessed and adverse effects and symptom severity were monitored weekly, by telephone interviews alternating with follow-up visits. Photographs of the participant’s face and ears were taken at two and four weeks into the intervention and used to calculate a photograph-based toxicity score, described elsewhere. At both the six-month and final study visits, participants were asked about study medication side effect severity, perceived study group assignment, and willingness to repeat treatment if shown to be effective in reducing future skin cancers.

Starting at the six-month visit, blinded study dermatologists performed full body skin exams semiannually throughout the study. The presence and any spot treatment of AKs were recorded. AKs were spot treated at the clinician’s discretion, almost always with cryotherapy. Additional AK spot treatments done outside of scheduled study visits were also recorded in the medical record.

To determine the primary study endpoint, all clinically suspicious lesions were biopsied both during the semiannual exams and in-between study visits. Histopathologic specimens from these biopsies were first read by local pathologists and later underwent central pathology review by a board-certified dermatopathologist blind to study group assignment for final diagnosis. High inter-rater reliability was documented with two other central board-certified dermatopathologists. Study dermatologists reported all KC treatments done after biopsy. Documentation of skin cancers diagnosed outside the VA, and associated surgeries, were systematically sought in all participants and confirmed with medical record review. Study endpoint was defined as a primary BCC or primary SCC that was removed surgically. Surgical removal was defined as removal by excision, Mohs surgery, electrodessication and curettage, or complete surgical removal with biopsy.

The follow-up assessments for this study terminated with consent withdrawal, HIPAA revocation, study end date of June 30, 2013, or death.

Statistical analysis
For each of the key endpoints (KC, BCC, and SCC) we analyzed time to event (diagnosis) for the entire four-year trial and risk of event in the first year after randomization as the key outcomes.
The VAKCC Trial was designed to have 90% statistical power for a two-sided log-rank test to detect a hazard ratio that exceeded 1.420 or was less than 0.704 for the first occurrence of surgically-treated primary KC in the 5-FU group compared to the control group, hence a sample size of 1000 participants allocated equally to two treatment arms. This two-sided log-rank test allowed for an overall type I error of 5%, one interim analysis, and 8% total attrition due to death or loss to follow-up.

After half of the randomized participants (250 in each study arm) were followed for at least two years, an interim survival analysis was conducted with a two-sided log-rank test that was powered at 90% statistical power with a 0.2% type I error. A hazard ratio that exceeded 2.39 or was less than 0.42 was efficacy stopping rules for harm or benefit, and conditional probabilities were estimated to evaluate futility.

The baseline characteristics of the 5-FU and the control groups were compared by a two-tailed Student’s t-test for continuous measures and \( \chi^2 \) test for discrete measures. We used the Kaplan-Meier life-table analysis to compare the time from randomization to the first surgically-treated KC in the 5-FU group to the control group, censoring at the earliest date of death, HIPAA revocation, consent withdrawal date, date of last visit for those lost to follow-up, or study end date of June 30, 2013.

All statistical analyses were conducted with SAS Version 9.2 (SAS Institute Inc., Cary, NC) and STATA Version 8.0 (Stata Corp, College Station, TX). Relative risks (and 95% confidence intervals (CI)) for the respective endpoints in the 5-FU group compared to the control group were estimated using Cochran Mantel Haenszel methods. Confirmatory Cox proportional hazards regression modeling was conducted to provide hazard ratios and 95% confidence intervals around the ratio. We tested the proportional hazards assumption by including a time-treatment interaction term in the model as well as by graphical methods.

Pearson’s correlation test was used to examine the relationship between two different measures of study medication side effects: 1) a photograph-based toxicity score based on photographs taken at two weeks into the intervention phase and 2) a retrospective self-report of side effects recorded at the six-month study visit.

**Ethics**

The VAKCC trial was approved by the Cooperative Studies Scientific Evaluation Committee and received initial and annual review by the VA Central Institutional Review Board as well as Research and Development committees at each of the 12 clinical sites. Additional oversight was provided by the Data Monitoring Committee, the Executive Committee, and the CSP Human Rights Committee. All participants gave written, informed consent, and Declaration of Helsinki Principles were followed.

**Role of the funding source**

This study was funded by the U.S. Department of Veterans Affairs, Cooperative Studies Program, which provided general guidelines for conducting the trial and approved the design, data collection, analysis, interpretation of data, and writing of this report, and the decision to submit this manuscript for publication. Almost all of the authors, including the first author, are employees of the U.S. Department of Veterans Affairs.
References


eTable 1. Study medication side effects reported by participants in the 5-fluorouracil (5-FU) and control groups at the six-month and final visits

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<thead>
<tr>
<th>Group</th>
<th>Event</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
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<tr>
<td>5-FU</td>
<td>6 month</td>
<td>60 /439 (14%)</td>
<td>111 /439 (25%)</td>
<td>176 /439 (40%)</td>
<td>92 /439 (21%)</td>
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<tr>
<td>5-FU</td>
<td>Final visit</td>
<td>66 /390 (17%)</td>
<td>89 /390 (23%)</td>
<td>138 /390 (35%)</td>
<td>97 /390 (25%)</td>
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<tr>
<td>Control</td>
<td>6 month</td>
<td>329 /432 (76%)</td>
<td>80 /432 (19%)</td>
<td>19 /432 (4%)</td>
<td>4 /432 (1%)</td>
</tr>
<tr>
<td>Control</td>
<td>Final visit</td>
<td>298 /396 (75%)</td>
<td>71 /396 (18%)</td>
<td>20 /396 (5%)</td>
<td>7 /396 (2%)</td>
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