

NON-MELANOMA SKIN CANCER PREVENTION: IMPACT OF NON-
STEROIDAL ANTI-INFLAMMATORY DRUGS, RETINOID DOSE RESPONSE
AND MEASUREMENT RELIABILITY

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Mary Catherine Clouser

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As members of the Dissertation Committee, we certify that we have read the dissertation

prepared by Mary Catherine Clouser

entitled Non-Melanoma Skin Cancer Prevention: Impact of Non-Steroidal Anti-Inflammatory Drugs, Retinoid Dose Response and Measurement Reliability

and recommend that it be accepted as fulfilling the dissertation requirement for the

Degree of Doctor of Philosophy

_____ Date: 04/07/09
Denise J. Roe, Dr. P.H.

_____ Date: 04/07/09
Robin B. Harris, Ph.D.

_____ Date: 04/07/09
Janet A. Foote, Ph.D.

_____ Date: 04/07/09
Daniel C. Malone, Ph.D.

_____ Date: 04/07/09
Grant H. Skrepnek, Ph.D., R.Ph.

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

_____ Date: 04/07/09
Dissertation Director: Denise J. Roe, Dr.P.H.

STATEMENT BY AUTHOR

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SIGNED: Mary Catherine Clouser

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To everyone who grew up playing outside under a bright sun.

Ten thousand flowers in spring, the moon in autumn,
a cool breeze in summer, snow in winter.
If your mind isn't clouded by unnecessary things,
this is the best season of your life.

Wu Men

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LIST OF ABBREVIATIONS

AK	actinic keratosis
ALT	alanine aminotransferase
ANOVA	analysis of variance
ASA	aspirin
AST	aspartate aminotransferase
BCC	basal cell carcinoma
C	cytosine
CA	California
CI	confidence interval
CIN	cervical intraepithelial neoplasia
COX	cyclooxygenase
COX-1	cyclooxygenase 1
COX-2	cyclooxygenase 2
DFMO	D,L—difluoromethylornithine
DN	dysplastic nevi
DNA	deoxyribonucleic acid
EcCa	eccrine carcinoma
FL	Florida
HaCaT	human keratinocyte cell line
HNSCC	head and neck squamous cell carcinoma
HR	hazard ratio
HSC-5	human skin squamous cell carcinoma cell line
ID	identification
IU	international units
K	kappa
kg	kilograms
MED	minimal erythematol dose
mg	milligrams
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMSC	non-melanoma skin cancer
NSAIDs	non-steroidal anti-inflammatory drugs
OR	odds ratio
PCNA	proliferating cell nuclear antigen
PGG ₂	prostaglandin G ₂
PGE ₂	prostaglandin E ₂
PGH ₂	prostaglandin H ₂
PTCH	patched gene
PUVA	psoriasis-oral methoxsalen photochemotherapy
RA	retinoic acid
RAR	retinoic acid receptors
RARγ	retinoic acid receptor gamma

LIST OF ABBREVIATIONS-Continued

RNA	ribonucleic acid
mRNA	mesenger ribonucleic acid
RR	relative risk
RXR	retinoid X receptors
RXR α	retinoid X receptor alpha
RXR β	retinoid X receptor beta
SCC	squamous cell carcinoma
SD	standard deviation
SEB	surrogate endpoint biomarkes
SEER	Surveillance Epidemiology and End Results
SENCAR	SENSitivity to CARcinogenesis
SIR	standardized incidence ratio
SKICAP	skin cancer prevention trials
SPF	skin protection factor
T	thymine
μ g	micrograms
US	United States
UV	ultraviolet
UVB	ultraviolet B
UVA	ultraviolet A
UVR	ultraviolet radiation
WBC	white blood cell count
WHS	Womens Health Study

ABSTRACT

Non-melanoma skin cancers (NMSC) are the most common malignant neoplasms in the White population, afflicting about 20% at some point in life (1, 2). The incidence of NMSC is increasing by two to three percent per year (2). Strategies for NMSC prevention are important because, although NMSC does not result in substantial mortality, it does have the ability to cause substantial morbidity, including disfigurement and loss of function, and treatment is costly (3).

The goals of the dissertation were to explore the reliability, clinical, epidemiological, pharmacoepidemiological and statistical issues that potentially affect studies of NMSC prevention. This dissertation utilized three studies designed to examine ways to prevent NMSC recurrence or identify early markers in the development of NMSC (SKICAP-AK trial, n=2,297, SKICAP-SCC/BCC trial, n=525, and Biomarkers 1 Study, n=91). The goals of this research were to examine the impact of non-steroidal anti-inflammatory drugs (NSAIDs) on NMSC recurrence, determine if there was a dose response for treatment with the drugs isotretinoin and retinol on the recurrence of NMSC, and examine factors related to reliability of NMSC risk assessment questionnaires often used in epidemiological and clinical studies.

Overall, the findings of this research indicated that NSAIDs likely play a role in reducing the risk of developing an SCC in those at high risk, and that the use of oral isotretinoin and retinol play little role in reducing the risk of

redevelopment of NMSC in those who have had a previous NMSC. In addition, there was evidence of substantial reproducibility for factors related to assignment into skin cancer risk group and self-reported history of skin lesions, with self-reported sun sensitivity questions being somewhat less reliable. More research should be done to address the role of NSAIDs in chemoprevention at both the basic science level and epidemiological level. Additional secondary analyses of large data sets that contain information on NMSC and NSAIDs use should be conducted in order to further strengthen the argument that NSAIDs play a role in reducing NMSC occurrence. Future work should focus on determining the specific populations that could benefit from NSAIDs use for the reduction of NMSC.

DISSERTATION FORMAT

The SKICAP-AK and SKICAP-BCC/SCC trials were companion NMSC chemoprevention studies conducted at the Arizona Cancer Center, University of Arizona from 1984-1990. The goal of the SKICAP-AK trial was to test the hypothesis that daily supplementation of retinol (25,000 IU) for 5 years reduces the incidence of NMSC in highly promoted individuals (those with a history of greater than ten diagnosed AK and no more than two prior SCC or BCC). The goal of the SKICAP-SCC/BCC trial was to examine the effect of retinol and synthetic equivalent, isotretinoin, on the incidence of NMSC in high-risk subjects, those who had at least four biopsy-proven skin cancers, one of which had to have occurred within 12 months of consenting to the study. These studies were funded by the Department of Health and Human Services, USPHS, and National Cancer Institute Grants CA-34256 and CA-27502 and conducted under the direction of Thomas E. Moon, Ph.D. The Biomarkers 1 study was conducted by the Arizona Cancer Center, University of Arizona over an approximately three month period and was designed to assess the reproducibility of various surrogate endpoint biomarkers (SEB), within the skin carcinogenesis pathway, specifically the variability of the SEBs (polyamine levels, p53 expression and proliferating cell nuclear antigen (PCNA) expression). This study was funded by grant P01 CA27502 from the National Cancer Institute and conducted by David S. Alberts, M.D.

The combination of these three studies allowed for the exploration of several questions related to NMSC chemoprevention and epidemiology. The Biomarkers 1 study allowed for the examination of reliability issues related to questions commonly used in epidemiological studies of skin cancer that define an individual's risk. Many of these same scales and questions were used in both the SKICAP-AK and SKICAP-BCC trials and were included in initial statistical models related to those projects. The SKICAP-AK trial allowed me to practice both my epidemiological and pharmcoepidemiological (part of my minor) skills by providing a large data base to examine how a particular medication (NSAIDs in this case) can potentially affect disease development (NMSC in this case). This project allowed me to add relevant information to the growing literature related to NSAIDs and chemoprevention of cancer. Intent to treat analyses are the standard for randomized clinical trials. The SKICAP-BCC/SCC trial allowed me the opportunity to examine a negative clinical trial in a dose response manner in order to see how analysis approach may affect the results.

I began work on the first of these projects in 2005. My responsibilities over the course of this work have included database management, extensive data cleanup, extensive data collection, additional data entry from case report forms and coding, quality assurance, statistical analysis and writing. The Epidemiology Ph.D. program established that three publishable manuscripts can contribute to the main body of the dissertation. All three of these papers contribute to the literature on NMSC. After completion of all necessary steps to

obtain data to investigate the hypotheses I developed, I have conducted the analyses, summarized results, drafted manuscripts for journal submission, and submitted manuscripts. One manuscript has been published (hypothesis #3), a second has also been accepted for publication (hypothesis #1), and a third (hypothesis #2) has been prepared for submission. These three papers were incorporated into the dissertation. Dr. Denise Roe provided assistance on analysis strategies and in the interpretation of the results. Dr. Robin Harris provided assistance with interpretation of the results as well as provided advice on additional data analysis methods. Dr. Foote provided expertise and historical reference for both the SKICAP-AK study and the SKICAP-BCC/SCC study. Drs. Roe, Harris and Foote provided editing and comments on the manuscripts produced from the SKICAP-AK and SKICAP-BCC/SCC studies, and Drs. Roe and Harris provided editing and comments on the manuscript produced from the Biomarkers 1 study. All manuscripts were submitted to committee members and co-authors for review and comments.

CHAPTER 1

INTRODUCTION

EXPLANATION OF THE PROBLEM

Section 1: Background and Significance

Skin cancer is the most common form of cancer in the United States (4). Fifty percent of Americans who live to be age 65 or older will have skin cancer at least once (5). Basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma are the three primary types, the first two being highly curable. Life time exposure to the sun's ultraviolet rays is the most important environmental factor involved in the development of skin cancer, with susceptibility to sunburn likely the most important genetic factor (6). Other risk factors include light skin color, hair color, eye color, family history of skin cancer, personal history of skin cancer, chronic exposure to the sun, history of sunburns early in life, certain types of moles or a large number of moles and freckles, chemical carcinogens, chronic ulceration or inflammation, immunosuppression, viral carcinogens and scarring dermatoses (3). For BCC and SCC, age likely due to cumulative sun exposure, is also a risk factor, with the incidence of skin cancer in those 55 to 75 years of age being four to eight times higher than those 20 years younger (3).

BCC and SCC are not cancers that are normally included in United States tumor registries and, therefore, sources regarding time trends in incidence are scarce. Incidence rates of non-melanoma skin cancer (SCC and BCC) were

examined in two United States geographic areas at two different time points (1971-1972 and 1977-1978) (7). Results from these surveys indicate that there were statistically significant increases in BCC, 18 percent or almost 3 percent per year, not dependent on age group (7). Increases for SCC were not statistically significant, but the survey was based on relatively few cases (7). Glass et al. examined the incidence of SCC over a 27 year period with data from a prepaid health plan in the northwestern United States, and found that SCC increased 2.6 times in men and 3.1 times in women during that period (8). A survey done in Switzerland from 1976 to 1985 confirms that the incidence of NMSC is on the rise (9). Although this survey is now quite old, it demonstrates that NMSC incidence is increasing. A survey done in Arizona for the years 1985-1996 did not demonstrate an increase in BCC or SCC over this time period, and suggested that for SCC in particular, there was a plateau or even what could be considered a decline (10). Multiple surveys suggest that NMSC incidence is increasing, however in some parts of the world, such as Southeastern Arizona, this may not hold true.

Based on isolated surveys in the United States, it is estimated that approximately 80 percent of skin cancers are BCC, approximately 16 percent are SCC, and approximately 4 percent are melanomas (11). BCC and SCC originate from epidermal keratinocytes (11). BCC is a slow growing tumor that rarely metastasizes (less than 1 per 4000 cases), but it can cause high amounts of morbidity (3, 11). Metastatic BCC most commonly occurs in middle-aged men on

the head or neck, with survival rates for metastatic BCC of approximately 1.5 years (3). In contrast, SCCs are more likely to metastasize; it is estimated that approximately 3 to 10 percent metastasize, although there have been reported rates as high as 30 percent (3, 11). Thickness of the tumor and level of invasion strongly predict SCC metastatic risk (3). Although the metastatic risk is low, survival from metastatic SCC is also low with five year survival rates of between 14 and 39 percent (3).

Although mortality from NMSC is rare, the most vulnerable population group is consistent with prevalence vulnerability, those 65 years and older. The proportion of skin cancer deaths in those age 65 to 84 during the period from 1979-1981 was 34 percent, in comparison to 16 percent mortality among those under 65 years (12). SCC was responsible for approximately 1200 deaths in 1998, a number equivalent to the yearly mortality attributed to Hodgkin's lymphoma (11). Although the mortality due to SCC is low, associated morbidity causes an important burden on the health care system, and will likely continue to increase, as the highest rate of physician visits for neoplasms of the skin is among those 65 years of age and older (13).

The goal of prevention is to reduce the incidence, morbidity and mortality due to a particular disease whether it be through sanitation, lifestyle intervention, behavior modification or a chemical (drug) intervention (14). As previously stated, preventing a disease decreases the morbidity and mortality associated with it, thereby decreasing health care costs. Diseases, such as cancer and

specifically certain types of skin cancer that have a known and identifiable precursor lesion lend themselves well to prevention activities. There are three types of prevention; primary, secondary and tertiary. Primary prevention is defined as methods designed to reduce the occurrence of disease and takes place during the period of prepathogenesis. Primary prevention can be active (vaccinations, wearing protective clothing, lifestyle changes) or passive (fluoride in water, fortifications of food) (15). Secondary prevention strategies seek to reduce the progress of disease during the pathogenesis phase, and include activities such as cancer screening programs and pre-malignant lesion removal (15). Lastly, tertiary prevention involves activities to reduce the morbidity of a disease such as physical therapy after a stroke (15). Primary methods for skin cancer prevention include behavioral modification such as changing behavior related to sun exposure activities and the use of sunscreens, both active types of prevention strategies. A single study in Australia examined trends in NMSC incidence between 1985 and 1995 through the use of random national household surveys of those over 13 years old (16). They found an increase in BCC rates of 19% between the years 1985 and 1995 and an increase of 93% for SCC over the same time period (16). Despite the raise in overall rates they did find a reduction in BCC for those in the younger age groups and report that as evidence for a beneficial effect of public health campaigns to reduce sun exposure (16). However, because modifying personal behaviors is often difficult for individuals to adopt and because the primary methods employed to date have not yet resulted

in a substantial decrease in NMSC incidence, additional strategies are required (17). Chemoprevention is the use of pharmaceuticals or nutraceuticals to reduce the incidence of disease. Chemoprevention strategies have been applied in other areas.

Because NMSC is a substantial public health burden due to increasing rates, and behavioral modification strategies have not been as effective as needed, it is a good target for chemoprevention and ongoing research. Although NMSC does not result in substantial mortality, NMSC does have the ability to cause substantial morbidity, including disfigurement and loss of function, and treatment is costly (3). Effective chemopreventative agents, which could be administered over long periods at low doses, could provide a decrease in incidence and subsequent reductions in morbidity and mortality without excessive toxicity.

Section 2: Hypothesis and Specific Aims

The purpose of this research was to assess several clinical and epidemiological issues in NMSC prevention. This work explores the effect of two compounds, NSAIDs and retinoids for skin cancer chemoprevention within the context of previously completed Skin Cancer Prevention (SKICAP) trials from the Arizona Cancer Center. These trials had been designed to evaluate whether retinoids could reduce the occurrence of new NMSCs in a population at high risk due to considerable sun damage. Since these SKICAP trials were conducted

between 1984 and 1990, new information related to NSAIDs and their potential role in skin cancer prevention has begun to emerge. Furthermore, information related to retinoids has remained equivocal. The SKICAP trials allow for the examination, in a large population, of NSAIDs use and its ability to reduce the risk of NMSC without conducting a full scale clinical trial. Additionally, because the original SKICAP BCC/SCC trial used an intent-to-treat analysis to determine if retinoid administration could reduce the re-occurrence of NMSC, a dose-response analysis was completed. The dose-response analysis determined whether those who adhered better to the study drug regimen (retinol and isotretinoin) benefited versus those who were poor adherers and thus received a lower dose of the intervention.

Finally, since intervention studies like SKICAP include epidemiological questions, determining the reliability of these questions is valuable. The Biomarkers 1 study, aimed at assessing markers of risk, was an ideal study for evaluating self risk assessment questions and reliability. The reliability of these risk assessments is important because chemoprevention trials often rely on risk assessment for recruitment purposes, risk group assignment and confounder assessment

Three primary hypotheses are examined in this dissertation:

- 1) There is no difference in development of first new NMSC (BCC or SCC) in subjects participating in the SKICAP-AK trial who reported taking NSAIDs versus those who did not. (Manuscript 1-

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- 2) Based on an analysis of total dose consumed, there is no dose response in new occurrence of BCC or SCC in those who took retinol, isotretinoin or placebo in the SKICAP-BCC/SCC trial. (Manuscript 2)

- 3) Assessment of risk group, skin lesion history and sun sensitivity in the Biomarker 1 study will be reliable. (Manuscript 3 “Risk Group, Skin Lesion History and Sun Sensitivity Reliability in Squamous Cell Skin Cancer Progression”-Cancer Epidemiology Biomarkers and Prevention 2006;15 (11) 2292-2297)

The above hypotheses were tested by exploring the following specific aims:

Specific Aim #1: To assess if participants who reported taking NSAIDs while participating in the SKICAP- AK trial had:

- a. Difference in diagnosis of a first new BCC or SCC development from study initiation to exit as compared to those who reported no NSAIDs use during the study.

- b. An interaction between NSAIDs use and the intervention (retinol).

- c. For those reporting use of aspirin only: Difference in first new SCC or BCC development from study initiation to exit as compared to those who reported no NSAIDs use.
- d. For those reporting use of NSAIDs only: Difference in first new SCC or BCC development from study initiation to exit as compared to those who reported no NSAIDs use.

Specific Aim #2: To determine if there is a dose response relationship between the total dose (mg/kg) of retinol or isotretinoin and new occurrence of SCC or BCC for participants in the SKICAP-BCC/SCC trial as compared to those on placebo.

Specific Aim #3: To explore the Biomarkers 1 study data in order to determine:

- a. If subject risk group assigned by telephone interviewers based on subject self-assessment is as reliable as risk group assignment by study dermatologist based on a skin examination.
- b. If study subjects reliably report skin lesion history at two different study time points.
- c. If study subjects reliably report sun sensitivity at two different study time points.

BACKGROUND AND LITERATURE REVIEW

Section 1: Skin Cancer Incidence and Mortality

Over 1,000,000 occurrences of new skin cancers are diagnosed yearly in the US, accounting for approximately 40% of all new cancer diagnoses (11). The incidence of skin cancer has been increasing in recent years and this increase is expected to continue as the population ages and larger amounts of ultraviolet radiation reach the earth's surface due to depletion of the ozone layer (3, 11, 13). While the incidence has been increasing, mortality rates decreased by 20 to 30 percent for both men and women and Whites and Blacks from the period 1969-1988 (18). NMSC occurs more frequently in White men than in White women (67% vs. 33%) and is more evenly distributed by sex in Latin Americans (51% males, 49% females) (19). In a study done in New Brunswick, Canada from 1991 to 2001 through the Provincial Cancer Registry, to which all reporting of cancer diagnosis is mandatory, age standardized incidence rates for BCC were 87 per 100,000 for males and 68 per 100,000 for females, with incidence for both BCC and SCC increasing over the study period (20). A study from Northern Jordan looking at NMSC trends from 1997 to 2001, reported age adjusted incidence rates per 100,000 for BCC of 23.3 (females) and 19.7 (males) (21). Incidence rates per 100,000 for SCC were 6.18 (females) and 14.2 (males), with incidence rates increasing for males and decreasing for females during the study period (21). Incidence of NMSC based on gender appears to vary by geographic location.

Despite the large number of people diagnosed with NMSC, patterns of mortality are poorly documented (18). The study of mortality patterns is severely limited by the exclusion of NMSC from large United States cancer registries. Additionally, routine death certification studies only provide a rough guide, because they include deaths from SCC of mucosal sites in the head and neck, and are also subject to the bias all studies using death certificates suffer (22). Weinstock et al. published several papers dealing with NMSC mortality in the 1990's, and these are some of the most current papers to describe mortality.

A study of NMSC mortality in the United States for the period 1969-1988, on the basis of routine death certification, found that approximately 1,200 deaths per year were attributable to NMSC (18). Weinstock et al. also examined the mortality due to NMSC from 1979 through 1987 in Rhode Island residents. They found that the age adjusted mortality rate was 0.44 per 100,000 per year, 65 percent of the deceased individuals were men, and that 59 percent were due to SCC and 20 percent due to BCC (22).

Among deaths from BCC, the mean age was 85 years and the median survival from the time of diagnosis was 5 years (22). The most recent study looking at mortality risk in SCC was done in 2005 on patients enrolled in a Texas skin cancer registry (23). This study found that the three year overall and disease-specific survival rates were 70 percent and 85 percent (23). The factors associated with adverse disease specific survival rates were local recurrence at presentation, invasion beyond subcutaneous tissues, perineural invasion, lesion

size and depth of invasion. Three year disease specific survival was 100 percent for patients who had none of these risk factors (23). Among deaths from SCC, the mean age was 73 years with 80 percent of the SCCs having metastasized and 47 percent having arisen on the nose (22). The median survival from the time of diagnosis was 2 years (22).

Melanoma is the most serious and lethal form of skin cancer and can metastasize to other parts of the body (24). The American Cancer Society estimates that in 2008, there will be 8,420 fatalities in the United States, 5,400 in men and 3,020 in women (25). The number of new cases of invasive melanoma is estimated at 62,480 annually (25). Melanoma incidence has increased from 5.7 cases per 100,000 in 1973 to 12.5 cases per 100,000 in 1994, while melanoma mortality has increased from 1.6 to 2.2 per 100,000 during the same period (26). Melanoma originates in melanocytes, the cells which produce the pigment melanin. The majority of melanomas are black or brown but some can be skin-colored, pink, red, purple, blue or white (24). Risk factors for melanoma include: sun exposure, number of moles on the skin, skin type and genetics (24). Those who have had a previous SCC or BCC are at increased risk for melanoma. An examination of Surveillance Epidemiology and End Results (SEER) data from 1973-1994 shows an increase in localized stage melanoma but no increase for distant stage melanoma, however mortality rates appear to have increased over time (26). Although, melanoma is a type of skin cancer, melanoma skin cancer is not the focus of the present work.

Section 2: Actinic Keratoses (AK)

Although the focus of this work is on SCC and BCC, AK cannot be ignored for a couple of reasons. Firstly, it is the known precursor lesion to SCC (27-29). Secondly, people were recruited into the SKICAP-AK study, the study from which the data for the NSAIDs hypothesis originates, based on a minimum number of AKs they had on their forearms as a marker of risk.

AK is the most common precancerous dermatosis (11). AK is typified by atypical epithelial proliferation that develops due to disregulated keratinocyte maturation in the epidermis (11, 28, 30). Clinically AK is a scaly or crusty bump that forms on the skin surface (30, 31). They range in size from very small to over an inch across and may be light or dark, tan, pink, red, a combination of these, or the same color as a persons' skin (30, 31). The scale or crust is horn-like, dry, and rough, and can often be felt easier than seen (31). AK appear on chronically sun exposed areas such as the face, ears and dorsal surfaces of the forearms and hands (11, 31). AK is attributed to UV radiation exposure, and other risk factors for AK include both host and environmental factors (30, 31). Aging, fair skin pigmentation, compromised immune function, and presence of genetic disorders of DNA repair are risk factors for AK (11). Skin types that burn easily and tan poorly (Types I and II) and those living at higher elevations are at greatest risk of developing AKs (32).

While most AKs do not progress to SCC, it is believed that AK represents SCC in situ at its earliest stages (11, 27-29). Evidence that AKs are the pre-

malignant precursors of SCC, but not BCC, include shared risk factors, a histologic continuum, and the presence of similar molecular/genetic alterations in both AK and SCC (11). An AK can: 1) go into spontaneous remission often with reduction in UV exposure, 2) remain stable, or 3) become an SCC (30).

Histologically, AKs are characterized by dysplasia of keratinocytes with loss of cellular polarity and nuclear atypia. The diagnosis of SCC is made when these atypical cells pass through the basement membrane, invading into the underlying dermis (11). AK is cytologically indistinguishable from SCC (11, 33, 34).

Several studies have gathered evidence to show that AK is the precursor lesion of SCC. In a study done to determine the risk of malignant transformation of AK to SCC, the risk for transformation within one year was found to be less than 0.1 percent (35). Where accurate mapping of both SCCs and pre-existing AKs was available, it was found that 60 percent of the SCCs arose from a lesion diagnosed clinically as an AK and 40 percent of SCCs developed on what had been clinically normal skin 12 months previously (35). In a study of 22 patients with metastatic SCC whose primary lesions were examined using light microscopy, AK was present in 44% of the lesions (34). In a 5 year longitudinal study done in Australia, 60 percent of SCCs arose from a preexisting AK (35). An Arizona study done in individuals with 10 or more AKs reported a cumulative probability of 14 percent for developing SCC within 5 years (11, 36).

Section 3: Risk Factors for Squamous Cell Skin Cancer

SCC is the second most common skin cancer and affects more than 200,000 Americans per year (37). SCC arises from the keratinocytes in the skin. Actinic keratoses (AK) are considered a precursor lesion to SCC. AKs arise from the epidermis and resemble the squamous cells that comprise most of the upper layer of skin (37). SCC occurs on all areas of the body including the mucous membranes, but most commonly occurs in sun exposed areas (37). In a small percentage of cases, SCCs metastasize and can be life threatening. Early stage SCC is relatively easily treated with methods such as radiation therapy or surgery; advanced disease requires multimodality therapy including surgery, radiation, chemoradiation, or a combination of these treatments (38). There has been little increase in long-term survival rates for advanced SCC over the past 30 years, with the case-fatality rate estimated to be only 1 percent (23, 38).

Prevalence, case-control and cohort studies have examined the etiology of SCC and appear to come to similar conclusions about risk. These studies are summarized in Table 1. Besides exposure to the sun, exposures to chimney soot, arsenic, insecticides, immunosuppression and human papilloma virus infection have been associated with SCC (3, 13). SCC usually appears as a single red, scaling plaque or nodule that is sharply demarcated and can be difficult to differentiate from eczema, psoriasis and Paget's disease (3).

The incidence of SCC increases with increasing proximity to the equator and is more strongly related to latitude or measured UVB radiation than is BCC

(39). SCC occurs almost exclusively on parts of the body that are usually exposed to sunlight (39). In addition the risk of SCC is associated with skin sensitivity with estimates of relative risk between 1.5 and 4.5 for comparisons of the least sensitive skin with the most sensitive skin (39, 40).

Exposure has been explored by many of the studies in Table 1. Sun burns during childhood and overall cumulative lifetime sun exposure may play a role in the risk of SCC development. However, not all studies have found this association. Total lifetime exposure to the sun has shown a strong dose-response relationship with SCC and there is increasing risk with increasing hours of occupational exposure (39, 41). Case-control data that evaluated childhood exposure should be viewed with caution as there may be bias by ability to recall or differential recall by case status.

A migration study of AK, the precursor lesion to SCC, done in Australian-born persons versus British born person who had migrated to Australia, found that the British immigrants showed a lower proportion of AK, providing additional evidence that sun exposure in childhood may play a primary role in risk of SCC in adulthood (42). In addition several migration studies done in Australia indicate that the risk of SCC may increase with increasing length of residence in an environment with high ambient solar radiation (43).

Strong associations have been seen with biological markers of long-term sun exposure, and include solar lentigines, facial telangiectasia, and elastosis of the neck and dorsum of the hands (39).

Pigmentary traits are strong factors for those who are more likely to have an SCC. Persons with light skin, red hair, light eyes and freckles are at higher risk than those with darker hair, eyes and skin. All studies that looked at age as a risk factor found that the older a person is, the more likely they are to develop an SCC.

A high percentage of patients with SCC develop a second primary skin cancer within 5 years (11). The benefit of early detection of SCC is that it avoids the small possibility of mortality, which has fallen considerably between 1950 and 1980 (2, 44).

Table 3.1: Epidemiological Studies of SCC

Source	No. of Patients Enrolled	Interventions and Design	Patient Population	Primary Result
B. Vitasa et al. (45)	808 (202 AK, 35 SCC, 33 BCC, 7 both BCC and SCC, 588 with no NMSC or AK)	Cross-sectional prevalence survey	Maryland watermen 30 years of age or older	<ul style="list-style-type: none"> • Those with SCC or AK had higher average annual UV-B doses than age-matched controls particularly in those younger than 60 years (11% and 8%) • Age most important risk factor (p=0.0002) • Those in upper quartile of cumulative UV-B exposure had RR=2.5 (95% CI 1.18-5.4) compared to lower 3 quartiles
M. Weinstock (46)	51 deaths (30 SCC)	Prevalence	All deaths reported 1979-1987	<ul style="list-style-type: none"> • Evidence of declining mortality

			attributed to NMSC in Rhode Island	<p>with increasing incidence</p> <ul style="list-style-type: none"> • 59% of deaths due to SCC (mean 72.5 years) • 70% men with metastases in 23%-median survival 2 years • Ear most common primary (47%) • Much misclassification of diagnosis
D. English et al. (40)	1163 (132 SCC)	Case Control Interviewed about lifetime sun exposure	Population Based	<ul style="list-style-type: none"> • Site specific exposure (OR=3.3, 95% CI 1.3-8.2) at 65,000 hours • Site specific stronger for sun exposure in childhood vs. adulthood (Max predicted OR in ages 8-14 years= 5.1 at 3.3 hr exposure/day) • Intermittent pattern of weekly exposure and vacation close to unity • Number of blistering sunburns to a site (3+ blistering burns OR= 2.1, 95% CI 1.0-4.3) • Use of sunscreen and hats inconsistent effects • Exposure during adolescence and childhood increases risk

R. Zanetti et al. (47)	3572 (cases= 1549 BCC, 228 SCC)	Case Control	Population and Hospital Based	<ul style="list-style-type: none"> • Head most common site (76.8% in men, 58.8% in women) • Second most common site lower limbs in women (25.5%) and upper limbs in men (7.9%) • Pigmentary traits (hair color stronger than eye, 2 fold increase in risk for those who never tan and always burn) • No association between number and age at first sunburn • Fair hair, blue/hazel or grey eyes and a tendency to burn (OR=5.4, 95% CI 10.72-271.86)
R. Gallagher et al. (48)	586 (180 SCC cases)	Case Control	<p>Cases = Males 25-79 in Alberta, Canada, new pathology reports</p> <p>Controls = Males from health care insurance plan matched on sex and age</p>	<ul style="list-style-type: none"> • Increased risk for subjects with light skin and red hair who burn rather than tan • Freckling in childhood OR=1.6 (95% CI 1.0-2.4) • Severe burns presence of two or more per year in childhood OR=10.5 (95% CI 2.9-38.0) • No association between recreational exposure during childhood and adolescence or mean annual

				<p>lifetime occupational exposure</p> <ul style="list-style-type: none"> • Suggestion of decreased risk with increasing lifetime recreational exposure • Occupational exposure over 10 years prior to diagnosis; OR=4.0 (95% CI 1.2-13.1) in highest exposure group • Southern European ancestry protective; other important factors were freckling on arm in childhood, forearm skin color and permanent color difference between neck and protected areas • Solar elastosis of neck strongest risk factor along with telangiectasia of face
F. Aubrey et al. (49)	311 SCC cases 610 controls	Case-control	Cases- Primary SCC of skin diagnosed between 1977-1978 Controls- other skin disease diagnosed between 1977-1978 matched on sex, age and	<ul style="list-style-type: none"> • Eye color, hair color, complexion, recent and non-occupational sun exposure found to be risk factors; RR=4.60 (95% CI 0.58-36.53) • Association with tobacco smoking RR=2.30 (95% CI 1.27-4.15) • Association with

			hospital of diagnosis	sunlamp use; RR=13.42 (95% CI 1.38-130.48)
F. Grodstein et al. (13)	107,900 (197 SCC)	Cohort-8 year follow-up	Female Nurses 30-35 years of age at baseline (1976) (Nurses' Health Study Cohort)	<ul style="list-style-type: none"> • Living in California RR=1.8 (95% CI 1.3-2.6) and Florida RR=2.1 (95% CI 1.1-3.9) compared to northeastern states • Living in CA and FL at birth and 15 years of age (RR=2.5, 95% CI 1.14-4.4) for CA, (RR=3.0, 95% CI 0.7-1.2) for FL • Red (RR=2.0, 95% CI 1.1-3.7) and light brown (RR=1.7, 95% CI 1.2-2.4) hair color • Burning after 2 hours of sun exposure as child compared to never burn (RR=1.5, 95% CI 0.9-2.5) and (RR=1.1, 95% CI 0.6-2.0) for painful burn • Number of severe burns (RR=2.4, 95% CI 1.5-4.0) for 6 or more • Current smokers (RR=1.5, 95% CI 1.1-2.1) vs. never smokers • Age related incidence: 10.9 per 100,000 person years 35-44, 21.0 of

				100,000 person years 45-54, 46.7 of 100,000 person years 60-69
S.Rosso et al. (50)	1549 BCC 228 SCC 1795 controls	Population based case-control	Southern European populations	<ul style="list-style-type: none"> • Increase risk of SCC with increase in sun exposure (>70,000 cumulated hours) • Inverse correlation with work and holiday exposure • Outdoor work associated with SCC (OR=1.6 for 54,000 cumulated hours) • Risk pattern differed by tanning ability
A. Kricger et al. (51)	226 BCC 45 SCC 1,015 controls	Population based case-control	Geraldton, Western Australia	<ul style="list-style-type: none"> • SCC risk higher in native born Australians • Southern European ancestry protective for SCC • Inability to tan strongest pigmentary risk factor for SCC • Freckling on arm in childhood associated with SCC • Forearm skin color and permanent color difference between neck and adjacent protected areas associated with SCC • Soar elastosis of neck risk factor for SCC

				<ul style="list-style-type: none"> • Telangiectasia of face associated with SCC • History of acne Protective for SCC
B. Armstrong et al. (43)	Various	Review Article	Various	<ul style="list-style-type: none"> • Incidence rates of SCC (1977-1978) increase across latitude gradient with increasing estimated ambient solar UV (10 US metropolitan populations –Seattle to New Orleans) • Risk increases with increasing average annual hours of bright sunlight at place of residence, regardless of migration; steep gradient for SCC • Lifetime potential for SCC determined by sun exposure in first 10 years of life- extent to which potential is realized determined by sun exposure later in life • Pattern and amount of UV exposure operate independently to determine risk – slope of increase with amount of exposure is greater for SCC as compared to BCC, and slope of increase, with

				intermittency not related to SCC <ul style="list-style-type: none"> • Risk of SCC equal in those who migrated to Australia in first 10 years of life to those born there; risk three fold less in those who migrated after first 10 years of life.
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OR= Odds Ratio, RR= Risk Ratio

Section 4: Risk Factors for Basal Cell Skin Cancer

Ninety percent of all skin cancers in the United States are BCC, approximately 800,000 cases per year (5, 52). BCC arises from the basal keratinocytes at the bottom of the epidermis and adnexal structures and occurs mostly on sun exposed areas of the skin such as face, ears, and neck (3, 52). The number of new cases of BCC has been increasing, and simultaneously the average age of onset has been decreasing (52). The most common treatments for BCC are primary resection, radiation therapy and micrographic surgery (38).

BCC is more common than SCC and is rare in non-White populations. In a US survey conducted in 1977-78, the incidence rate of SCC and BCC combined was 232.6 per 100,000 person-years in Whites but only 3.4 among Blacks (39). Risk of BCC appears to increase with increasing intensity of ambient solar radiation. In Australia and the US, incidence is greatest in regions closest to the equator. BCC is categorized both morphologically and

histologically into subtypes (3). Classification includes nodular ulcerative (45%-60%), superficial (15%-35%), morpheaform, infiltrating and pigmented (1%-2%) with each subtype having a different appearance and clinical course (3).

Solar exposure has been shown to be the most important risk factor for BCC, however this association is not completely clear (41). It appears that recreational sun exposure may have some association to BCC but occupational does not (50, 53). BCC is significantly associated with non-occupational exposure to the sun measured as summer holiday or weekend exposure, and risk increases significantly with lifetime measures of sunburn (50, 53). Curiously, studies have shown that the risk of BCC was 40 percent higher in those who regularly spent time outdoors and used sunscreen than in those who regularly spent time outdoors and did not use sunscreen (39). The majority of evidence indicates that there is no association with occupation. In addition, sunburns in childhood, freckling on the arms and number of moles on the back seem to be associated with BCC risk. It may be that the timing of sun exposure rather than total dose is the more important factor for BCC development.

The highest density of BCC is found on the usually sun exposed body sites and the lowest on the rarely exposed sites (39). Based on the many epidemiologic studies that have examined the risk for BCC, there appears to be a relationship between pigmentary traits, with those with fair skin and light hair color being at higher risk. Relative risks of 2.0 or more have been found for BCC for skin that burns rather than tans, and light skin color is also significantly

associated with BCC (39, 41). Moderate associations between BCC and telangiectasia and solar elastosis, other non-neoplastic skin lesions have been found (39). The benefit of early BCC detection is that lesions found at a smaller size reduce the risk of scarring and reduce the cost of therapy (2).

Migration studies indicate that the risk of BCC may increase with increasing length of residence in an environment with high ambient solar radiation, but may also suggest that exposure to solar radiation early in life may be particularly important in increasing the risk of skin cancer (43).

Table 4.1: Epidemiological Studies of BCC

Source	No. of Patients Enrolled	Interventions and Design	Patient Population	Primary Result
B. Vitasa et al. (45)	808 (202 AK, 35 SCC, 33 BCC, 7 both BCC and SCC, and 588 with no NMSC or AK)	Cross-sectional prevalence survey	Maryland watermen 30 years of age or older	<ul style="list-style-type: none"> • Ease of sun burning associated with BCC and AK but not SCC • No established clear relationship of BCC to cumulative ultraviolet-B exposure • BCC had 8% less UV-B exposure • Age most important risk factor (p=0.0001)
M. Weinstock	51deaths (10 BCC)	Prevalence	All deaths reported 1979-	<ul style="list-style-type: none"> • Evidence of declining

(46)			1987 attributed to NMSC in Rhode Island	<p>mortality with increasing incidence</p> <ul style="list-style-type: none"> • 20% of deaths due to BCC (mean 72.5 years) • Half of fatal BCC cases in men with only 1 metastasis (other deaths due to direct extension of BCC into vital structures)- median survival 5 years
R. Zanetti et al. (47)	3572 (cases= 1549 BCC, 228 SCC)	Case Control	Population and Hospital Based	<ul style="list-style-type: none"> • Head most common site for BCC (78.1% in men, 76.9% in women) • Second most common site the trunk in both men (14.1%) and women (10.1%) • Pigmentary traits (hair color stronger than eye, 2 fold increase in risk for those who never tan and always burn) • Number of

				<p>sunburns before age 15 (controlled for by pigmentary traits) OR=1.68 (95% CI 1.17-2.39)</p> <ul style="list-style-type: none"> Subjects with fair hair and tendency to burn showed a 5-fold to 10-fold increase in risk for BCC if they had sunburns before age 15 (sunburn and never tan, red hair and blue eyes RR = 9.61 (95% CI 5.74-16.07)
R. Gallagher et al. (53)	632 (226 BCC cases)	Case Control	<p>Cases =Males 25-79 in Alberta, Canada, new pathology reports</p> <p>Controls=Male s from health care insurance plan matched on sex and age</p>	<ul style="list-style-type: none"> Men with light skin color (OR=4.0, 95% CI 1.4-11.3), red hair (OR=2.1, 95% CI 0.7-2.20) and burn, never tan (OR=1.6, 95% CI 0.8-3.2) Freckling in childhood (OR=1.8, 95% CI 1.2-2.5) Severe childhood sunburn (OR=4.5, 95% CI 1.7-12.3)

				<ul style="list-style-type: none">• Mean recreational sun exposure 0-19y (OR=2.6, 95% CI 1.1-6.5)• Mean annual lifetime recreational exposure was protective even for those in highest category of exposure (OR=0.4, 95% CI 0.2-1.0)• No elevated risk from occupational exposure for any decade of employment from age 20• Mean annual lifetime occupational exposure showed non significant slight gradient of risk.• The relationship with childhood sun exposure was most pronounced among sun-sensitive subjects whose skin tended to burn
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				<p>rather than tan in the sun (OR=3.1, 95% CI 1.10-10.4)</p> <ul style="list-style-type: none"> • These findings suggest that timing and character of solar exposure may be more important than cumulative dose in predicting adult BCC risk among people with sun-sensitive skin.
S. Rosso et al. (50)	1549 BCC 228 SCC 1795 controls	Population based case-control	Southern European populations	<ul style="list-style-type: none"> • Inverse correlation with work and holiday exposure • BCC: 2 fold increase of risk for lower exposure (8000-10,000 cumulated hours) • Recreational activities associated with BCC (OR=1.6 for 2600 cumulated hours) • Risk pattern differed by tanning ability
A. Kricger et al. (51)	226 BCC 45 SCC	Population based case-	Geraldton, Western	<ul style="list-style-type: none"> • BCC risk higher in native

	1,015 controls	control	Australia	<p>born Australians</p> <ul style="list-style-type: none"> • BCC decreased with increasing age at arrival in Australia • Southern European ancestry protective for BCC • Inability to tan strongest pigmentary risk factor for BCC • Freckling on arm in childhood associated with BCC • Number of moles on back associated with BCC • Soar elastosis of neck risk factor for BCC • Loss of fine texture of skin on back of hands associated with BCC • History of acne and warts protective for BCC
P. Vitaliano et al. (41)	366 BCC 58 SCC 294 controls	Case-Control	Tumor Clinic of the Skin and Cancer Hospital	<ul style="list-style-type: none"> • Solar exposure the most important factor for BCC (p<.001)

				<ul style="list-style-type: none"> • Ability to tan shown to be of special importance even at low levels of exposure ($p < .001$) • Subjects over 60 at higher risk ($p < .005$) • Complexion significant for BCC ($P < .025$)
B. Armstrong et al. (43)	Various	Review Article	Various	<ul style="list-style-type: none"> • Incidence rates of BCC (1977-1978) increase across latitude gradient with increasing estimated ambient solar UV (10 US metropolitan populations – Seattle to New Orleans) • Lifetime potential for BCC determined by sun exposure in first 10 years of life-extent to which potential is realized determined by sun exposure later in life. • Pattern and amount of UV exposure operate

				<p>independently to determine risk –slope of increase with amount of exposure is less for BCC as compared to SCC and slope of increase with intermittency being intermediate for BCC</p> <ul style="list-style-type: none"> • Risk of BCC equal in those who migrated to Australia in first 10 years of life to those born there; risk three fold less in those who migrated after first 10 years of life.
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Section 5: Biological Basis for Sunlight and NMSC

SCC and BCC occur primarily on sun-exposed areas of the body and have been strongly associated with chronic sun exposure. Mechanisms by which UV radiation can cause carcinogenesis include causing DNA damage and/or modification of immune function. Shorter wavelength UVB is thought to be primarily responsible for the DNA damage associated with UV exposure, and the effects on cells, causing formation of reactive oxygen species that likely play a role in the promotion phase of UV-induced skin carcinogenesis (11).

Evidence is strong that UV radiation (sun exposure) is likely one of the major causal factors in NMSC development. Cancers produced by exposure to UV light constitute nearly 50 percent of cancers diagnosed in the United States, and 90 percent of the new cases of skin cancer are attributable to UV light irradiation (54). UV light from sun exposure has been documented as a complete carcinogen responsible for initiation and promotion of both BCC and SCC (54). In 1992 the International Agency for Research on Cancer reviewed all the evidence linking carcinogenesis and solar and ultraviolet radiation and concluded: "There is sufficient evidence in humans for the carcinogenicity of solar radiation." (43).

Six categories of epidemiologic evidence have been outlined that are relevant to the idea that sunlight is a causal agent in development of NMSC: 1. NMSC is more frequent in residents of areas with high ambient solar irradiance; 2. NMSC is more frequent in sun-sensitive people; 3. NMSC occurs mainly on sun-exposed body sites; 4. NMSC is more frequent in people with high sun exposure; 5. NMSC is more frequent in people with benign sun related skin conditions; 6. NMSC risk is reduced by protecting the skin against the sun (39).

UV radiation produces a number of photoproducts in DNA, with the most common being cyclobutane-type pyrimidine dimers and pyrimidine-pyrimidone photoproducts formed between adjacent pyrimidines (cytosine (C) and thymine (T)) (39). UV photoproducts are mutagenic if they are not repaired before cell division occurs, with the most common UV-induced mutations being a C to T

transition which occurs at dipyrimidine sites. Tandem transitions CC to TT which also occur, are almost specific to UV (39). High proportions of NMSC in humans have been found to have this C to T or CC to TT mutation, which is the UVB signature of mutations in the p53 gene (43).

p53, a tumor suppressor gene, is essential in maintaining genomic integrity by blocking DNA replication in response to DNA damage, from exposure to agents like UV light (27, 55, 56). p53 is present at low levels in normal cells but expressed at higher levels in response to DNA damage. This increase in p53 expression has been found to be related to the dose and wavelength of UV irradiation. UVA irradiation induces p53 in all layers of the skin. Mutations in p53 have been identified in chronically sun-damaged skin, AK, SCC (90%) and BCC (50%), and over expression has been observed throughout the sequence of UV induced skin carcinogenesis (39-41). In addition people with an inherited disorder, xeroderma pigmentosum, affecting their ability to repair DNA photoproducts, develop high numbers of BCC and SCC at a young age on body sites commonly exposed to the sun, adding to the evidence that unrepaired photoproducts in DNA are the genesis of NMSC (39).

Erythema (i.e. sunburn), an inflammatory response, occurs in both UV irradiated rodent and human skin. UVB (280-315 nm) is much more effective at producing cancer in animals, erythema in humans, and DNA damage, than is UVA (315-400 nm). Wavelengths of about 340 nm or more are less than 0.1 percent times as potent in causing cancer in experimental animals as

wavelengths of about 295 nm (39). Studies of NMSC in humans are strongly supported by experimental evidence of the capacity of UV radiation to cause SCC and melanoma in animal models (43).

Section 6: The Carcinogenesis Pathway for BCC and SCC

The molecular basis for the differences between BCC and SCC are not yet completely clear, although they are clinically and pathologically distinct lesions (57). Patterns of chromosome loss are different between BCC and SCC with the pattern of loss largely confined to a single chromosome arm for BCC and being more widespread for SCC (57).

Tumor suppressor genes regulate normal cell growth and differentiation (55). If these tumor suppressor genes become disabled then the development and progression of neoplasms can occur (55). Patched gene (PTCH) is a tumor suppressor gene, and its job is to convey extracellular growth regulatory signals to the cell nucleus (55). PTCH appears to be involved in the development of BCC and may also be involved in the development of SCC (55). PTCH mutations are frequent in BCC and are specific to UV transitions (C:T) (55). PTCH represents earlier events in the development of BCCs than subsequent p53 gene alterations (55).

Both BCC and SCC express p53 protein (55, 58). p53 is a tumor suppressor gene that is involved with both BCC and SCC photocarcinogenesis (55). p53 is a transcription factor involved in the maintenance of genomic stability either through induction of apoptosis or cell-cycle arrest (55). The p53

tumor suppressor protein has been proposed to function in many diverse cellular processes, such as apoptosis, cell-cycle arrest, DNA repair, recombination and cellular differentiation (59). p53 mutations allow the propagation of damaged abnormal cells by UV (59). When the skin is exposed to UV, p53 gene activity is induced and p53 rapidly increases in the keratinocytes. This p53 overexpression is associated with p53 gene inactivation which in turn reduces the appearance of sunburn cells which represent apoptotic keratinocytes generated by UVB (55).

The development of SCC is a multistep process. SCC is strongly related to cumulative sun exposure (60). Because high p53 protein expression is seen in SCC and AKs this may indicate that its expression is related to the effect of UV radiation. It may be an early event in the evolution of SCC and p53 positive cells in AK may progress to SCCs (55).

BCCs express p53 protein and mutations of p53 have been documented in 40% to 50% of studied BCCs, with 72% of those mutations bearing the signature of UV light induction (56, 58, 60). Many of these mutations are C to T and CC to TT transitions, the signature mutations of exposure to UVB radiation (60). That being said, the importance of p53 mutations for BCC growth remains to be demonstrated because without genetic damage, p53 activation will not occur (56). In addition aberrant mitosis, one of the hallmarks of p53 dysfunction has yet to be observed in BCC (56).

In the case of both BCC and SCC, overexpression of COX-2 is the basis for treatment regimens using NSAIDs since both BCC and SCC exhibit p53

mutations and this in turn is tied into the expression of COX (61). Thus examining NSAIDs use in a cohort of participants at high risk for NMSC to see if those who use NSAIDs have a reduction in BCC and SCC is a valid research question. Since p53 mutations are greater in SCC, there is an expectation that NSAIDs would be more effective in preventing these lesions as compared to BCC. Both retinoids and p53 have a role in cell differentiation and therefore the belief that retinoids could prevent BCC and SCC is also a valid research question. When p53 becomes disabled, for example due to UV radiation, cells do not differentiate correctly. Since retinoids are necessary for cell differentiation, supplementation with these compounds may prevent neoplastic cells from forming or help to repair damage that has already occurred.

Section 7: Risk of Redeveloping NMSC after a Previous NMSC

Prevention of NMSC is important because once a person is diagnosed with NMSC they are at an increased risk for developing another NMSC. If it were possible to find interventions that would prevent the recurrence of NMSC, much morbidity could be prevented and health care dollars could be saved. In approximately 50 percent of people with a history of NMSC, a new NMSC will develop in the first five years, with risk being highest during the year after treatment (3). Marcil et al., in a study examining the risk of recurrent NMSC, found that the 3 year cumulative risk of a subsequent SCC after an initial SCC was 18 percent and for BCC was 44 percent. Both of these cumulative risks

were a 10 fold increase in incidence compared with the incidence of first tumors in a comparable general population (2). However, it would appear that the risk of developing a BCC in patients with a prior SCC is about equal to the risk among persons with a prior BCC, but the risk of developing an SCC in patients with a prior BCC is low at 6 percent (2).

Approximately one third of recurrent BCC develop in the first year after treatment of a primary BCC, and two thirds develop within three years, with the risk of a second recurrence after treatment higher than the risk of a first (3). Recurrence rates for BCC are dependent on location, size, histologic type of the primary tumor and the initial therapy (3). BCC on the head and face have the highest risk of recurrence, and the neck, trunk and extremities have the lowest risk (3). Large BCC recur more frequently than smaller ones and those BCC that demonstrate infiltration, sclerosis, or multifocality histologic patterns also having a higher rate of recurrence (3). SCCs that are associated with chronic inflammation or scars and occurrence on lower extremities are more likely to recur (3).

Section 8: NSAIDs and Chemoprevention

Aspirin has been used for over a century as an anti-inflammatory drug, analgesic, and antipyretic and along with other NSAIDs has emerged as a potential agent for prevention of cancer (62). The class of drugs known as NSAIDs include salicylates (aspirin, salsalate, difunisal), propionic acids

(flurbiprofen, ibuprofen, ketoprofen, naproxen, oxaprozin), acetic acids (diclofenac, etodolac, indomethacin, ketorolac, nabumetone, sulindac, tolmetin), fenamates (meclofenamate), and the oxicams (piroxicam) (63). Exact mechanisms by which NSAIDs exhibit their chemopreventative action are still unknown, but these drugs do have the ability to inhibit cell proliferation and angiogenesis and also to induce apoptosis (63).

Chronic inflammation caused by bacterial, viral, and parasitic infections, non-digestible particles, chemical irritants, UV radiation and other factors, correlates with increased risk of developing cancer in an affected organ (64, 65). Arachidonic acid metabolism initiates the inflammatory process and this metabolism along with prostaglandin synthesis is linked with the promotion and progression of cancers (65). The metabolic pathway for arachidonic acid is linked to the cyclooxygenase (COX) pathway.

The importance of elevated prostaglandins in tumor promotion has been deduced through the use of inhibitors such as NSAIDs, which show activity against both COX isoforms (COX-1 and COX-2) (64). NSAIDs can thus be classified according to cyclooxygenase isoform selectivity with non selective NSAIDs inhibiting both COX-1 and COX-2 (63). UVB irradiation of the skin leads to visible erythema, a local sign of inflammation, by turning on the arachidonic acid cascade which the prostaglandins are involved in. Thus COX is potentially linked to NMSC by virtue of this pathway (66, 67).

8.1: Mechanisms for Effect of NSAIDs

NSAIDs are among the most commonly and successfully used medications in the world. However, their use is frequently limited by gastrointestinal side effects ranging from dyspeptic symptoms, perforation of gastroduodenal ulcers, to life-threatening bleeding (68, 69). NSAIDs inhibit the synthesis of prostaglandins, which play a role in the control of cell proliferation, neoplasia, and immune response (70, 71). There is also evidence that the antineoplastic effects of NSAIDs may be independent of COX as NSAIDs have been found to inhibit proliferation of colon cancer cell lines that do not express COX and do not produce prostaglandins (62).

In the skin, the pathway leading to prostaglandin synthesis liberates oxygen-derived free radicals, which may cause tissue damage during the inflammatory response, and in addition, prostaglandins may promote the retention of UV-damaged cells via inhibition of apoptosis (72). Inflammatory properties of prostaglandins are a result of their ability to increase vascular permeability, induce vasodilation, and potentiate the inflammatory effects of histamine and bradykinin (72). Prostaglandin synthesis is catalyzed by prostaglandin endoperoxide synthase, also known as COX (73). NSAIDs block prostaglandin production by inhibiting COX. Three broad classes of COX inhibitors exist; aspirin synthesized from salicylic acid; indomethacin and other NSAIDs; and the first selective COX-2 inhibitors- the coxibs (e.g., celecoxib and rofecoxib) (74).

8.2: Cyclooxygenase (COX)

COX activity is induced by growth factors, cytokines, and tumor promoters (75). COX are bifunctional hemoproteins and the key enzyme in arachidonate metabolism (76). Cells use COX to catalyze the first two steps in the biosynthesis of prostaglandins, the conversion of arachidonic acid to prostaglandin G_2 (PGG₂) and the bisoxygenation of arachidonic acid to prostaglandin H_2 (PGH₂), which in turn is converted to physiologically active prostaglandins, prostacyclins, and thromboxanes, collectively known as prostanoids (71, 75-77). Prostaglandins have the undesirable ability to induce pain, fever, and symptoms associated with the inflammatory response (78). In inflamed tissues, the highest levels of immunoreactive COX antigen are found in monocytes, endothelial cells, and synovial fibroblasts (75).

COX-1 and COX-2 share a 60 percent amino acid homology, similar tertiary structures, and similar but not identical active sites (79). COX consist of a long narrow channel with a hairpin bend at the end and are both membrane-associated (80). Arachidonic acid released from damaged membranes adjacent to the opening of the enzyme channel, which is largely hydrophobic, is sucked in and twisted around the hairpin bend (80). Two oxygens are inserted, and a free radical extracted, resulting in the five-carbon ring that characterizes prostaglandins (80). Although the catalytic activities and tertiary structures of COX-1 and COX-2 are remarkably similar, COX-2 has a broader affinity for substrates because the hydrophobic channel leading to the active site of this

enzyme is more accommodating (74). COX-1 is considered a constitutive enzyme, thus it is present in nearly all cell types at a constant level (81). COX-1 plays a role in gastric mucosal protection, regulation of renal blood flow and platelet aggregation (63).

A study evaluating the expression of COX in human and mouse skin biopsy found that COX-1 is observed throughout the epidermis whereas COX-2 increases in the more differentiated suprabasilar keratinocytes (82). Basal cell carcinomas express little if any COX-1 or COX-2 whereas both COX isoenzymes are strongly expressed in SCC deriving from a more differentiated layer of the epidermis (82). COX-2 expression in human keratinocytes appears to be related to differentiation of these cells both in vivo and in culture (82).

The COX-2 enzyme was identified in 1991 (71). COX-2 is a cytokine-induced isoenzyme, meaning it requires inducing stimuli such as pro-inflammatory cytokines and growth factors, tumor necrosis factor, and even infection of intestinal epithelial cells with invasive bacteria such as Salmonella or a local injury (71, 83, 84). COX-2 produces the prostaglandins responsible for mediation of pain and inflammation. It is normally absent from cells, and when induced, the protein levels increase and decrease in a matter of hours after a single stimulus (81).

COX-2 is expressed in specialized tissues including the neurons in the cerebral cortex and limbic region of the brain, testes, ovary, and the macula densa region of the kidney (77, 79). Inflammation and other stresses can

increase COX-2 expression at sites including rheumatoid synoviocytes, macrophages, fibroblasts, and polymorphonuclear leukocytes (71, 79, 80). Levels of COX-2 have been found to be very low and restricted to a few keratinocytes of interfollicular and follicular epidermis in normal skin but in AK, SCC and keratoacanthomas, levels of COX-2 are elevated (72). COX-2 mediates mitogenic growth factor signaling and down-regulates apoptosis, thus promoting tumor growth (85). COX-2 inhibition is time dependent, with selectivity developing over 15-30 minutes and is thereafter essentially irreversible (80).

8.3: NSAIDs and Chemoprevention-Epidemiological Studies

It is unclear whether aspirin (ASA) and other NSAIDs can reduce the risk of cancer. Many epidemiological studies and several randomized clinical trials have been conducted using NSAIDs and ASA or evaluated the use of NSAIDs and ASA in various cancers with mixed results. Several meta-analyses have been performed. Some of the most convincing results are in the colon cancer/polyp prevention arena. Multiple studies using ASA or NSAIDs in colorectal polyp (the precursor lesion to colon cancer) and colorectal cancer prevention have reported positive results (86, 87, 87-98). Many of these colon cancer studies have not had adequate data on dose and duration of NSAIDs use to fully evaluate the effect of NSAIDs on colorectal cancer risk. However, several studies have suggested that duration of NSAIDs use is an important factor in lowering colorectal cancer, with longer use being associated with decreased risk

(62, 92, 95, 96). The protective effect of NSAIDs and ASA in preventing adenomatous polyps is evident regardless of other underlying risk factors including gender, age, smoking history, alcohol consumption, family history of colorectal cancer, body mass, and dietary fiber intake (99). Research on colorectal cancer and adenoma indicates that the protective effect of NSAIDs on neoplasia requires continued use with infrequent or previous use not being associated with reduced risk. The neoplastic effect of NSAIDs rapidly reverse after cessation of use (62).

The use of ASA has also been consistently associated with a reduced risk of esophageal and stomach cancer (100, 101). A case control study of breast cancer found that frequent use of NSAIDs and/or COX-2 inhibitors was associated with a lower risk of breast cancer (OR= 0.75, 95% CI 0.64-0.89), with similar results for COX-2 inhibitors (OR= 0.81, 95% CI 0.69-0.97) and NSAIDs (OR= 0.65, 95% CI 0.43-0.99) when assessed separately (102). Only the frequent use of ASA at doses >100 mg/day was associated with a lower risk of breast cancer (OR= 0.75, 95% CI 0.64-0.89) (102). A population-based analysis of the North Jutland Prescription Database and the Danish Cancer Registry compared cancer incidence among 172,057 individuals prescribed non-aspirin NSAIDs, with expected incidence (based on county specific rates) of cancer during a 9 year study period, and found that the standardized incidence ratio (SIR) was 1.1, 95 percent CI 1.0-1.1 among NSAIDs users (103). When SIRs were examined for individual cancers in people who had 10 or more prescriptions

for non-aspirin NSAIDs they found reduced risk estimates for stomach (SIR= 0.7, 95% CI 0.4-1.1), ovarian cancer (SIR 0.7, 95% CI 0.4-1.0), colon (SIR= 0.7, 95% CI 0.6-0.9), and rectum (SIR= 0.6, 95% CI 0.4-0.9) (103). Increased SIRs were found for lung, kidney and prostate cancers with SIRs between 1.3 and 1.6 (103). The SIR for NMSC was 1.1 (95% CI 1.0-1.2) (103). When the same analysis was done looking at those who used low dose aspirin the overall SIR was 1.09, 95% CI 1.05-1.13 (104). There were no risk reductions for colon or rectum or for other site specific cancers. Increased SIRs were seen for kidney and brain (SIR= 1.4, 95% CI 1.1-1.7 and SIR= 1.7, 95% CI 1.3-2.2, respectively) (104).

Other studies have not found a reduction in risk. The Women's Health Study (WHS) used a 2 x 2 factorial design to evaluate the effects of low-dose ASA (100 mg) taken every other day and 600 IU of vitamin E (in the form of natural source alpha-tocopherol), also taken every other day for a period of 10 years. ASA did not reduce overall cancer incidence or mortality, however it was found to reduce stroke risk (100). This study was of a relatively large size, long duration, and had a substantial number of outcome events (2865 cases of cancer overall). Thus, it was not likely to have missed any important effects on overall cancer incidence or overall cancer mortality (100).

8.4: NSAIDs and NMSC

Topical agents such as 3.0 percent diclofenac sodium gel have been successfully used to treat AKs. One study showed that after a 90 day treatment

period, 78 percent of the patients had greater than or equal to 75 percent AK lesion clearance based on the target lesion number score, with this improving to 85 percent of patients at day 120 (105). A second study showed that a higher proportion of those treated with 3.0 percent diclofenac gel had a target lesion number score of zero compared to the placebo group (50% versus 20%; $p < 0.0001$) (106). In addition, a higher proportion of treated patients had a cumulative lesion number score of zero compared to the placebo group (47% versus 19%; $p < 0.001$) (106). A third study examining AK treatment with 3.0 percent diclofenac in 2.5 percent hyaluronan gel found almost identical results with patients in the active treatment group showing significant improvement in target lesion scores, cumulative lesion number scores and lesion total thickness scores, all within the same magnitude as the previously mentioned studies (107). In a study of patients with a history of Chronic Lymphocytic Leukemia who were immunosuppressed and had Bowen's Disease (SCC in-situ), the combination of an immune modulator (5% imiquimod) and a COX inhibitor (sulindac), was found to produce clinical resolution and histologic clearing of SCC tumors after 16 weeks of therapy (108). Topical indomethacin applied after an erythemogenic dose of UVB light has been shown to suppress the development of erythema by inhibition of the prostaglandin synthesis cascade (66). In addition, topical indomethacin has been shown to act as a potent UVB and UVA photoabsorption filter when applied topically prior to UV exposure in humans (66).

Until recently there was virtually no epidemiological evidence in the literature regarding NMSC and NSAIDs. To date, there are now a couple of studies in addition to the in vitro and animal model studies.

8.4.1: In Vitro Studies

Prostaglandin E₂ (PGE₂) generation can regulate epidermal cell proliferation in vitro. Elevated levels of PGE₂ have been observed in SCC and BCC of the skin. These may correlate with an increased propensity for metastatic and invasive behavior (67). COX-2 expression and PGE₂ production have been studied in human skin epidermal cancer cell lines, cutaneous SCC (HSC-5) and eccrine carcinoma (EcCa), and found to be enhanced in the cancer cell lines compared with the non-tumorigenic human keratinocyte cell line (HaCaT) (109). When COX-2 was suppressed, cell growth in the cancer cell lines was also suppressed.

It has also been demonstrated that acute exposure of human keratinocytes to UVB irradiation results in increased production of PGE₂. When human subjects were irradiated on sun-protected skin with up to four times their minimal erythema dosage and biopsied 24 hours later, up-regulation of COX-2 protein expression was not observed (67). Human SCC biopsies exhibited strongly enhanced staining for COX-2 protein via immunohistochemistry as compared to normal non-sun-exposed control skin (67). Both of these facts demonstrate acute up-regulation of COX-2 via UVB irradiation.

The main source of prostaglandin generation in the SCC tumors appears to be resulting from the keratinocytes. These data may further suggest that COX-2 activation is increased prior to the development of overt malignancy and may therefore play a role in tumor promotion. In addition to the cyclooxygenase pathway, a series of potential new molecular targets for NSAIDs, involved mainly in signaling pathways, such as extracellular signal-regulated kinases, activator protein 1, NFkB, and peroxisome proliferators-activated receptor signaling pathways have been identified (63).

8.4.2: Animal Model Studies

As previously stated, it is believed that NSAIDs act to prevent carcinogenesis by inhibiting one or both of the known forms of COX (COX-1 or COX-2). In a study using the wild type and COX deficient mice, it was found that the mice deficient in either COX-1 or COX-2 developed 75 percent fewer skin papillomas as compared to the wild type mice (110). This study also showed that it may not be apoptosis that COX inhibition is affecting but rather the early stages of keratinocyte differentiation (110). Apoptosis, a cellular process associated with NSAIDs induced inhibition of tumorigenesis, was not significantly altered in the epidermis or in the papillomas of the COX-deficient mice. It would appear that both COX-1 and COX-2 have roles in keratinocyte differentiation, and that the absence of either isoform causes premature terminal differentiation of initiated keratinocytes and reduced tumor formation (110). Because loricin, a

protein expressed in the later stages of keratinocyte differentiation, was not significantly altered by COX deficiency, it was suggested that COX-1 and COX-2 are involved in the early stages of differentiation, and that COX-1 and COX-2 contribute to skin tumor growth by overlapping but non-identical mechanisms (110).

UV radiation has DNA-damaging, tumor initiation and tumor-promotion activity. The combination of damage to the skin resulting from chronic UVB exposure and the inflammatory response it induces, is a major source of skin cancer development (111). In UV carcinogenesis mouse models, celecoxib treatment has been found to reduce tumor multiplicities by nearly 90 percent, and that intervention late in the UV carcinogenesis process reduced subsequent tumor multiplicity and size (64). In a study by Fischer et al., hairless mice irradiated with UV and then treated with D,L- α -difluoromethylornithine (DFMO), celecoxib, or DFMO and celecoxib were found to have tumor regression and a reduction in tumor number. The combination of celecoxib and DFMO showed the greatest regression, with an 89 percent reduction in tumor number as compared to the control group (64). In the celecoxib group there was a 25 percent reduction in tumor number suggesting that celecoxib has a minor effect on tumor regression, but a significant effect in preventing new tumor development (64).

Another study by Fisher et al. evaluated the ability of celecoxib and indomethacin to block UV induced skin tumor development in hairless mice

(112). Mice fed with celecoxib showed a dose-dependent reduction (60%-89%) in tumor yield and mice fed indomethacin reduced tumor yield by 78 percent (112). They reported that acute and chronic UV exposure increased cell proliferation and edema and that neither compound reduced these parameters, while UV induced prostaglandin synthesis in the epidermis was blocked by both compounds (112). UV induced increases in COX-2 expression in skin were not altered in either treatment group, and tumors that constitutively express high levels of COX-2 displayed no reduction by treatment with either celecoxib or indomethacin (112). Indomethacin has also been shown to affect tumor initiation and promotion by UV radiation in another mouse model. When indomethacin was given to mice during photocarcinogenesis induction, the probability of remaining tumor free was increased and the average tumor multiplicity was decreased (113). When indomethacin was administered only during the initiation period, there was a reduction in tumor multiplicity and the progression of tumors to malignant SCC, and when it was administered in the post-irradiation promotion period, there was an increase in the probability of remaining tumor free (113).

In a study of the inhibitory effects of sodium salicylate and acetylsalicylic acid on UVB-induced mouse carcinogenesis, the higher dose of ASA (40 μ mol) significantly inhibited the rate of tumor formation ($p < 0.05$) and the lower dose (10 μ mol) had no inhibitory effect as compared to the control (114). Further investigation revealed that ASA does not inhibit UVB-induced thymine dimer

formation and the moderate inhibition of ASA is likely due to a molecular event, such as the inhibition of various UVB signaling pathways (114).

Topical treatment with celecoxib following UVB irradiation has been shown to inhibit several parameters of acute inflammation, including vascular permeability, the infiltration and activation of neutrophils, and the production of PGE₂ (111). In addition it has been shown to inhibit acute oxidative damage, and in long-term studies to reduce chronic inflammation and UVB-induced papilloma/carcinoma formation (111).

Pentland et al. conducted a study looking at the differences in tumor number and multiplicity in mice previously irradiated with UV and then treated with oral celecoxib or placebo (54). There was a 56 percent reduction in tumor number and multiplicity in the drug treated group showing that celecoxib was able to prevent new tumor formation after the onset of photocarcinogenesis (54).

In general, the animal model studies indicate that COX-1 and COX-2 work by blocking prostaglandin synthesis, and likely have different but overlapping mechanisms by which they promote tumor growth. NSAIDs appear to reduce the size and multiplicity of tumors both when used before, during and after irradiation and before and after onset of photocarcinogenesis. NSAIDs action has been observed when given weeks after carcinogen administration or during the early promotion or late initiation phases of carcinogenesis (62). The anticancer action they exert appears to be reversible in that tumor occurrence increases shortly after discontinuation of the agent (62).

8.4.3: NSAIDs in Dermatologic Therapy

NSAIDs have long been in use to treat many different dermatological problems including acne, psoriasis, sunburn, erythema nodosum, cryoglobulinemia, Sweet's syndrome, systemic mastocytosis, urticarial, livedoid and nodular vasculitis to name a few (72). As previously stated, NSAIDs act mainly by inhibiting prostaglandin synthesis via the COX pathway. A small randomized, double-blind crossover study of 10 healthy women has shown that NSAIDs (ASA and indomethacin) can be protective against UV induced erythema development (66). However, there are no data confirming that inhibiting sunburn with NSAIDs use can prevent chronic long-term sunburn effects (66).

8.4.4: NSAIDs in Skin Cancer Prevention

There have only been two previous studies of the role of NSAIDs in skin cancer prevention. First, Grau et al. published a study in 2006 which examined the association of NSAIDs use with the risk of BCC and SCC using data from the Skin Cancer Chemoprevention Study (115). The Skin Cancer Chemoprevention Study was a 1,805 subject study that randomized people with a recent history of NMSC to placebo or 50 mg a day of β -carotene for approximately five years. In addition, subjects were asked about their NSAIDs use. They defined NSAIDs exposure in two ways. The first was a binary variable that indicated the report of NSAIDs use in any of the questionnaires for a given study period. The second was as an ordered categorical variable that attempted to account for duration of

exposure as the questionnaires, administered every 4 months during the study, did not ask information about dose or frequency of use. The three categories were none, sporadic use (less than half of questionnaires positive for NSAIDs use), and frequent use (more than half of the questionnaires positive).

Covariates in the full models included age, sex, center, skin type, number of nonmelanoma skin cancers prior to study entry and number of completed questionnaires. They found that for BCC, NSAIDs exhibited a weak protective effect in crude analyses, which attenuated after adjustment. For SCC, the use of NSAIDs in the year previous to diagnosis reduced the odds by almost 30 percent (adjusted OR=0.71, 95% CI 0.48-1.04) (115). When they attempted to account for frequency of use, the results for BCC and SCC were not striking.

A second study by Butler et al., published in 2006, was a nested case-control study looking at the risk of AK and SCC in those using NSAIDs, who were part of the Nambour Skin Cancer Prevention Trial (116). The original cohort members were randomly selected from the Nambour community in Queensland, Australia and were randomized in a 2x2 factorial design to daily sunscreen vs. discretionary use of sunscreen and beta carotene supplements vs. placebo. For the case-control study, there were 86 SCC cases who were eligible, available and agreed. Control subjects were active cohort member with no diagnosis of SCC. Past and current NSAIDs use was assessed by a structured face-to-face interview with a single medical practitioner blinded to the subject's skin cancer status. Participants were asked whether they had ever used any of the NSAIDs

provided on a list along with color photographs of packaging. Subjects who indicated use were then asked about age at first and last use, as well as regularity, intensity of use and typical dose for the time periods, last year, 1-5 years ago and greater than five years ago. Subjects were categorized as either never users, regular users or other users of NSAIDs. Regular users were defined as subjects who had taken any type of NSAIDs two or more times per week for at least one year. Within the regular use category, there were two frequency categories: those who used a lower dose (ingestion of at least one tablet of any type of NSAIDs greater than or equal to two times per week) and higher (ingestion of at least one tablet of any type of NSAIDs greater than or equal to eight times per week). Subjects who took NSAIDs infrequently or for less than one year at the given frequency threshold were considered other users, and never users were those who had not reported any use. The authors found that those who used NSAIDs eight or more times per week for more than one year had a substantially lower incidence of SCC (OR=0.07, 95% CI 0.01-0.71), and that those who used full dose NSAIDs two or more times per week for more than five years also had a lower incidence (OR=0.20, 95% CI 0.04-0.96) (116). The analysis of AK was restricted to the control group only. Furthermore, results indicated that control subjects who were regular users of NSAIDs in the past year had lower AK counts than never users (rate ratio=0.67, 95% CI 0.36-1.23). After adjustment for age, sex, skin color, tanning ability, occupation, clinical solar elastosis, hip or knee pain and sunscreen intervention arm, the association

between regular full dose use and AK counts still remained (rate ratio=0.52, 95% CI 0.30-0.91).

Section 9: Retinoids and Chemoprevention

Vitamin A and all of its synthetic derivatives are referred to as retinoids. Vitamin A is necessary for the normal development of all epithelial tissues (117-119). The retinoids exert a variety of effects and are vital for embryogenesis, reproduction, vision, glycoprotein production and regulation of inflammation, growth, and differentiation of normal and neoplastic cells in vertebrates, and tend to accumulate preferentially in the skin (119-123). Vitamin A appears to induce proliferation and differentiation of the basal germinative layer of epithelial cells (123).

There are two dietary forms of Vitamin A: provitamins or the carotenes, which are derived exclusively from plant sources but not synthesized by humans, and the alcohol and aldehyde forms or preformed vitamins and their esters found in milk, eggs, and meat (122, 124). Vitamin A is stored in the liver in lipocytes as retinyl esters (122). Excreted vitamin A can be reabsorbed and recycled, to meet a portion of the daily requirements. Thus vitamin A can be conserved when stores are low and excreted when stores are high (124). Serum vitamin A levels are also affected by numerous common conditions including the menstrual cycle, use of oral contraceptives, and some disease states such as chronic pancreatitis, bile acid insufficiency, hyperthyroidism, Lyme disease and nephrosis (124).

One of the major drawbacks of the retinoids is the toxicity associated with taking excessive amounts. The first and classic reported cases of acute hypervitaminosis A involved Eskimos and Arctic explorers ingesting polar bear or seal liver meat (117). These individuals developed severe headaches, drowsiness, irritability, nausea and vomiting, erythema and desquamation of the face, trunk, palms and soles of the feet with symptoms resolving in 7-10 days (117). Toxic effects can differ depending on the retinoid being used. These side effects have always been an issue in chemoprevention studies because there is a fine balance between acceptable toxicity and the desired preventative outcome. Side effects of the synthetic retinoids involve mucocutaneous drying and chapping leading to cheilitis, facial dermatitis, conjunctivitis, dryness of the nasal mucosa with minor nosebleeds, dry mouth with thirst, xerosis, hair loss, palmoplantar desquamation, stratum corneum fragility or easy peeling due to minor frictional trauma and scratching, paronychia and nail abnormalities (125).

Since 1968, approximately 1500 retinoids have been synthesized by modifying either the ring structure, side chain or terminal group of the molecule (125, 126). Retinoids are biologically tested to show varying degrees of activity, measured by the criteria of papilloma regression, hypervitaminosis A, and therapeutic index (126).

Isotretinoin is the stereoisomer of the naturally occurring vitamin A acid, and a first generation analog considered a nonaromatic retinoid developed for systemic and topical treatment of various skin disorders (120, 127). The

pharmacokinetics of isotretinoin have been characterized in both healthy subjects, subjects with dermatologic disorders, and subjects with preneoplastic lesions or cancer and are similar for all groups (127). Isotretinoin is absorbed by the gastrointestinal tract but has low absolute bioavailability; the drug is degraded in the gut and absorbed or excreted in the stool either as a metabolite or parent drug or in the urine as a metabolite (127). Isotretinoin has a low potential for hepatic toxicity, and hepatic concentrations peak and fall with serum concentrations with no marked hepatic accumulation (127). There are both long term and short term toxicities associated with isotretinoin (127). These include abnormal spermatogenesis or embryonic development, lipid changes (increase in triglycerides but not cholesterol), hyperostosis and bone fractures, bone and joint pain, and diffuse idiopathic skeletal hyperostosis (127).

9.1 Retinoids Mechanism

Retinoids produce their biological effects through gene transcription altering by binding to nuclear receptors known as RA receptors and retinoid X receptors (RXRs) (120, 121). They all belong to the super-family of ligand-inducible transcriptional regulators that include steroid hormone receptors, thyroid hormone receptors and vitamin D₃ receptors (120, 121). The anticancer effects of vitamin A are thought to work via changes in gene expression through activation of a signal transduction pathway in which nuclear retinoid acid receptors (RARs) play a role. Natural and synthetic retinoids have been found to

activate different retinoid receptors (128). RARs can be divided into two types: RARs and RXRs, with each type including three sub types: α , β and γ (129, 130). RARs and RXRs can either activate or suppress gene expression by binding to the cis-acting DNA response elements (130). RAR γ and RXR α are the predominant receptors found in the skin (128). RAR γ is abundant in the epidermis and skin where it may play a role in maintenance and differentiation (130). RXR α and RXR β are localized to the basal cell layer of the epidermis (130).

The expression pattern of these aforementioned receptors has been investigated in an attempt to understand their involvement in skin carcinogenesis (130). RAR α messenger RNA (mRNA) was found to be down regulated in papillomas and carcinomas, while RXR α mRNA was up regulated in carcinomas. This up-regulation of RXR α in carcinomas suggests that certain nuclear signal transduction pathways requiring RXR for heterodimer formation may be elevated because of the growth advantages and metastatic potential of these cells (130).

Decreased expression of nuclear RARs and their induction by retinoids have been reported in skin cancers and premalignant lesions (129). In a study looking at SCC tumors 94 percent of the specimens tested had decreased RARs α and β and 88 percent had a reduction in RXR α while there was no receptor loss in BCC tumors (128). The Alberts et al. study of vitamin A dose escalation showed that vitamin A increases expression of RARs and RXR α and decreased karyometric scores in sun damaged skin, especially at doses $\geq 50,000$ IU. This

suggests that vitamin A works through the mediation of nuclear retinoid receptors and in a dose-response manner (129).

Retinoid excess can lead to an increase proliferation while deficiency leads to decreased proliferation, along with enhance squamous differentiation in the basal cell layer of the skin (128). Evidence indicates that it is the promotion and progression steps of carcinogenesis that can be inhibited by retinoids (128)

9.2 Retinoids and NMSC

9.2.1 In Vitro Studies

Studies of tumor cells grown in vitro, showed that the addition of retinoic acid to the culture medium reduced proliferation and hinted that the retinoids may be useful in the prevention or treatment of cancer (121). In cell cultures retinoids inhibit the growth of various cell lines by directly inhibiting cell proliferation (126). In organ cultures of prostate, trachea, and fetal lung, they prevent or reverse hyperplasia and metaplasia induced by vitamin A deficiency or carcinogenetic agents (126). Studies have also shown that retinoids can prevent and reverse malignant transformation of cells, probably by restoring the properties of non-transformed cells and normal growth control, suggesting that retinoids suppress the phenotypic expression of malignancy (126).

9.2.2 Animal Model Studies

Vitamin A was first recognized in 1909. In 1925, Wolbach and Howe demonstrated that a vitamin A deficient diet led to metaplasia of the gastrointestinal, respiratory, and urogenital epithelia in rats and Fujumaki et al. discovered that carcinoma developed in the stomach of rats deprived of adequate dietary vitamin A (122, 131). In addition squamous metaplasia developed in the epithelia of the eye, nasal mucosa, respiratory tract, salivary gland, tracheal epithelium, urinary bladder and pancreatic ducts of animals deficient in vitamin A, where normal mucus-secreting columnar epithelium was replaced by keratinizing squamous cells (123, 132). Many additional early animal studies established a connection between vitamin A deficiency and susceptibility to cancer (121, 123).

Since many malignancies are epithelial in origin, control of epithelial cell differentiation is an issue. Because retinoids are known to play a role in differentiation, it is not surprising that there is such a large body of work in the area of retinoids and cancer (133). Retinoids have been successfully used to prevent cancer of the skin, lung, bladder and breast in experimental animals (134). All-trans-retinoic acid was one of the first retinoids tested, apart from vitamin A and it was found to prevent chemical induction of skin papillomas and carcinomas of mice. It also exerted a therapeutic effect, leading to pronounced regression of established papillomas and squamous cell carcinomas (126).

Early mice studies by Bollag found that when retinoids are administered during the promotion phase of carcinogenesis, there is a reduction of papilloma and carcinoma multiplicity, as well as a decrease in carcinoma incidence with papilloma growth being retarded and papilloma regression enhanced (135). This led to the idea that there is a dual effect of retinoids in skin carcinogenesis: a prophylactic or preventative effect (delayed appearance and reduced incidence), as well as a therapeutic effect, manifested by the retarded growth and enhanced regression of the papillomas. In animal experiments where retinoid administration was delayed until several well established papillomas were present, the administration of a retinol resulted in regression of the established papillomas (135). Additional animal studies in SENCAR mice have found that dietary retinoic acid and topical retinoic acid can inhibit both chemical and ultraviolet B radiation induced papillomas from conversion to carcinomas without affecting papilloma incidence (136, 137).

9.2.3 Retinoids and Cancer

In 1941 Abels et al. provided the first scientific data that associated vitamin A deficiency with cancer in humans (122). The recognition that vitamin A deficiency leads to hyperkeratosis of the skin and to squamous metaplasia of mucous membranes, and that retinoids rapidly reverse skin metaplasias eventually led to the establishment of retinoic acid treatment for many skin diseases including Darier's Disease, Lamellar Ichthyosis, Pityriasis Rubra Pilaris,

and Cystic Acne (125, 125, 138-140). In addition, an early epidemiological study of over 8000 Norwegian men revealed that a low dietary intake of vitamin A was correlated with a high incidence of lung cancer after matching for equivalent smoking habits (141). Retinoids have also been used in the treatment of advanced cancer with partial responses and responses of short duration seen in patients with advanced melanoma, non-small cell lung cancer, head and neck cancers and ovarian cancer (142).

Both prospective and retrospective dietary intake studies of vitamin A and cancer (lung, colon, stomach, prostate, cervix, breast, larynx, esophagus, oral, intestinal, gastrointestinal, etc.) generally show that there are statistically significant differences when comparing the lowest vitamin A intake group to the highest vitamin A intake group, or at a minimum an inverse association between consumption of foods high in vitamin A and cancer (124, 135). Dietary intake studies of the association between retinol and BCC and SCC have shown inconsistent results (143). Of the 11 dietary studies reviewed, only one hospital based case control study by Wei et al. showed an inverse association between the use of vitamin A supplements and the development of BCC (OR=0.20, 95% CI 0.06-0.62).

Serum retinol levels have been studied to determine their association with risk of various cancers in an attempt to understand the relationship further. A review of 13 serum retinol level studies, both prospective and retrospective, showed that low vitamin A levels existed in the cancer population and potentially

played a role in incidence, basically suggesting that the lower the serum vitamin A level, the greater the cancer risk (124). Another case control study evaluating a community sample of 3102 individuals and their stored serum, found that persons that eventually developed cancer had significantly lower mean serum retinol levels at least 12 months before the cancer diagnosis when compared with controls. The association was the same for all four race-sex groups and consistent for various cancer sites and cell types (144).

Wald et al. evaluated the retinol concentration in stored serum samples in 227 cancer cases and 454 controls (145). Those who developed cancer within one year since the time the blood was collected had lower serum retinol concentrations than those who developed cancer one to two years after blood was collected. Controls had higher levels of serum retinol even when compared to those whose cancer developed three or more years after the blood was collected. This indicated that low serum retinol may be a metabolic consequence of cancer rather than a precursor of cancer (145).

The majority of primary human cancers arise in epithelial tissues that are dependent on retinoids for normal cellular differentiation (131). The potential chemopreventative properties of retinoids have been studied extensively in various animal models, epidemiologic studies and clinical trials, and have shown their activity in treatment or prevention of skin malignancy. Actinic keratoses were the first skin lesions to be successfully treated with topical all-trans-retinoic acid (120, 146). Retinoids have also been used to successfully treat

Keratoacanthoma, BCC, Epidermodysplasia Verruciformis, and dysplastic nevus syndrome (117, 146, 147). Retinoids have also been investigated as therapeutic anticancer agents and been found to be effective for cutaneous BCC, cutaneous SCC, and malignant eccrine poroma (117).

Several studies of serum retinol levels have focused specifically on skin cancer. Kune et al. found that the mean level of serum β -carotene and vitamin A were statistically significantly lower in cases than controls and that this difference was present for both BCC and SCC. In a dose response analysis of β -carotene, there was decreasing NMSC rates with increasing levels of serum β -carotene. However, Kargas et al. found no association between plasma concentrations of retinol and SCC in a nested case-control study (148).

9.2.4 Retinoids and Dermatologic Therapy

Isotretinoin, also known as 13-cis-retinoic acid, is administered either as a 0.05 percent topical cream or in an oral form (0.25-1.0 mg/kg/d) and is used to treat cystic acne, recalcitrant nodular acne, rosacea, gram-negative folliculitis, pyoderma faciale, hidradenitis suppurativa and also for cancer prevention (120). Oral retinoid therapy seems to prevent or delay development of cutaneous carcinomas in some cutaneous diseases with a high risk of malignancy, such as xeroderma pigmentosum, Mibelli's porokeratosis, Gorlin's disease, Ferguson-Smith disease and solar keratosis (149).

9.2.5 Chemoprevention Studies

The chemopreventative effects of retinoic acid are thought to occur at the promotion stage rather than the initiation stage (121). The biological activity of retinoids is acquired through their irreversible conversion to retinoic acid, which is a natural product derived by the oxidation of retinol (119). Because of the role retinoids play in cellular differentiation and proliferation in many cell types including ectodermal, endodermal, and mesodermal; epithelial, fibroblastic, and mesenchymal; and neoplastic, preneoplastic and non-neoplastic, there is reason to believe they may have chemopreventive effects (122, 150). In addition retinoids have been shown to be chemopreventative in several cancer progression pathways including promyelocytic leukemia, SCC and cancers of the head and neck (121).

Retinol and its analogues have been studied and continue to be studied as chemopreventative agents in a wide range of cancers, including skin, with mixed results. Non-dermatological cancers that retinoids have been used successfully as chemopreventative agents include leukoplakia, bronchial metaplasia, laryngeal papillomatosis, cervical dysplasia, myelodysplastic syndromes, and some bladder tumors (117, 131, 151). Isotretinoin, one of the analogues of retinol has been shown to prevent recurrence of BCC at new sites in patients previously diagnosed with BCC (152). Acitretin another analogue of retinol has been successfully used to prevent/reduce NMSC in renal transplant patients (153-156). Topical retinoids have also been found to be no more

effective than placebo to treat biopsy proven cervical intraepithelial neoplasia (CIN) II/III (157).

Several studies of isotretinoin given for one to two years to prevent recurrence of second primary tumors in patients treated for SCC of the head and neck (HNSCC) found the treatment was ineffective (158, 159). In addition in a randomized, 8 year trial (3 years on intervention and 4 years of follow-up) in 1,190 patients with a history of stage I or II HNSCC, low dose isotretinoin was found to be no more effective in reducing the rate of second primary tumors than placebo (HR=1.06, 95% CI 0.83-1.35) or increasing the rate of survival (HR=1.03, 95% CI 0.81-1.32) Table 9.1 summarizes the skin cancer chemoprevention studies that have used retinol and Table 9.2 summarizes epidemiological studies.

Table 9.1: Retinol Skin Cancer Chemoprevention Studies

Source	No. of Patients Enrolled	Interventions and Design	Patient Population	Primary Result
W. Hong et al. (160)	103	Randomized-isotretinoin 50-100 mg per square meter or placebo	Patients who had undergone primary treatment for squamous cell cancers of the larynx, pharynx, or oral cavity	<ul style="list-style-type: none"> • No difference between groups based on recurrences of the primary cancers • Isotretinoin group had significantly fewer second primary tumors
L. Goldberg	2	Case Study-twins	Twins with basal cell	<ul style="list-style-type: none"> • Isotretinoin at 0.4 mg/kg/day

et al. (161)		Isotretinoin 0.4 mg/kg/day and 0.2 mg/kg/day	nevus syndrome	<p>was effective in preventing the formation of the majority of new BCCs and reduced rate of growth of existing lesions</p> <ul style="list-style-type: none"> • Isotretinoin at 0.2 mg/kg/day was less effective
G. Peck et al. (162)	12	Prospective 3.1 mg/kg/day isotretinoin	Patients with multiple basal cell carcinomas resulting from varying causes	<ul style="list-style-type: none"> • Of 270 tumors 8% underwent complete clinical remission • All patients had toxicity • Lower doses 0.25 to 1.5 mg/kg/day were ineffective for chemotherapy but demonstrated a chemopreventative effect in 3 patients who took it for 3 to 8 years • Need for long-term maintenance therapy with isotretinoin for chemoprevention of BCC depends on underlying cause of NMSC
G. Peck et al. (163)	3	Prospective 1.5 mg/kg/day isotretinoin for	Patients with multiple BCCs due to	<ul style="list-style-type: none"> • 9 of 65 identified lesion underwent complete clinical

		2.5-4 years	excessive sunlight, nevoid BCC syndrome or arsenical insecticide exposure	<p>regression</p> <ul style="list-style-type: none"> No tumors enlarged in 2 patients A few tumors enlarged slightly in a third patient No new lesions in any of the patients
G. Goodman et al. (164)	13	100,000 units/m ² -350,000 units/m ² Retinol	Cancer patients	<ul style="list-style-type: none"> Mixed responses Dose of 200,000 U/m²/day is recommended
G. Peck et al. (165)	11	Average max dose 4.7 mg/kg/day 13-cis-retinoic acid	Patients with multiple basal cell carcinoma	<ul style="list-style-type: none"> Of 248 tumors 16% underwent complete clinical regression 23% of tumors 3-5 mm and 20% of tumors 6-10 mm underwent complete clinical regression Of 248 tumors 65% decreased in size Of 248 tumors 19% were unchanged
F. Meyskens et al. (118)	117	Phase II 3mg/kg/day isotretinoin for patients with advanced cancer 2 mg/kg/day for patients with preneoplastic lesions	Patients with advanced cancer or aggressive preneoplastic conditions of squamous cell histology	<ul style="list-style-type: none"> Considerable activity seen in patients with squamous cell epithelial disease 6 of 14 with squamous cell epithelial cancer had objective regressions of

				<p>skin or subcutaneous metastases</p> <ul style="list-style-type: none"> • 3 of 5 patients with preneoplastic lesions had objective responses
S. Lippman et al. (166)	4	Case series trial Isotretinoin 1 mg/kg	Patients with refractory advanced squamous cell carcinoma of the skin	<ul style="list-style-type: none"> • Impressive response in all 4 patients • Retinoids may be an effective and well-tolerated therapy for refractory advanced SCC of the skin
G. Peck (167)	2 cases	Case study Oral isotretinoin high dose (2.0-3.0 mg/kg/day) and low dose 1.5-0.25 mg/kg/day Total of 7-8 years	Patient 1 with nevoid basal cell carcinoma syndrome Patient 2 with arsenical insecticide exposure	<ul style="list-style-type: none"> • Treated with high dose for chemotherapy-15% of lesions underwent complete clinical regression • Low dose aimed at chemoprevention and no new lesions in arsenical exposure patient • After discontinuation new lesions in arsenical exposure patient appeared in 17 months • NBCCS patient developed tumors within 13 months

				<ul style="list-style-type: none"> • Long term therapy is necessary for continuation of cancer chemopreventive effect
A. Sankowski et al. (168)	21 patients	15 treated with 0.6% 13-cis-retinoic acid in an ointment for 21 days	BCC of the face	<ul style="list-style-type: none"> • 2 had complete regression • 13 had moderate decrease of tumor size
J. Tangrea et al. (152)	981	Randomized - 10 mg isotretinoin or placebo	2 or more previously confirmed BCC	<ul style="list-style-type: none"> • After 36 months of treatment, no significant difference in either cumulative percent of patients with an occurrence of BCC at a new site or the annual rate of BCC formation • Significant adverse systemic effects
K, Kraemer et al. (169)	5	Prospective 3 year of oral isotretinoin	Xeroderma pigmentosum with a history of multiple NMSC	<ul style="list-style-type: none"> • During two years of treatment average reduction in NMSC of 63% (p=0.019) • Mean 8.5 fold increase in number of NMSC after treatment discontinuation over frequency during treatment (p=0.007) • High dose oral

				isotretinoin was effective in chemoprevention of NMSC in patients with xeroderma pigmentosum
F. Khuri et al. (170)	1190	Randomized control trial (isotretinoin)	Patients previous treated for stage I or II HNSCC	<ul style="list-style-type: none"> • Randomized, 8 year trial (3 years on intervention and 4 years of follow-up), low dose isotretinoin was found to be no more effective in reducing the rate of second primary tumors than placebo (HR=1.06, 95% CI 0.83-1.35) or increasing the rate of survival (HR=1.03, 95% CI 0.81-1.32)
S. Toma et al. (158)	267	Randomized control trial (isotretinoin)	Patients previously treated for stage III and IV HNSCC	<ul style="list-style-type: none"> • 5 year actuarial survival =58.9% for intervention and 57.2% control (p=0.94) • Disease progression 36.6% in treatment and 33.9% in control
J. Bavinck et al. (156)	44	Randomized control trial (acitretin)	Renal transplant patients with > 10 AK	<ul style="list-style-type: none"> • 11% in treatment group reported new SCC and 47% in control group (p=.01) • Relative decrease in

				number of keratotic skin lesions in treatment group =13.4% as compared to relative increase of 28.2% in control group (difference=41.6%, 95% CI 11.5-71.7)
D. McKenna et al. (155)	16	Open label subjects act as own control (acitretin)	Renal transplant patients having had at least 2 NMSC	<ul style="list-style-type: none"> • Significant reduction in the total number of tumors excised during period of therapy as compared with pretreatment interval • 50% remained tumor free while on study drug
G. Gibson et al. (154)	11	Open label subjects act as own control (acitretin)	Renal transplant patients having skin lesions	<ul style="list-style-type: none"> • Significant reduction in number of skin cancers during treatment compared with pre-treatment (3 and 6 months of treatment $p<0.001$) and trend towards fewer skin cancers in the 12 to 18 month treatment period
R. George et al. (153)	23	Open randomized crossover (acitretin)	Renal transplant patients	<ul style="list-style-type: none"> • Number of SCC in treatment was significantly lower than in the

				<p>drug-free period (p=0.002)</p> <ul style="list-style-type: none"> • Benefit of treatment did not continue into the drug-free period
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Table 9.2: Retinol Epidemiologic Studies

Source	No. of Patients Enrolled	Interventions and Design	Patient Population	Primary Result
G. Kune et al. (171)	88 controls 88 cases (NMSC)	Case-control	Male patients admitted for surgical removal of NMSC	<ul style="list-style-type: none"> • Inverse relationship for fish intake (p=0.05), vegetables (p<0.001); beans, lentils, peas (p<0.001), carrots, silverbeet, pumpkin (p<0.001), β-carotene, vitamin C foods (p=0.004) • Cases has lower mean serum levels of β-carotene (p<0.001) and vitamin A (p=0.02) • Incidence of NMSC inversely related to level of serum β-carotene (p<0.0001)
Q. Wei et al. (172)	131 cases 200 controls	Clinic based case-control	Patients with histopathologically confirmed primary BCC	<ul style="list-style-type: none"> • Regular intake of vitamin supplements was associated with a nearly 70%

			and 200 cancer free controls with non-premalignant skin disorders	<p>reduced risk for BCC (OR=0.3, 95% CI 0.2-0.6)</p> <ul style="list-style-type: none"> • Lowest OR's associated with vitamin A (OR=0.2, 95% CI 0.06-0.62) • Low response rate issue with selection and recall bias
T. Nijsten & R. Stern (105)	135	Nested cohort	Patients in the PUVA follow-up study who reported at least 1 year of substantial retinoid use between 1985 and 2000	<ul style="list-style-type: none"> • Comparison of a patients own tumor experience while using and not using retinoids • Retinoid use associated with a 30% reduction in SCC (p=0.02) • Incidence of SCC significantly decreased during years of substantial retinoid use (IR=0.79, 95% CI 0.65-0.95) • No association between BCC incidence and oral retinoid use

Section 10: Epidemiological Assessment of NMSC

Epidemiologic studies often use personal interviews or self-administered questionnaires as a sole source of exposure information (173). Self-administered questionnaires do not generate the same information, particularly

for less severe or transient disease, as personal interviews (174). Agreement between self-report from the interview and from the questionnaire was highest ($\kappa=0.83-0.88$) for myocardial infarction, cancer and diabetes mellitus (174). In addition it has been suggested that accurate reporting is more likely for diseases that have clear and unambiguous diagnostic criteria (173). If patients feel that NMSC and AK's are not severe, they may be less likely to reliably report them.

In a study comparing patient self reported history of skin cancer to the gold standard of chart documentation, patients were found to correctly identify their BCC status in 84.3 percent of the cases, their SCC status in 81.5 percent of cases, their overall NMSC status in 91.8 percent of cases, and their melanoma skin cancer status in 94.8 percent of cases, again showing that the more serious lesions elicited greater validity (175). However, an Australian pilot study ($n=63$ eligible) that recruited subjects with known NMSC from the Tazmanian cancer registry and then examined the validity of subject self-report did not find that people accurately reported their NMSC history and that false negative reporting was high (176). Of the 22 subjects who reported a history of NMSC the researchers were able to identify treating physicians in only 18 of those cases. Of the 18 for whom they could identify the treating physician they were able to obtain the chart for confirmation on only 15. Of the 15 for whom they were able to obtain a chart, they could only confirm four previous NMSCs for a false positive rate of 61 percent (11/15) (176). The results of this study should be

viewed with caution as the entire sample size was 63 and the researchers were only able to access the charts of a small percentage of those who reported having a previous NMSC.

In a study examining test-retest of self-reported sun sensitivity, a survey was administered two to four weeks apart (177). Weighted kappa was used for ordered categories to give “partial credit” for small error versus large error using Cicchetti-Allison weights (177). Reliability for potential confounders, such as sun sensitivity and history of sunburns, was found to be substantial, ranging from $k=0.62$ for tendency to burn to $k=0.78$ for skin color and prior skin cancer diagnosis (177). Skin type (of Fitzpatrick), a 4-point scale based on historical ability to tan and susceptibility to sunburn, was found to be associated with the minimal erythema dose of ultraviolet B radiation required to produce visibility reddened skin (178). Color of untanned skin and hair were also independent predictors and the combination of items yielded a more accurate predictor of sun sensitivity than any one or two individual response variables (178).

Weinstock et al. reported that test-retest reliability of questions on tanning in a case-control study of melanoma nested in the Nurse’s Health Study cohort was high in the prevalent case group (Spearman’s $r=0.78$) and control group (Spearman’s $r=0.76$), but lower in the incident case group (Spearman’s $r=0.59$) (179). However, the questions were not worded identically in the two questionnaires (179). Among women diagnosed with melanoma, after the first questionnaire and before the second, there was a substantial shift toward

reporting a reduced ability to tan when participants were questioned after the diagnosis of melanoma ($p=0.035$) (179). Test-retest reliability of the hair color assessment by questionnaire was high with the Spearman correlation coefficient between 0.76 and 0.87. Sun sensitivity may be subject to recall bias when assessed by ability to tan, but not when assessed by hair color (179).

The concept of sun-reactive skin typing was created in 1975 for a specific need: to be able to classify persons with white skin in order to select the correct initial doses of ultraviolet A (UVA) (in joules per cubic centimeter) in the application of the then newly developed technique for the treatment of psoriasis-oral methoxsalen photochemotherapy (PUVA) (180). Fitzpatrick introduced the concept of skin typing as a clinical classification system based on a patient's historical assessment of his or her acute skin response to natural sunlight, with respect to the development of erythema and the ability to tan (181). In this system a lower skin type number (I or II) denotes a person who tans poorly and sunburns easily, and a higher number (III or IV) denotes persons who tan easily and seldom sunburn (181). The "Fitzpatrick skin typing system" has been used by the US Food and Drug Administration in its guidelines for sunscreen products for over-the-counter human use (180). Rampen et al. investigated burning and tanning histories in 790 students, 18 to 30 years old with a self-administered questionnaire classifying them into skin types based on the Fitzpatrick scheme (182). The minimal erythemal dose (MED) was then measured in a subgroup. The investigators found no significant correlation with the self-reported burning

tendency and the MED. However tanning ability showed a better correlation with skin complexion characteristics than burning tendency. The authors concluded that self-reported burning-tanning histories form an unreliable means of skin typing (182). Skin typing on the basis of self-reported burning tendency and tanning ability may be rather subjective. In this study subjects tended to over-record no burning and under record no tanning.

The importance of the tanning history as opposed to the burning history is further emphasized by the fact that in this study, the correlation with biologic complexion factors like hair and eye color and freckling tendency was somewhat better for the self-reported tanning than for the burning propensity (182). The Fitzpatrick classification does not quantify the degrees of burning and tanning but rather their frequencies (always burn, never tan and vice versa). These two variables are not necessarily synonymous if complexion traits have to be deduced from self-reported reaction patterns to sunlight. The use of the tanning ability only seems to be the preferred method in questionnaires (182). In a case-control study of reported sunburn history in melanoma, Berwick et al. found that reported sunburn history (including ever burned, number of burns, and time of first and last burns), skin reaction to sunlight and history of freckling were not highly repeatable with the poorest reliability being for ever burned among cases and ever freckled among controls (183).

Stern et al. compared skin type with eye and hair color as a risk factor for the development of cutaneous carcinoma in 1,380 patients with psoriasis who

had enrolled in a prospective study of PUVA therapy for psoriasis (181). They found that the relative risk of cutaneous cancer after exposure to PUVA increased monotonically with decreasing skin type number (181). Skin typing based on a patient's historical judgment of his or her response to sunlight, is a useful and convenient means of predicting the patient's risk of skin cancer rather than such characteristics as eye and hair color (181). That being said the skin types were assigned by the investigator not the study participants themselves.

CHAPTER 2

PRESENT STUDY

The methods, results and conclusions of these studies are presented in the papers appended to this dissertation. Due to space limitations in the publications, further information about methods, results and analyses are included here.

Section 1: Can NSAIDs Affect NMSC Recurrence (SKICAP-AK Trial)?

The SKICAP-AK trial was one of two trials that was part of the retinoid skin cancer prevention trials. SKICAP-AK was a double blind, randomized, placebo-controlled trial testing the hypothesis that daily supplementation of retinol (25,000 IU) for 5 years reduces the incidence of skin cancer in high risk individuals. High risk individuals were defined as those with a history of greater than ten clinically or pathologically diagnosed AK and no more than two prior pathologically confirmed SCC or BCC. A full description of the study design and methods have been previously published (36, 184). The SKICAP-AK study had two clinical centers which recruited and maintained subjects; one in Phoenix and the second in Tucson, Arizona.

Enrollment of patients began in June 1984 and continued until November 1988. Eligible subjects had to have a history of greater than 10 AKs diagnosed clinically or pathologically, the most recent of which had to have been diagnosed within the preceding year, and/or a pathologically confirmed record of at most,

two prior SCC or BCC. Over 11,000 subjects were screened prior to enrollment and 25 percent were found to be eligible. Prior to randomization, all subjects had a 3-month placebo run-in period to evaluate their ability and willingness to adhere to the study protocol. All subjects who achieved at least 75 percent capsule-count adherence were randomized. Some 2297 subjects were randomized to receive either 25,000 IU of retinol per day or placebo.

At baseline, subjects completed a questionnaire regarding demographics, sun sensitivity, residential history, amount of time spent in the sun per week, use of sunscreen, sunburn history, health habits (alcohol consumption, smoking), leisure time activities, health history, medical radiation exposure, family history of disease, occupational and environmental exposures, and dietary intake. Each subject was evaluated by a study dermatologist at their initial visit.

Subjects were scheduled for a return clinic visit one month after randomization and then every six months. At each follow-up visit, subjects were evaluated for any clinical sign and symptoms of toxicity, asked if they had seen a dermatologist since their last visit and had any biopsies or diagnosis of NMSC and had a mole and freckle count done by the interviewer.

The first occurrence and total number of SCCs and BCCs pathologically diagnosed after participants were randomized were the primary study endpoints. All skin biopsies performed after randomization were identified by participant self report at study follow-up visits, by review of pathology records of dermatologists, pathology laboratories, and the southeast Arizona skin cancer registry.

Diagnostic pathology slides were requested for all biopsies and centrally reviewed by the trial dermatopathologist. If diagnostic pathology slides were not available, the community pathologist diagnosis was accepted.

Participants were examined for skin lesions by a study dermatologist or their own dermatologist at least once each year and skin lesions suspicious for skin cancer were referred for biopsy and possible treatment to the subject's dermatologist. Participants unable to return for a follow-up visit were examined and had a blood specimen collected by a non-study dermatologist, and written documentation was provided. Termination and close-out procedures included scheduling all randomized participants for an exit interview and full-body skin examination conducted by a study dermatologist.

All skin lesions suspicious for skin cancer were recorded, and the subject was referred for biopsy and treatment. Subjects were followed by study staff to ensure that biopsies were performed and diagnoses were obtained along with diagnostic pathology slides for review by the study dermatopathologist. At the exit interview, subjects skin sensitivity was again assessed, along with sun exposure, sunscreen use, smoking status, health history including cancer history, hospitalization history and use of prescription or over the counter medication including name of the medication, reason for use, daily dose and length of time used in months.

A total of 526 subjects had a first new SCC or BCC that was confirmed microscopically by the study dermatopathologist, representing 96 percent of all

reported first new skin cancers. Of these, 140 subjects had both a first new SCC and first new BCC. There were 249 subjects with a first new SCC and 417 subjects with a first new BCC. Of the 249 with a first new SCC, 113 were diagnosed among the retinol group and 136 among the placebo group (36).

In the original study, the median age of subjects was 63 years, 70 percent were men, and all participants had at most two prior skin cancers (36). Serum retinyl palmitate levels taken during the study showed very similar levels and an 8-fold increase in median serum retinyl palmitate levels in the group assigned to receive retinol (36).

Clinical symptoms reported were similar between the two groups with the retinol group having a higher total serum cholesterol value, higher aspartate aminotransferase (AST) or alanine aminotransferase (ALT), lower white blood cell count (WBC) and lower hemoglobin throughout the intervention (36). The Cox analysis hazard ratio (HR) of first new SCC was 0.74 for participants who received retinol relative to the placebo group (95% CI, 0.56-0.99) when adjusted for the pre-selected characteristics (36). There was no significant difference between retinol and placebo groups for BCC.

The study also evaluated the probability of first new SCC and BCC based on subgroups of study subjects and found that there was higher risk in older subjects, men, subjects with one or more prior skin cancer, subjects with eight or more prior moles and freckles, larger number of hours in the sun, and a skin type that always or usually burns with the probability of first new skin cancer being

higher for BCC than SCC in all sub-groups (36). The intervention effect showed variation across strata. There was a suggested reduced risk of first new SCC for subjects that had eight or more moles and freckles on their arms, although intervention with retinol did not significantly lower the risk of first new BCC in any subgroup (36).

1.1: Description of the Medication Data and How it was Collected

Concomitant medication information for the SKICAP subjects was coded only for medications reported on the exit interview form. The exit interview form collected the following information about concomitant medications: 1) Are you currently taking or using any prescription or over the counter medications?; and 2) The form then asked them to list the names of the medications in a table along with reason they were taking it, daily dose, and length of time the medication was taken in months. Participants filled out this form on their own unless help was needed from the interviewer.

Medications reported on this form were coded according to the Drug Codes by Number coding system developed by the Skin Cancer Prevention Program. These codes were kept and consistently updated by the Data Manager and are used by all the Arizona Cancer Center Skin Cancer Prevention trials. Codes are organized by category of medication. Each category or type had a 6 or 7 digit code that references the category and a 4 digit code that references the specific medication to equal a 10 or 11 digit code. Upon exploration of the

electronic database for the SKICAP trials, it was discovered that the medications had only been entered for these trials at the broad category level and therefore individual medications could not be identified.

Because of the nature of the NSAIDs analysis and the manner in which the drug categories were organized, it became necessary to pull all the charts of the subjects who reported taking any medications in either the NSAIDs category or the anticoagulant category. Depending on the reason given for taking the medication, an NSAID could have been coded into either category. For example, if a subject was taking aspirin for pain this would be coded under the NSAIDs category, but if they were taking aspirin for stroke prevention then it would likely end up in the anticoagulant category.

There were 188 identification (ID) numbers which indicated there was anticoagulant use (medication code 4002010000). Of the 188 ID numbers, there was one duplicate for a total of 187 subjects who reported the use of an anticoagulant. All 187 of these charts were requested from the University of Arizona archival documents warehouse. Of the 187 subjects using an anticoagulant, 30 (16%) (including one of the duplicates) turned out to be using Coumadin, 1 chart was unlocatable (0.5%) and the rest, 157 (84%) reported using aspirin or another NSAIDs. The page from the exit visit form containing the concomitant medication information was copied from each chart and data entered so the specific medication being taken could be ascertained.

1.1.1: NSAIDs Use

There were 382 ID numbers which indicated at the exit visit that there was NSAIDs use (medication code 13001010000). Of the 382 ID numbers, there were 18 duplicates and 2 triplicates for a total of 360 unique subjects who reported the use of NSAIDs. All 360 of these patient charts were requested from the University of Arizona archival documents warehouse. All 360 charts were located and all were found to have recorded NSAIDs use. The page from the exit visit form with the concomitant medication information was copied from each chart and data entered so that the specific medication being taken could be ascertained. The data below is the result of this data extraction.

In total there are 551 entries for NSAIDs use including the duplicates and triplicates. Table 1, Appendix D lists subjects who reported taking more than one NSAIDs concomitantly. There are 18 duplicates from the NSAIDs medication code category and 2 triplicates. In addition 6 subjects had an NSAID listed in the anticoagulant group and one in the NSAIDs group.

Table 2, Appendix D provides the reason codes for all NSAIDs use. Some 201 (36%) report using NSAIDs for osteoarthritis, 80 (15%) report using for pain, 79 (14%) report use for heart condition and 67 (12%) report use for anticoagulation.

Table 3, Appendix D shows the dose of all NSAIDs use with the last two digits representing the units of use. Some 92 (16.7%) reported taking 325mg, 27

(4.9%) reported taking 400 mg and 26 (4.7%) reported taking 5 grains. Dose and units varied extremely widely for the population.

Table 4, Appendix D is the summary for all NSAIDs use in months. The mean length of use is 58 months or close to 5 years. The minimum is approximately half a month and the maximum is 600 months or 50 years. There are 7 subjects missing information on length of use. Table 5, appendix D shows a categorization of length of NSAIDs use. Figure 1, Appendix D shows a histogram of use with use clustered more toward the shorter length.

1.1.2 Aspirin Use

There were 293 reports of medications falling into the 4002010023/13001010023 category (i.e. Aspirin, etc.), (Table 6, Appendix D), the largest category of use (53%). The next most frequently used were the 1300101003 (i.e. Advil, etc.) category with 86 reports of use (16%). The majority of people taking NSAIDs in the aspirin category reported taking them for a heart condition 78 (25%), followed by anti-coagulation blood thinner 67 (23%) and osteoarthritis 46 (16%) (Table 7, Appendix D).

Table 8, Appendix D provides a summary of the length of time for people taking NSAIDs in the aspirin category. The mean is 63 months or approximately 5 years with the maximum being 600 or 50 years and minimum being 1 month.

Table 9, Appendix D provides a tabulation of length of time in months for people who reported use in the aspirin category. Not unexpectedly, there is a

spike around the intervals related to years. People were asked to self-report their time of use and can easily estimate years relative to months. Table 10, Appendix D provides daily reported doses for the aspirin category of NSAIDs. Again the doses were quite varied with 91 (31.06%) taking 325 mg.

Medication data was prepared for analysis by first rectifying inconsistencies related to data entry error and secondly by removing the duplicates. The criteria, for which medication to remove, were based on length of time in months. In a case where a subject reported concomitantly taking two NSAIDs, the NSAIDs they reported taking for the longest time in months was kept in the data base and the other was removed. This left a total of 523 unique entries. Longest time use was chosen as the criteria for which NSAIDs entry was kept based on the hypothesis that it is chronic NSAIDs use that will be important rather than more sporadic short periods of use (98).

1.2: Patient Characteristic Variables

Patient characteristic variables were examined and it was determined that there should be collapsing of groups, especially in groups where there are very few subjects. Tables 11-14, Appendix D provide the patient characteristic variables that were used for basic demographic analysis as well as potential confounders in the main analysis. For eye color it was decided that -9="." (missing) and the one subject who reported having black eyes should be placed

in the dark brown eye category (6=5). There were ample subjects in each category of hair color and therefore no collapsing of categories was required.

Because the literature reports that ancestry of Southern European origin can be protective in skin cancer (olive skin), that people of pigmentation are at a reduced risk of skin cancer and small numbers in some categories, the following decisions were made to re-code Mother's ethnicity. 0 was changed to "." (missing), 1 (American Indian), 2 (Asian or Pacific Islander), 5 (Hispanic) and 3 (Black) were combined together to =1. Eastern European was re-coded to 2, Northern European was re-coded to =3, Southern European was re-coded to =4 and Other was re-coded to =5. The following decisions were made to re-code Father's ethnicity. 0 was changed to "." (missing), 1 (American Indian), 5 (Hispanic) and 3 (Black) were combined to =1. Eastern European was re-coded to 2, Northern European was re-coded to =3, Southern European was re-coded to =4 and Other was re-coded to =5.

1.3: Removal and Redefinition of Subjects for Analysis Data Set

The first analysis that was completed was a logistic regression for which an abstract was generated and submitted to the International Society of Pharmacoepidemiology meeting. There were 2297 consented subjects in the SKICAP-AK trial. For the logistic analysis, only those who completed an exit form where medications were recorded were included. There were 402 subjects with no exit form leaving 1895 subjects in the analysis.

There were 15 subjects who had a report of melanoma and they were removed from the analysis pool, since they have a higher recurrence risk than those who have never had a melanoma. For the same reason, 651 subjects who reported a history of NMSC prior to randomization were also removed from the analysis set leaving 1410. To make a distinction between the chronic NSAIDs users and subjects who had only used for a short time, NSAIDs use was defined in the following way: non-user, meant the subject reported no NSAIDs use on the exit questionnaire, new users were those who reported use of NSAIDs but not equal to their length of time on study and continuous users reported use of NSAIDs equal to or longer than an individual subjects time on study.

Because the medication information was obtained at the exit visit, a “start date” for NSAIDs use was generated. This date was based on the number of months a subject reported using NSAIDs. Using this date, it was determined who had been diagnosed with an NMSC prior to starting NSAIDs use. Any subject who had an NMSC diagnosis prior to NSAIDs use was removed from the NSAIDs user category and re-coded as a non-user. There were 31 subjects re-coded as non-users.

Seven subjects reported no length of time for their NSAIDs use and they were removed from the data, since there was no way to categorize their use. In addition, there were 15 subjects who reported that their use was as needed (PRN). Table 15 and Table 16, appendix D describes each of the subjects in regard to their retention in the analysis data set.

1.4: Statistical Analysis

All analyses were performed using intercooled STATA version 9.0 (StataCorp, 4905 Lakeway Drive, College Station, Texas). Chi square tests compared basic demographic characteristics between NSAIDs users and non-NSAIDs users, ASA only users and non-NSAIDs users, and non-ASA NSAIDs users and non-NSAIDs users. Two sided Fisher's exact tests compared characteristics of the NSAIDs duration groups (new versus continuous users) and compared characteristics of those with no diagnosis of NMSC, diagnosis of SCC and diagnosis of BCC. Two sample independent t-tests were used to compare means.

To examine the relationship between NSAIDs use and development of NMSC, hazard ratios and 95% confidence intervals were estimated. Cox proportional hazards models modeled time to the development of first BCC or SCC and adjusted for treatment group, age and gender. Schoenfeld residuals were used to test that the proportionality assumption was met for all risk factors in each of the models. Participants who developed both first new BCC and SCC during the study period were included in both the BCC and the SCC groups.

In initial analyses multiple baseline variables commonly associated with risk for NMSC were included as potential confounders. Total reported usual weekly sun exposure was a combination of self-reported total weekday hours and weekend hours of sun exposure dichotomized to 0-10 hours or greater than 10 hours. The variables skin reaction to the sun, eye color, hair color, mother's

ethnic origin and father's ethnic origin were categorized according to known phenotypic traits that would make an individual at higher or lower risk of NMSC. Skin reaction to the sun was split into three categories: always burn (tan little/usually burn-tan minimally), burn moderately (tan average/burn minimally-tan easily), rarely burn (tan easily/never burn). Both eye color (blue/green, grey, light/dark brown) and hair color (blonde, light/dark brown, black, red) were categorized. Mother and Father's ethnic origin were categorized into four categories related to risk (American Indian, Alaska Native, Asian, Pacific Islander, Black, Hispanic), (Eastern/Northern European), (Southern European), (Other). In addition smoking status (never smoker, former smoker, current smoker) was also considered as a potential confounder.

Because AK is the known precursor lesion to SCC, history of AK diagnosis (yes or no) prior to first SCC/BCC diagnosis was also looked at. There were two clinic sites for this study (Tucson versus Phoenix) and this variable was also considered. Only age, treatment and gender remained in the final model. None of the other variables resulted in greater than a 10% change in the hazard ratios, so were not included in subsequent analyses.

1.5: Results

For the 1,402 subjects included in these analyses, the mean time on study was 60.7 months (standard deviation (SD) = 11.5, range 31.0-91.9). There were 1081 subjects classified as non-users and 321 who reported use. Of the 321

subjects who reported use, 170 reported ASA only and 151 NSAIDs only. NSAIDs users tended to be older, with 68.2% of the NSAIDs user group being greater than 60 years of age (mean 63.4 years) compared to 50.1% of the non-NSAIDs users group (mean 58.8 years). This difference in age was present regardless of whether subjects were ASA only users or non-ASA NSAIDs users. Proportionally more women reported using non-ASA NSAIDs compared to men, 41.3% versus 31.7% respectively ($p=0.02$).

Continuous users of NSAIDs tended to be older compared to new users (64.7 versus 62.1 years, $p=0.078$). For new users, the median time of use in the ASA only group was 30 months ($SD \pm 19.2$) and for continuous users it was 134 months ($SD \pm 118.8$). For new users taking non-ASA NSAIDs, median time of use was 23 months ($SD \pm 19.2$) and for the continuous non-ASA NSAIDs users it was 135 months ($SD \pm 92.1$). Furthermore there were no significant differences for duration of use related to gender, clinic, type of NSAIDs used or the stated reason for taking the medication (data not shown).

There were few differences between the NMSC outcomes based on gender, usual weekly sun exposure in hours, skin reaction to the sun, or smoking status. Age was the only statistically significant characteristic between outcome groups, with participants greater than 60 being more likely to be diagnosed with NMSC than those under 60 years of age ($p=0.011$).

Multiple variables (total weekly sun exposure, skin reaction to the sun, phenotypic traits, etc.) were added one at a time to the model to see if the hazard

ratios changed to assess for potential confounders. In the final models only age, treatment and gender were included. Potential interaction between treatment group and NSAIDs use was assessed. For all NSAIDs (yes or no), the likelihood ratio test p-value for SCC and BCC was 0.43 and 0.87, respectively. For new and continuous users (versus non-users) the p-value was 0.77 (SCC) and 0.98 (BCC). There was a statistically significant protective effect for BCC among those who reported any NSAIDs use (HR=0.58, 95% CI 0.39-0.85) as compared to those reporting no NSAIDs use. When NSAIDs use was stratified into new users versus continuous users, there was a protective effect only for new users versus the nonusers (HR=0.43, 95% CI 0.25-0.73). For continuous users, the relationship was less protective and not statistically significant (HR=0.88, 95% CI 0.52-1.50). Similarly, there was a statistically significant protective effect for development of an SCC only for the new user NSAIDs group (HR=0.49, 95% CI 0.28-0.87). The continuous user group showed no effect for SCC development (HR=1.11, 95% CI 0.65-1.92) compared to the nonusers.

NSAIDs use was further categorized by ASA use only or use of a non-ASA NSAIDs. Although the use of ASA was protective for both BCC and SCC, the relationships were not statistically significant (HR=0.64, 95% CI 0.38-1.06, HR=0.71, 95% CI 0.41-1.22, respectively). New use of either ASA only or non-ASA NSAIDs was likewise protective for both BCC and SCC but not statistically significant (HR=0.58, 95% CI 0.30-1.10, HR=0.51, 95% CI 0.24-1.10). Among continuous users, there was a non-significant protective effect for BCC only

(HR=0.74, 95% CI 0.35-1.6 for BCC and HR=1.08, 95% CI 0.52-2.22 for SCC).

Use of non-ASA NSAIDs was associated with being protective for BCC only in the new users group (HR=0.33, 95% CI 0.13-0.80).

1.6: Discussion

In these analyses of data from participants in a skin cancer prevention trial who were at high risk for skin cancer, we found a protective effect for NSAIDs use on the development of a first BCC or SCC among participants who were more recent initiators of NSAIDs use versus those reporting continuous use. The protective effect for initial BCC was most evident for non-ASA NSAIDs use, whereas protection for development of an initial SCC was not confined to a specific NSAIDs category. Although typical dose-response relationships suggest that longer duration of NSAIDs use enhances protection, long-term NSAIDs use was not significantly protective for either NMSC outcome. In addition, NSAIDs use was expected to be more protective with regards to SCC rather than BCC, but these analyses did not confirm that expectation. However, these results are consistent with the results of a recently published study by Grau et al. (115).

Many studies have looked at the use of NSAIDs and ASA in various cancers with mixed results. Some of the most convincing studies are from the colon cancer/polyp prevention arena where multiple studies using ASA or NSAIDs for prevention have reported protective effects (86, 87, 87-97). Use of NSAIDs has also been associated with a reduced risk of esophageal, stomach,

ovarian and breast cancers and increased risk for lung, kidney and prostate cancers (100, 103).

The effect of NSAIDs use on skin cancer has been examined in vitro, in multiple animal models, as a topical agent to treat certain skin conditions, and in a few epidemiological studies. There have been no prospective randomized clinical trials reported to date. Grau and colleagues published a study in 2006 looking at the association of self-reported NSAIDs use with the risk of BCC and SCC using data from the randomized, Skin Cancer Chemoprevention Study of oral β -carotene (115). The three response categories were no use, sporadic use (less or half of study visit questionnaires positive for NSAIDs use) or frequent use (more than half of the study visit questionnaires positive). NSAIDs use exhibited a weak protective effect in crude analyses for BCC development, which attenuated after adjustment. However, use of NSAIDs in the year previous to diagnosis reduced the odds for development of SCC by almost 30% (adjusted OR=0.71, 95% CI 0.48-1.04) (115).

Butler et al. published a nested case control study looking at the risk of AK and SCC in those reporting NSAIDs use who were part of the randomized, Queensland, Australia Nambour Skin Cancer Prevention Trial comparing daily sunscreen vs. discretionary use of sunscreen and β -carotene supplements vs. placebo (116). Participants who used NSAIDs eight or more times weekly for more than one year had a lower incidence of SCC (OR=0.07, 95% CI 0.01-0.71)

and those who used full dose NSAIDs two or more times per week for more than five years also had a lower SCC incidence (OR=0.20, 95% CI 0.04-0.96) (116).

Chronic inflammation correlates with increased risk of developing cancer in the affected organ and can be caused by UV radiation and other factors (64, 65). Arachidonic acid metabolism, which can be triggered by the effect of UV on the cell membrane in the case of skin, initiates the inflammatory process. This metabolism along with prostaglandin synthesis and the cyclooxygenase pathway is linked with the promotion and progression of cancers (65). The importance of elevated prostaglandins in tumor promotion has been studied through the use of inhibitors such as NSAIDs, which block prostaglandin production by inhibiting both COX isoforms (COX-1 and COX-2) (64). In the skin, the pathway leading to prostaglandin synthesis liberates oxygen-derived free radicals, which may cause tissue damage during the inflammatory response, and in addition prostaglandins may promote the retention of UV-damaged cells via inhibition of apoptosis (72).

Three classes of COX inhibitors exist; aspirin synthesized from salicylic acid, indomethacin and other NSAIDs, and the first selective COX-2 inhibitors-the coxibs (e.g., celecoxib and rofecoxib) (74). A study evaluating the expression of COX in human and mouse skin biopsy has shown COX-1 is observed throughout the epidermis whereas COX-2 increases in the more differentiated suprabasilar keratinocytes (82). BCC expresses little if any COX-1 or COX-2; whereas both COX isozymes are strongly expressed in SCC deriving from a more differentiated layer of the epidermis (82). COX-2 expression in human

keratinocytes appears to be related to differentiation of these cells both in vivo and in culture (82). Based on the metabolic pathway, NSAIDs are potentially good chemopreventative agents for skin cancer, specifically SCC.

Although the present SKICAP-AK study captured information about duration of NSAIDs use, the information is self-reported on an exit survey and is only an estimate of the total duration of use. Our validation study comparing self-report of NSAIDs use over the course of clinic visits as compared to the exit survey demonstrated probable misclassification. It is likely there were more true NSAIDs users than what was captured via the exit survey, thus causing our estimates to be biased toward the null.

Similar to both the Grau and Butler studies, the present analyses requires decisions about how to categorize NSAIDs use (115, 116). The literature currently lacks a consistent definition of what constitutes chronic use. There is also a lack of evidence suggesting the amount of time one may need to use NSAIDs in order to see a protective effect, along with the effect that timing of cumulative or intense UV insult may have on NSAIDs-derived protection. Variability in NSAIDs dosing and dosage relative to other physiological factors, such as body fat and skin photo-sensitivity and thickness, add complexities that could not be addressed in the current study.

One of the main eligibility criteria for the parent prevention trial was that patients had to have at least ten clinically apparent actinic lesions present at the time of recruitment, making this a highly promoted and at risk population.

Results from the current analyses are most relevant, therefore, to those populations at high risk for NMSC.

Bias is a concern for all epidemiological studies and must always be considered when interpreting results. In these analyses, it is possible, but unlikely, that participants who developed an NMSC have differential recall of their medications compared to those who did not develop an NMSC. At the time this study was conducted, 1984-1988, there was little if any information in the scientific literature and none in the popular literature related to NSAIDs use and the possibility of reducing one's risk for NMSC. We might expect to see some recall bias for variables like total sun exposure or skin reaction to the sun as these variables were well known as being related to skin cancer development at the time the study was done. However, even for these variables we found little evidence for variation between endpoint groups. This lack of evidence bolsters our belief that there was little recall bias in medication recalls within the study population.

Bias may also be caused by indication but because the protective effect was stronger in recent initiators and not continuous users it is likely that this did not affect the estimates. In addition there may be other unmeasured/unknown bias affecting these results including the length of time of follow-up for the study and some non-cases becoming cases after the end of the study period. While this is possible, no information is available for this study population about events that occurred after study end. Our analysis sample was younger and included

more women than the original SKICAP-AK population; both younger age and being female are related to a reduced risk of NMSC possibly leading to the lack of risk seen for SCC.

Other biological factors which affect the COX pathway may need further investigation at the basic science level. Our findings suggest that the shorter duration or perhaps more realistically, more recent use is more protective for development of first NMSC. Tolerization may be one mechanism playing a role in this finding. Chronic use of NSAIDs may lead to receptor over expression and alternate signaling pathway activation.

We observed some potential variation in the magnitude of the protective effect by type of NSAIDs. Statistically significantly protective effects were found primarily for non-ASA NSAIDs use and for BCC development. However, the collected data and the small sample size did not allow evaluation of more specific types of NSAIDs. Each type of non-ASA NSAIDs provides different levels of COX-1 and COX-2 inhibition and some NSAIDs may be better skin carcinogenesis chemopreventative agents than others. This remains yet another area of investigation. In addition this was a secondary analysis of data and therefore we cannot rule out statistical error as a potential explanation for our reported observations.

These analyses have several strengths. This cohort was well monitored with regular in-person study visits and exams by a study dermatologist on an annual basis. Chart reviews were completed to ensure acquisition of all skin-

related biopsy or treatment information. Diagnosis of NMSC was verified through acquisition of the specimen slide and central pathology review.

These results are counter to the idea that longer duration of NSAIDs use is more protective, an idea that permeates the literature on NSAIDs and chemoprevention, although the shorter duration may be a surrogate for recent and new use of the agents. The results are also counter to the idea that the major effect will be for SCC development. This counterintuitive finding may indicate that NSAIDs can have biological effects in other ways than currently known and that NSAIDs block different signaling pathways in carcinogenesis processes for BCC development versus SCC. It is clear that more research at the basic science level remains to be done, as well as more controlled prospective studies in order to understand the role of NSAIDs in the chemoprevention of NMSC.

Section 2: Dose Response Versus Intent To Treat (SKICAP SCC/BCC Trial)

The objective of this study was to examine the effect of retinol and isotretinoin on the incidence of NMSC in high-risk subjects. A full description of the study design and methods have been previously published (184, 185). Eligible participants had to have had at least four biopsy-proven skin cancers, one of which had to have occurred within 12 months of consenting to the study and no diagnosis of melanoma within the past year, if female, not of childbearing

potential, and willing to make visits to the study clinic and to either the study dermatologist or his or her own dermatologist for at least the next three years.

Between January 1985 and June 1990, 719 people consented to the study. Of the 719 consented participants, 525 men and women with a history of at least four BCCs and/or SCCs in the past year successfully competed run-in by achieving a 75 percent adherence level and were randomized (185). Study clinics were in Tucson, Phoenix, and Yuma, Arizona and San Diego, California. Of the 194 participants who were not randomized, 71 chose to cease participation, 78 were ineligible because they failed to meet one or more of the inclusion criteria, 37 had clinical symptoms, 6 were insufficiently compliant with adherence to the protocol and 2 were lost to follow-up (185).

Thirty-three percent were randomly assigned to 25,000 IU oral retinol, thirty-three percent to isotretinoin (5 mg for those under 145 lbs. and 10 mg for those equal to or greater than 145 lbs.) and thirty-three percent to placebo daily for three years (185). Eligibility criteria included: 1) being between 21 and 85 years of age; 2) ambulatory and capable of self-care; 3) no diagnosis of a life-threatening condition or internal cancer in the past year; 4) near normal or normal laboratory values in a routine screening panel of tests; 5) planning to live continuously in Arizona for the succeeding 3 years (185). The following excluded participation in the study: 1) Diagnosis of basal cell nevus syndrome; 2) Diagnosis of xeroderma pigmentosum (185).

At the first visit each participant provided signed consent, and was given a detailed explanation of the protocol requirements. Information on diet, medical history, socioeconomic status, and a baseline blood chemistry were obtained. For participants not referred to the study by a community dermatologist and who had recent written documentation of a skin examination, a study dermatologist provided a detailed skin examination (185). Participants returned for clinic visits 1 month after randomization and every 6 months thereafter in order to monitor adherence and possible toxicity.

The time to first occurrence of SCC and time to first occurrence of BCC after randomization, pathologically confirmed by the study pathologist, were the primary outcome measures. Per protocol, skin cancers that were diagnosed after study closeout or after the participant's third study follow-up year (36 months post-randomization) were not included (185). A skin examination was performed by a study dermatologist or by the participant's dermatologist at least once every 6 months and all clinically suspicious skin lesions were biopsied. Skin biopsies were identified by subject self-report, review of pathology records, and a regional skin cancer registry with all pathology slides of all diagnostic biopsies requested and centrally reviewed (185).

2.1: Compliance

Participants were given a six month capsule supply based on weight. Persons weighing under 145 lbs. took one capsule from supply A and one

capsule from supply B and persons weighing over 145 lbs. took two capsules from supply A and one capsule from supply B. Those who weighed greater than 145 lbs. got six bottles of 100 and those who weighed less than equal to 145 lbs. got four bottles of 100 capsules. At each follow-up visit, a new six months supply of medication was provided and a six month calendar log was also given. The skin cancer prevention medication calendar asked participants to mark down, for each day, if and when they took their capsules. The calendar also provided an extra line allowing for comments. In addition to marking down whether they took their capsules, the calendar allowed them to record side effects and asked that the month, date, and description of the side effect be included. Participants were to bring the calendar along with any unused capsules to each clinic visit.

A computer program that calculated adherence was used by each individual clinic to standardize results. Percent adherence is the number of pills taken divided by the number of days between visit intervals. Each dispensed bottle contained 100 pills. On the post randomization checklist, the date the participant started taking the most recently issued medication was entered along with if the pills were taken, the date of the visit, and the number. The number of capsules in the full bottle was recorded along with number of empty bottles, capsules left in opened bottles, and number of no's marked on the calendars. From this information, a percent compliance and a percent calendar compliance were calculated. Pending capsule count compliance was coded as a -7 and when it is permanently unavailable, coded as a -9.

Instructions for participating in the study included: 1) Do not donate blood while participating in this study as the study medication is potentially harmful if the donated blood is given to a pregnant woman; 2) Store your bottles of medication at 59 to 86 degrees Fahrenheit in a dark place. Your pill reminder may be placed in a convenient location as long as it is within the 59 to 86 degree temperature range; 3) Take capsules every day. If you forget to take a capsule, do not take a double dose the next day. Your medication is prescribed by weight, so please let us know if your weight goes above or below 145 lbs.; 4) Take capsules with food if possible; 5) Record on your calendar whether you took the capsules or not, the time at which you took your medication, and any questions or comments; 6) If you are having trouble remembering to take your capsules, please call us, as we can suggest ways to jog your memory; 7) Call your dermatologist if you experience any medical problems. If you cannot reach him or her, call us; 8) Please do not take more than 10,000 international units of Vitamin A daily in addition to what may be in your capsule; and 9) When you return for your next appointment to the Skin Cancer Prevention Program clinic, please bring: a. Empty medication bottles and any capsules not taken b. Calendars.

2.2: Toxicity Monitoring

If a side effect was identified at a follow-up visit, a compliance call, or the participant called in with a complaint, a Clinical Monitoring form was completed.

Detailed instructions regarding the problem, duration and dose-modification were included on the form. For all randomized subjects, the subject and interviewer agreed to the amount and length of any proposed modification if the problem was not mandated by the following protocol requirements. A progress note was written in the participant's chart with the problem, answer, and any dose-modification given and the participant was encouraged to record any changes on his or her calendar.

Protocol requirements: Any participant complaining of a level-2 clinical symptom will drop to a half dose for at least one month. After one month the interviewer contacts the participant to assess the status of the complaint. If the condition has disappeared or improved to level 1, the participant will be asked to return to full dose. If the complaint has improved to a level 1 or better but the participant wishes to remain at half-dose, he is allowed to do so. If the problem has not gone away but the participant now attributes it to something other than the study medication, the participant may return to full dose. If there is no improvement after three months at half-dose and the participant attributes complaint to retinol/isotretinoin, the participant will be asked to discontinue the study medication and will be contacted in one month to assess the status of the complaint. The participant can continue on study at any dose as long as protocol restrictions for toxicity are adhered to. A second clinical monitoring form is completed indicating any adjustments that have been made and the process is continued with another report generated at a future date.

The clinical monitoring questionnaire requests the interviewer to ask “Have you been experiencing any health problems possibly related to taking the capsules since we last saw you?” if so, what and duration. In addition specific questions were asked pertaining to isotretinoin and retinol and included whether someone was experiencing alopecia, cheilitis, conjunctivitis, dry skin, dysuria, epistaxis, exanthema, fatigue, headache, menstrual changes, musculoskeletal stiffness/pain, nausea/vomiting, peeling palms/soles, skin infection along with severity of the symptom and duration. In addition blood specimens were analyzed to monitor levels of cholesterol, triglyceride, hemoglobin, blood cell count, platelet count and liver function.

2.3: Intent To Treat

The current standard for analysis of clinical trial data is intent to treat analysis (also known as use-effectiveness) which seeks to answer the question “Is it better to adopt a policy of treatment A if possible, with deviations if necessary, or a policy of treatment B if possible, with deviations if necessary, for patients with a particular medical condition?” (186, 187). The intent to treat analysis requires inclusion of all randomized patients, regardless of whether they remain on protocol for the duration of the study, when the study is analyzed in their originally randomized assignment groups (188). The SKICAP-BCC/SCC study was analyzed using the intent to treat principle.

Arguments for use of intent to treat are many. Intent to treat analysis reduces differences among treatments making it more difficult to demonstrate that one treatment is better than the other, prevents a researcher from obscuring the facts in favor of their own theories, and is a very conservative analysis (186, 189). Because the randomization process helps control for potential confounders, only the original assignment retains any guarantee of this and therefore inferences made from a study should be based only on the original treatment assignments (186, 187, 190). If subjects who deviate from the protocol because of toxicity, age, disease severity, etc. are omitted, then groups may not be comparable and the biases originally controlled for through randomization such as patient characteristics may affect the outcome of the analyses.

Not using the intent to treat analysis and removing those who deviate from the protocol or withdraw prematurely can reduce the sample size and thus the power of the trial to detect differences among treatments. This undermines the reason for randomization and can introduce bias into the analysis. Analysis by treatment received can be biased because there is potential for the compliance variable to be related to outcome or because it is confounded by some other factor (190). In addition the argument has been made that it is not reasonable to generalize study results to only compliant participants with similar disorders because the majority of people are noncompliant under normal circumstances and do not adhere to a prescribed regimen (186, 190).

There are also arguments against the exclusive use of intent to treat for clinical trial data. Sheiner et al. argue that intent-to-treat does not provide valid significance levels, estimates, or interval estimates either for use-effectiveness in regular medical practice or for the effect of the actually administered therapy (method-effectiveness) (187). They argue that method-effectiveness maybe be more relevant to medical decision making than use-effectiveness and trials should be designed and analyzed to provide both. Because specific information about compliance is not utilized, it may either be discarded or not collected (187). Understanding potential benefit given full compliance and expected benefit averaging over rates of compliance in a particular trial are felt to be important especially for clinicians (187). The intent to treat estimate only provides an average prognosis of a mixture of compliers and of people who may not have taken the drug at all or who left the study prematurely and this average prognosis may not apply to all patients. For this reason, understanding method-effectiveness is important. Substantial deviations from the protocol such as loss to follow-up or drop out can dilute the data and diminish the ability of a trial to detect a difference between effects of treatment (187, 190).

Intent to treat analysis reveals the answer to the question “Is a treatment effective?” while an explanatory analysis seeks to answer the efficacy question “Can this treatment work?”. The secondary analysis of this data in a dose response manner seeks to understand the question “Can this treatment work?”

because it looks at the study from a compliance point of view and takes into consideration total dose of intervention consumed by each individual participant.

2.4: Dose Response Analysis

For the dose response analysis, total number of tablets/capsules taken by each participant was calculated based on the adherence percentages generated by physical pill count, available in the electronic data base. Anyone with compliance over 100 percent was set to equal 100 percent. Total tablets/capsules were then converted to total dose per kilogram for each participant. Supplemental Vitamin A intake information was collected at baseline and monitored throughout the study. Very few study participants reported supplemental Vitamin A intake (9.3%) at baseline and a comparison of supplemental vitamin A intake at baseline among the three treatment groups via chi-square revealed no difference among the groups ($p=0.49$). Therefore, no attempt was made to add supplemental Vitamin A use into the total dose per kilogram. In addition available patient study records did not record actual dose of vitamin A supplementation but did indicate if they were supplementing or not.

All skin cancer end points were monitored after randomization and confirmed by pathology. Participants were examined for skin lesions by a study dermatologist or their own dermatologist at least once a year and also underwent a full-body skin examination by a study dermatologist when they exited the study. Participants with suspicious skin lesions were referred for biopsy and treatment

and were closely followed by study staff to ensure that biopsies were performed and a diagnosis obtained. In addition, endpoints were identified by participant self-report at study follow-up visits, by review of pathology records of dermatologists, pathology laboratories and the Southeast Arizona Skin Cancer Registry. Diagnostic pathology slides were obtained on all biopsies, centrally reviewed and confirmed by the study dermatopathologist.

The original intent to treat analysis was then reproduced as best as possible in a dose response manner. Quartiles of total intervention use were generated for the retinol and isotretinoin group. The original intent to treat analysis adjusted for moles and freckles but the current analysis does not since it has become known that the mole and freckle data is not reliable or complete. In addition the original paper reported less history of skin cancers than these analyses. This is because the current study considered several types of precursor lesions as if they were cancer, including Bowen's, SCC in situ, and Keratoacanthoma, because these lesions are far enough along the skin cancer progression pathway.

All analyses were performed using intercooled STATA version 9.0 (StataCorp, 4905 Lakeway Drive, College Station, Texas). Chi square tests compared basic demographic characteristics between the quartiles of use for each of the interventions and one way analysis of variance was used to compare means. T-tests were used to compare both the mean dose of retinol and

isotretinoin in the first quartile to the placebo group. A Kruskal-Wallis test was used to compare median time on study for those taking retinol by quartiles.

To examine the relationships between quartiles of total retinol dose received versus placebo and total isotretinoin dose received versus placebo on the development of NMSC, hazard ratios and 95% confidence intervals were estimated. Cox proportional hazards models estimated time to the development of BCC or SCC occurrence. Participants who developed both a BCC and SCC occurrence during the study period were included in both the BCC and the SCC analysis. Test for trend was accomplished by estimating Cox proportional hazards models without creation of dummy variables for the dose variable. Potential confounding factors were evaluated in initial models by comparing the adjusted and unadjusted hazard ratios. Factors were included in the final models only if the adjusted hazards ratio changed by greater than 10%. Months on study was evaluated as a potential effect modifier by creating an interaction term including months on study and dose. Likelihood ratio tests were used to assess the significance of this interaction term.

The examination included three modeling approaches: a crude model, a model adjusted for age, gender and months on study, and a model adjusted for age, gender, skin reaction to the sun, total time in sun, months on study and history of NMSC. In addition, three alternative modeling approaches were performed in an attempt to try and understand the increased risk associated with the first dose quartile of retinol and SCC. These models were as follows: 1) A

model was constructed where all subjects who had not been on study at least six months were removed from the data set; 2) All events that occurred in the first six months were removed from the data set but all subjects were kept; and 3) Retinol dose was converted to mg ($1\mu\text{g}$ retinol=3.33 IU vitamin A activity) and retinol and isotretinoin dose were added together, converted into mg/kg and analyzed as one treatment group.

2.5 Results:

Of the 525 participants in the study, 102 participants had no diagnosis of either BCC or SCC after randomization. Two-hundred had at least one SCC diagnosed after randomization, and 132 had at least one diagnosis of BCC after randomization. One-hundred-seventy participants were diagnosed with both a BCC and SCC after randomization. Median time on study was 30.4 months for all participants (30.4 months for those on retinol, 29.7 months for isotretinoin and 31.3 for placebo).

The baseline characteristics did not differ by retinol dose quartiles with the exception of age. Those in the highest quartile of dose were younger with a mean age of 61 years ($p=0.0009$) as compared to participants in the other dose quartiles (68, 69 or 67 years) or placebo (65 years). The distribution of baseline characteristics for participants by quartiles of isotretinoin dose, reveals no differences between the dose groups with the exception of gender ($p=0.011$). The two groups with the highest intake of the isotretinoin were 80% and 84%

male, while the two lowest intake groups were 53% and 66% male compared to the placebo group that was 72% male.

For SCC, the crude and partially adjusted models show a statistically significant increase in risk of developing an SCC for the first dose quartile with the fully adjusted model just meeting statistical significance at the $p=0.05$ level. Test for trend was not significant. In the crude model those in the first quartile of retinol dose have a HR of 2.92 (95% CI 1.57-5.10) and this increased HR also remains in the adjusted models, 2.06 (95% CI 1.06-4.80) and 1.95 (95% CI 1.00-3.80) respectively. None of the three models showed any significance at any level for BCC first occurrence.

The relationships between the synthetic retinoid isotretinoin dose level and occurrence of BCC or SCC revealed a different outcome. Only the crude model for SCC showed a statistically significant increased HR in the first dose quartile, 2.38 (95% CI 1.35-4.19). For both the partially and fully adjusted model, there was no statistically significant increase in risk for participants in the first dose quartile, 1.17 (95% CI 0.724-1.88) and 1.69 (95% CI 0.866-3.31), respectively. Test for trend was not significant in any of the models. Again there were no significant results for BCC occurrence.

In an attempt to identify potential alternate explanations for the increased risk of SCC in the first retinol quartile, additional comparisons were made. The mean dose of retinol in the first quartile (48,481.1 mg/kg) indicated that the patients received a dose statistically significantly greater than those in the

placebo group ($p < 0.0001$). Not unexpectedly, median time on study for those in the first quartile was less than for those in the other three quartiles. The median time on study for those taking retinol in quartile one was 6.6 months, quartile two was 25.1 months, quartile three 38.8 months and quartile four 53.4 months ($p = 0.0001$). In addition, alternate analysis approaches involving the removal of all participants not on study for at least six months, removal of all NMSC diagnosis occurring in the first six months and conversion of the intervention doses in order to create a total dose for one intervention group did not significantly change the conclusions.

Off-study reasons were investigated by quartile of dose received. For both retinol and isotretinoin the major reason participants left the study in the lowest quartile were for toxicity consistent with the interventions (retinol=46.5%, isotretinoin=52.0%) and unwillingness to continue (retinol=30.3%, isotretinoin=31.9%). For quartile two the majority of subjects completed the study (retinol=62.8%, isotretinoin=53.3%) and a smaller percentage were unwilling to continue (retinol=18.6%, isotretinoin=24.4%). For quartiles three and four the majority completed the study, with fewer numbers unwilling to continue.

2.6: Discussion

These analyses evaluating dose response confirm the findings of the original intent to treat analysis. The daily regimen of 25,000 IU of retinol or 5 or 10 mg of isotretinoin were not effective at preventing new occurrences of SCC or

BCC among a population with histories of multiple cutaneous cancers. In the current analysis all models, crude and adjusted, evaluating SCC occurrence and actual retinol dose level received showed that there was a statistically significant increase in risk of occurrence for those in the first dose quartile, even after adjustment for potential confounders such as age, gender, skin reaction to sun, total time in sun, months on study and history of NMSC. Test for trend was not significant either for protection or for increased risk.

In the SKICAP-BCC/SCC study no protective effect for development of SCC or BCC was found for either isotretinoin or retinol using the intent to treat analyses. The original study reported fairly good compliance for those who remained on study but also indicated that the attrition rates were high in all groups. During conduct of the SKICAP-BCC/SCC study, new toxicity information about one of the intervention agents surfaced (185). This information was conveyed to all participants because of the double blinded nature of the trial design. This strong message of potential side effects given to each individual participant by the study interviewers may have increased the attrition rate over what would have been expected. The study population became sensitized to slight deviations from their normal health and could have ascribed it to their study medication and stopped the study. This increased attrition diminished the ability of the trial to detect a difference between the placebo and treatment groups and thus, the trial was not fully evaluable (191).

An additional limitation in longer studies such as SKICAP, is in separating the effects of normal aging and increased prevalence of chronic disease with potential adverse effects consistent with intervention. By recruiting a population with histories of multiple cutaneous cancers, the mean age of the study group was slightly above the typical retirement age of 65 years. Substantial changes in lifestyle and perceived health can accompany the changes associated with this stage regardless of participation in intervention trials.

The current standard for analysis of clinical trial data is intent to treat analysis (also known as use-effectiveness). Intent to treat analysis seeks to answer the question “Is it better to adopt a policy of treatment A if possible, with deviations if necessary, or a policy of treatment B if possible, with deviations if necessary, for patients with a particular medical condition?” (186, 187). The intent to treat analysis requires inclusion of all randomized patients in the analysis, in their originally randomized assignment groups, regardless of whether they remained on protocol for the duration of the study (188).

Arguments for use of intent to treat are many. Intent to treat analysis, in the presence of non compliance, reduces differences among treatments making it more difficult to demonstrate that one is better than the other, retains the original sample size and power, controls for bias, prevents a researcher from obscuring the facts in favor of their own theories, and maintains a very conservative analysis (186, 189). Because the randomization process helps control for potential confounders, measured and unknown, it is believed that only

the original assignment retains this guarantee (186, 187, 190). If subjects who deviate from the protocol because of toxicity, age, disease severity, etc. are omitted, then groups may not be comparable and the biases that were originally controlled for through randomization may affect the outcome of the analyses. In addition, the argument has been made that it is not reasonable to generalize study results to only compliant participants with similar disorders, because the majority of people are noncompliant under normal circumstances and do not adhere to a prescribed regimen (186, 190).

There are also arguments against the exclusive use of intent to treat analysis for clinical trial data. Sheiner et al. argue that intent to treat analyses do not provide valid significance levels, estimates, or interval estimates either for use-effectiveness in regular medical practice or for the effect of the actually administered therapy (method effectiveness) (187). These researchers argue that method-effectiveness maybe be more relevant to medical decision making than use-effectiveness and that trials should be designed and analyzed to provide both results. Understanding potential benefit given full compliance and the expected benefit averaged over rates of compliance in a particular trial are considered to provide important information, especially for clinicians (187). The intent to treat estimate only provides an average prognosis of a mixture of compliers and of people who may not have taken the drug at all or who left the study prematurely. This average prognosis may not apply to any individual patient. Substantial deviations from the protocol through either loss to follow-up

or drop out can dilute the data and diminish the ability of a trial to detect a difference between effects of treatment (187, 190).

Intent to treat analysis reveals the answer to the question “Is a treatment effective?” while an explanatory analysis seeks to answer the efficacy question “Can this treatment work?”. The secondary analysis of these data using a dose response approach seeks to answer the question “Can this treatment work?” because it examines the study from a compliance point of view, and takes into consideration the total dose of intervention consumed by each individual participant.

Overall reported toxicity of level two or greater in the SKICAP BCC/SCC study was mild, and the isotretinoin treated group reported more side effects than either the placebo or retinol group. The majority of toxicities reported were in the mucocutaneous category (185). However, by year 2 (28 months) of the study (visit 5), only 63% of the original 175 subjects remained on study in the isotretinoin treatment group, 68% of the 173 remained on study in the retinol group, and 72% of the 173 in the placebo group remained on study.

One of the major drawbacks of the retinoids is the toxicity associated with the physiologic accumulation of excessive amounts. The first and classical reported cases of acute hypervitaminosis A involve Eskimos and Arctic explorers ingesting polar bear or seal liver meat, and then developing severe headaches, drowsiness, irritability, nausea, and vomiting, erythema and desquamation of the face, trunk, palms and soles of the feet with symptoms resolving in 7 to 10

days (117). Absorption differences and variability in the risk of accumulation and toxic effects can differ depending on the retinoid being used. Synthetic forms such as isotretinoin are thought to distribute more uniformly in the body and are thought to be less likely to cause the severe adverse effects related to the accumulation of excessive levels of retinol in the liver. Unfortunately, the various synthetic retinoids are associated with additional adverse effects, rather than eliminating toxicity related to high retinoid intake. Side effects of the synthetic retinoids include mucocutaneous drying and chapping leading to cheilitis, facial dermatitis, conjunctivitis, dryness of the nasal mucosa with minor nosebleeds, dry mouth with thirst, xerosis, hair loss, palmoplantar desquamation, stratum corneum fragility or easy peeling due to minor frictional trauma and scratching, paronychia and nail abnormalities (125).

Our dose response analysis evaluated participants by original group assignment but based on total dose per kilogram body weight; therefore, the characteristics of the groups based on the original randomization remain intact. Additionally we looked at the characteristics of subjects in each treatment group by dose quartile, and there were few differences by quartile. Those in the highest quartile of retinol appeared to be younger, possibly indicating that those participants who were younger were able to stay on study longer or more able to tolerate the intervention. However, many of the potential adverse effects of the retinoid dosing such as skin and mucous membrane dryness and hair loss are also common occurrences with aging. In the retinoid intervention study with its

older age population it was not possible to discern some of the potential intervention related adverse effects from age related changes. For isotretinoin there were differences in quartile by gender indicating that men may have stayed on study longer.

Models with potential confounders such as skin reaction to the sun, total time in the sun, months on study, history of NMSC, age and gender were assessed in our dose response analyses. Based on the fully adjusted models these analyses indicate that there may be an increase in risk of recurrent SCC for those in the lowest quartile of retinol. In addition three alternative analyses were done in an attempt to better assess the increased risk in the first quartile for retinol and SCC. Unfortunately, none of the three alternative analyses of the data set provided an outcome significantly different from the original dose response analysis.

In order to try and explain why participants in the lowest quartile of total retinol were at increased risk for developing SCC, we compared time on study, mean dose, and off study reasons, by quartile of dose. Those in the lower quartile of dose were on study for less time than those in the upper quartiles; however this is to be expected as the total amount of intervention a participant took would be heavily dependent on the amount of time they were on study. In addition, mean dose between the placebo group and the first quartile was significantly different so participants in the first quartile did receive drug. The reasons why participants in the first quartile went off study were quite different

from those in the other quartiles. First quartile participants most frequently reported their reason for going off study as toxicity consistent with the intervention, and in the other three quartiles participants went off because they were exiting or unwilling to continue (small percentage). Median time on study for those with and without a BCC or SCC occurrence during the study was compared by quartile of dose and showed no evidence that those who developed an NMSC had a shorter time on study versus those who did not.

The present project examined the efficacy of retinoid interventions in preventing the development of new NMSC among a population with a history of multiple and recent cutaneous cancers. Side effects were more prevalent and greater attrition was associated with the isotretinoin. A review of 13 serum retinol studies, both prospective and retrospective, and a large case control study showed that low vitamin A levels existed in the cancer populations and potentially played a role for incidence, basically suggesting that the lower the serum vitamin A level, the greater the cancer risk (124, 144). However other studies have suggested that low serum retinol may be a metabolic consequence of cancer rather than a precursor of cancer (145).

Dietary intake studies of the association between retinol have shown inconsistent results for BCC and SCC (143). Of the 11 dietary studies reviewed only one hospital based case control study by Wei et al. showed an inverse association between the use of vitamin A supplements in the last 5 years and the development of BCC (OR=0.20, 95% CI 0.06-0.62) (172).

Oral retinoid therapy seems to prevent or delay development of cutaneous carcinomas for patients with some cutaneous diseases with a high risk of malignancy, such as xeroderma pigmentosum, Mibelli's porokeratosis, Gorlin's disease, Ferguson-Smith disease and solar keratosis (149). In addition, several small studies that treated patients with other cutaneous diseases who also already had BCC with high dose retinoids, oral or topical, reported that the tumors responded well to the treatment and, in many cases, the treatment reduced the number of existing tumors or prevented onset of new tumors (161-163, 165-168). Thus there is substantial literature indicating that retinoids may have the ability to prevent BCC.

A nested cohort study compared patients' own tumor experience while using and not using retinoids for patients participating in a Psoralen + UVA treatment (PUVA) follow-up study, who reported at least 1 year of substantial retinoid use between 1985 and 2000. They found that retinoid use was associated with a 30% reduction in SCC ($p=0.02$) (105). The incidence of SCC significantly decreased during years of substantial retinoid use (Incidence rate ratio=0.79, 95% CI 0.65-0.95), but there was no association between BCC incidence and oral retinoid use (105). In a study of 981 subjects who had two or more previously confirmed BCCs randomized to 10 mg isotretinoin or placebo, there was no significant difference in the cumulative percentage of patients with an occurrence of BCC at a new site or the annual rate of BCC formation after 36 months. Subjects reported significant adverse systemic effects (152). The

combined results of these two studies indicate no effect of retinoids on BCC development but significant reduction on cutaneous SCC development associated with retinoid intervention. In addition, studies done with the retinoid acitretin and etretinate in renal transplant recipients have shown that these agents are effective in reducing NMSC and AK incidence in this high risk population (153-156).

There has been little recent research on retinoids and skin cancer prevention. Recent studies of head and neck squamous cell cancer (HNSCC) have been null. In a randomized, 8 year trial (3 years on intervention and 4 years of follow-up) in 1,190 patients with a history of stage I or II HNSCC, low dose isotretinoin was found to be no more effective in reducing the rate of second primary tumors than placebo (HR=1.06, 95% CI 0.83-1.35) or increasing the rate of survival (HR=1.03, 95% CI 0.81-1.32) (170). In addition Toma et al. found no advantage for patients previously treated for Stage II and IV HNSCC who took isotretinoin as compared to those in the control group; 5 year actuarial survival was 58.9% for those in treatment versus 57.2% in the control group (p=0.94) (158).

These analyses confirm the results of the original intent to treat analyses and in addition raise an interesting question related to patients in the first quartile of dose for retinol and increased risk. Multiple attempts were made to understand if this increase in risk could be explained by different characteristics of the study population to no avail. History provides that other vitamin

compounds that have been investigated by chemoprevention studies were found to increase the risk for cancer rather than protect from cancer, however there is nothing in the current literature to suggest this is the case with retinols.

Section 3: Reliability of Risk Assessment Questionnaire Data (Biomarkers 1 Study)

Data for the reliability manuscript resulted from a clinical trial called the Biomarkers 1 study. The Biomarkers 1 Study was conducted by the University of Arizona Cancer Center over an approximately three month period. The study was designed to assess the reproducibility of various surrogate endpoint biomarkers (SEB), within the skin carcinogenesis pathway, specifically the variability of the SEBs (polyamine levels, p53 expression and PCNA expression). Subjects were recruited from dermatology clinics at the University of Arizona Medical Center, local dermatologist's offices and clinics, and via advertisements.

Eligible subjects were males and females of at least 18 years of age who were willing to use skin protector factor (SPF) 50 sunscreen applied daily. Subjects were recruited from 4 different risk groups. The first risk group was composed of people who had sun damaged forearms with no visible actinic keratoses (AK's) or other clinical lesions which is called the Pre-AK group. The second group included persons with visible AKs and is called the AK group. The third risk group was composed of people with a history of resected SCC in the last 12 months, the SCC group. The fourth group was composed of persons who

had dysplastic nevi (DN). Information on the design and some results of the study have been previously published (192, 193). In order to test the reliability of the previously mentioned SEBs, skin biopsies were collected at baseline and again 3 months later. The study sought to recruit approximately 30 participants per group, except for the Pre-AK group, to which 60 participants were recruited.

Because it is standard practice at the Arizona Cancer Center to require the use of study-provided sunscreen during chemoprevention trials, an attempt was made to assess the effects of this practice on SEBs by randomizing half of the Pre-AK group to sunscreen use. Those in the sun-screen group were asked to use the provided sunscreen (solbar, SPF = 50) on their forearms. The AK, SCC and DN groups were also asked to use study provided sunscreen.

Throughout the three month study period, participants filled out various questionnaires and forms providing information about history of NMSC, sun damage to skin and sun sensitivity. Risk group determination was initially made by staff members who administered the eligibility form via the telephone.

The Biomarkers 1 study screened a total of 880 potential subjects 342 of whom were found to be eligible. Of these 222 consented to participate in the study and 164 started the study with 143 completing the study. Figure 1, Appendix E shows how participants progressed through the study. Twenty-one subjects did not complete the study, 14 in the Pre-AK group 7 of whom were male and 7 of whom were female, five in the AK group all being male and three in the SCC group one being male and two being female. Table 1, appendix E

explains what forms were completed at each visit and the time points between visits.

3.1: Kappa

In epidemiologic research the interpretability of observed results depends on the accuracy of the measurements (194). In many situations the “truth” is not known, and in order to judge the quality of measurement one must settle for an assessment of agreement between multiple imperfect sources of information or between multiple measurements using a single imperfect source of information (194). Simple percent agreement between two binary classifications has been recognized as a misleading index because of the chance agreement that occurs, even if there is no systematic tendency for the two measurements to classify the same individual similarly (194).

The kappa statistic is an index of reproducibility utilized to compare similar questions with categorical answers on different questionnaires and corrects for agreement by chance (Does the same question yield consistent results at different time points?) (195, 196). It is used when there is no clear-cut or gold standard and when it is appropriate to give equal weight to both sets or readings (195). A kappa statistic less than zero indicates poor agreement, 0 to 0.20 slight, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial, 0.81 to 1.00 almost perfect agreement (196, 197).

Kappa is most frequently used as a measure of reliability (i.e. agreement, reproducibility consistency, or interchangeability of observers) in a single sample, however, under certain conditions kappa can be interpreted in the context of validity of the odds ratio when comparing two samples (194). Kappa is directly analogous to the intra-class correlation coefficient obtained from analysis of variance (ANOVA) models for quantitative measurements and can be used as a measure of the reliability of multiple determinations on the same subjects (197). Kappa indicates the extent to which the observational probability of agreement is in excess of the probability of agreement hypothetically expected under the baseline constraints (197).

For any given value of kappa, the degree of misclassification/bias that results from using the corresponding instrument/reader can depend on the prevalence of the condition and distribution of the marginals (195). An important limitation of kappa when comparing reliability of diagnostic procedures in different populations is its dependence on the prevalence of true “positivity” in each population (195). Thus a population subgroup with the larger of two kappas may appear to reflect better measurement, when in fact sensitivity and specificity could well be lower than for the other subgroup (194). However, strong dependence of kappa on prevalence can be considered a strength because kappa can be used as an index of the correspondence between the observed odds ratio and the odds ratio that would have been obtained had there been no

misclassification (194). For fixed sensitivity and specificity levels, kappa tends toward 0 as the prevalence approaches either 0 or 1 (194, 195).

When the marginals of the contingency table are unbalanced, high values of kappa may result. Kappa is higher when the positivity prevalence is different between observers than when both observers report similar prevalence, and kappa unduly rewards a differential assessment of positivity between observers (195).

If misclassification is non-differential, kappa reflects how free from bias the odds ratio is likely to be with a kappa of 0.0 indicating complete attenuation of the odds ratio and a value of 1.0 indicating a total lack of bias. For ordinal data the values for kappa can be greatly influenced by the number of categories so a four-category kappa for ordinal data cannot be compared with a three-category kappa (198). When using kappa with ordinal data an intermediate category will often be subject to more misclassification than an extreme category because there are two directions in which to err away from an intermediate position but only one direction in which to err away from the extremes (198).

With nominal data, there are no extremes since there is no directionality, so no individual kappa will have an inherent tendency to be larger or smaller than any other (198).

3.2: Statistical Analysis

Chi square tests were used to compare basic demographic characteristics between the three final risk groups. ANOVA and Bonferroni multiple comparisons were used to look at potential differences in mean age between groups.

To test the reliability between independent groups, Cohen's kappa was utilized. For comparison between the multiple levels of self-reported sun sensitivity, Weighted kappa was estimated. This statistic took advantage of the ordered categories, so that partial credit was given to small error versus large error (195). Because there was no clear-cut "gold" standard for any of the interviews or questionnaires, equal weight was applied to both sets or readings (195). A kappa statistic less than zero would suggest poor agreement, 0 to 0.20 slight, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial, 0.81 to 1.00 almost perfect (196, 197). Confidence intervals were calculated for the kappa statistic using the STATA command "kapci". STATA utilizes an analytical method for simple two by two comparisons and a bootstrap method in the case of dichotomous variables. When the bootstrap method was utilized, STATA was specified to perform 1000 repetitions.

3.3: Results

There were few differences between assigned risk groups, with the exception of gender. There were more men in both the AK (75.8%) and SCC

(62.5%) groups than in the Pre-AK group (33.3%). In addition those in the SCC group tended to be older, mean age 65.8 years compared to 58.1 years in the Pre-AK group. Similarly there were few differences for phenotypic characteristics, skin characteristics and sun protective habits with the exception of “ever use of sunlamps”. Self-reported usage was very low in the AK group (3.0%) compared to the other groups.

Agreement between risk group assignment was substantial ($kappa= 0.76$, 95% CI 0.65-0.85) and there were no differences in agreement by gender of the participant. The telephone interviewers misclassified 12, (17.4%) of true Pre-AK subjects as AK. Only 5 (15.6%) of the true AK subjects were misclassified, 4 as Pre-AK and 1 as SCC. A total of 3 (9.7%) SCC subjects were misclassified and all three were placed in the AK group instead of the SCC group.

Questions on the interviewer “Telephone Recruitment Form” and the self-administered “Participant Profile” asked subjects to describe what happens to their skin after a specified amount of sun exposure. Eligible responses were from one of 5 categories related to burning and tanning. There was an average of 43 days between the administrations of the two instruments. Using weighted kappa, agreement was moderate ($kappa\ weighted=0.46$, 95% CI 0.36-0.56), with higher agreement for women ($kappa\ weighted =0.53$, versus 0.36).

The kappa value for AK history was $kappa=0.66$, 95% CI 0.54-0.78, for SCC $kappa=0.78$, 95% CI 0.65-0.91 and for BCC $kappa=0.75$, 95% CI 0.55-0.94, all considered to be substantial agreement and all significantly different than

zero. Kappa values differed by gender but the differences were not significant as there is overlap in the confidence intervals. In addition individuals were more likely to report a diagnosis of NMSC at the telephone recruitment than on the self-reported health history.

3.4: Discussion

In epidemiologic research, as in any scientific endeavor, the interpretability of observed results depends to a considerable extent on the accuracy of the measurements (194). In most situations, “truth” is not known and, in order to judge the quality of measurement, one must settle for an assessment of agreement between multiple imperfect sources of information or between multiple measurements using a single imperfect source of information (194).

In this study, the objectives of the analyses were three-fold. We sought to determine how reliably participants were placed into risk groups by trained telephone interviewers compared with a study dermatologist’s assessment. Secondly, the consistency between self-reported sun sensitivity was assessed. Lastly, we examined the consistency of participant self-reported history of skin lesions, specifically NMSC and AK. We noted extremely good agreement ($kappa= 0.76$, 95% CI 0.65-0.85) between the classification of potential study participants into risk groups by trained telephone interviewers and the final assignment by a study dermatologist. During the recruitment phase of a clinical study, large numbers of people are often screened to find the few who qualify.

Recruitment and screening is a time consuming process and study costs increase dramatically if study dermatologist time is necessary for initial screening. In addition, if the study seeks to recruit specific numbers into each risk group, participant's risk group must be immediately and accurately identified.

Despite the level of good agreement, misclassification did exist, and not surprisingly, this misclassification centered on assignment of risk groups Pre-AK and AK. The telephone interviewers misclassified 17.4% of true Pre-AK subjects as AK. Similarly 15.6% of the true AK subjects were misclassified, with 4 people (1.3%) classified by the screener as Pre-AK and 1 (3.1%) as an SCC. Among the final SCC group 3 SCC subjects (9.7%) were misclassified and all three had been placed in the AK group instead of the SCC group by the screener. The screeners placed subjects into risk groups based on information they collected during the interview while the dermatologists made group assignments based on skin examinations. Since the telephone interviewers based their decisions on participant report, it would appear that subjects were more likely to report having AKs when they did not actually have any. This could be due to lack of knowledge pertaining to identification of an AK, or the difficulty of an untrained person with sun damaged skin to differentiate an AK from other sun damage. It may also be true that potential participants exaggerated their skin damage on the telephone because they had a strong desire to be eligible and participate in the study.

As risk group classification increased in seriousness, misclassification decreased. There was more misclassification in the Pre-AK risk group and much

less in the SCC risk group. If only the telephone interviewer was used to classify subjects into risk groups, there would be more true Pre-AK subjects in the AK group. This could then make it more difficult to distinguish between groups during analysis of biomarkers. For example, if there was a specific biomarker associated with development of SCC, this type of misclassification could decrease the likelihood of detecting any gradient between the disease groups.

In our study, agreement was not as strong for self-reported sun sensitivity measures (κ weighted=0.46, 95% CI 0.36-0.56). One caveat needs to be highlighted. The sun sensitivity questions being compared on the two forms were not worded in precisely the same manner. The question on the interviewer administered "Telephone Recruitment Form" and the self-administered "Participant Profile" differed slightly. The "Telephone Recruitment Form" was more focused toward assessment of whether an individual's untanned skin burns in the sun, and the self-reported "Participant Profile" focused on descriptions of tanning in addition to burning.

The concept of sun-reactive skin typing was created in 1975 to classify persons with white skin in order to select the correct initial doses of ultraviolet A (UVA) (in joules per cubic centimeter) for the treatment of psoriasis-oral methoxsalen photochemotherapy (PUVA) (180). It was decided that a brief personal interview regarding the history of the person's sunburn and suntan experience was one approach to estimate the skin tolerance to ultraviolet radiation (UVR) exposure and the Fitzpatrick skin typing system was created

(180). The “Fitzpatrick skin typing system” has been used by the US Food and Drug Administration in its guidelines for sunscreen products for over-the-counter human use (180).

Self-reported sun sensitivity is used to assess skin type and, therefore, risk for skin cancer. Only a few studies looking at the reliability of these measures are available in the literature and report better reliability than our study. Reliability, assessed by comparing answers to the same question at different time points, is utilized because the measures do not have a “gold standard”. In the multi-centre South European case-control study, a sub-sample of participants were re-interviewed and reaction to sun exposure was assessed on a four level scale (199). Weighted kappa for skin reaction to sun exposure was 0.61 (95% CI 0.53-0.70) which is slightly higher than the five level weighted kappa from our current study ($kappa=0.46$, 95% CI 0.36-0.56). (Recall that the value of kappa is affected by the number of categories) In a case-control study of melanoma that included test-retest reliability of self-reported exposure to sun sensitivity, there was good consistency with kappa values for ability to tan and tendency to burn of 0.66 and 0.62 respectively (177).

In a case-control study nested within the Nurse’s Health Study cohort, Weinstock et al. reported that test-retest reliability of tanning questions was high in the prevalent case group (Spearman’s $r=0.78$) and control group (Spearman’s $r=0.76$), but lower in the incident case group (Spearman’s $r=0.59$) (178). Their study had a similar caveat in that the questions were not worded identically

between the two questionnaires. Weinstock et al. also found that, among women diagnosed with melanoma after the first questionnaire and before the second, there was a substantial shift toward reporting a reduced ability to tan (179).

This highlights an important issue for development of study questionnaires. The issue of burnability and tannability are separate issues to subjects and need to be considered separately. In a study by Rampen et al. neither tannability nor burnability were linked very closely to the minimal erythemal dose (MED) which would be the “gold standard” of sun sensitivity (182). Rampen et al. investigated burning and tanning histories in 790 white students, 18 to 30 years old, with a self-administered questionnaire to classify them into skin types based on the Fitzpatrick scheme (burning tendency after one hour of sun exposure in early summer and the tanning ability after regular sun exposure during summer were recorded as follows: 0, none; 1, mild; 2, moderate; and 3, severe/intense) (182). MED was measured in a subgroup of this population. There was no statistically significant correlation with the self-reported burning tendency and the MED. Skin typing on the basis of self-reported burning tendency and tanning ability may be subjective because subjects tended to over-record no burning and under record no tanning. The correlation with biologic complexion factors like hair and eye color and freckling tendency was somewhat better for self-reported tanning than for the burning propensity (182).

The authors concluded that self-reported burning-tanning histories do not provide a valid means of skin typing, when compared with the MED (182). It may be that a better way to characterize sun sensitivity would be through proxy measures such as hair and eye color and freckling tendency which appear to be more reliably reported by subjects. Weinstock et al. found test-retest reliability of hair color assessment by questionnaire was high with the Spearman correlation coefficient between 0.76 and 0.87. Sun sensitivity may be subject to recall bias when assessed by ability to tan, but not when assessed by hair color (179). There is a need for further studies to look at the issue of skin type classification more closely.

Based on Weinstock et al.'s results, we might have expected that the SCC risk group would have been more reliable reporters of sun sensitivity or that there would be a gradient of response with the Pre-AK group being the less reliable reporters than the AK or SCC groups (178). However, we found that all risk groups were equally as reliable when reporting sun sensitivity (data not shown). Results from previous studies are consistent with our findings on agreement for self-reported history of NMSC. We found more serious skin conditions had higher agreement (for AK history $\kappa=0.66$, 95% CI 0.54-0.78 for SCC $\kappa=0.78$, 95% CI 0.65-0.91 and for BCC $\kappa=0.75$, 95% CI 0.55-0.94). In a study by Ming et al., self-reported history of skin cancer was compared with the gold standard of chart documentation. Patients were found to recall their cancer history quite well, with correct identification highest for melanoma (95% of cases)

and lowest for basal cell carcinoma (84% of cases) (175). In a study by Bergmann et al., assessing agreement of self-reported medical history using an in-person interview versus a self-administered questionnaire, kappa values of 0.83-0.88 were found for cancer reporting. Lower values were found for less severe or more transient disease, with the disease being reported at the interview but not on the questionnaire (174). Our study also found that NMSC diagnosis was more likely to be reported to the phone interviewer than on the self-administered "Health History Form".

The results of our study suggest that women may be more reliable reporters than men; however, the literature does not always support this finding. In an Australian study of ocular melanoma, that gathered information on sun exposure in the first four decades of a person's life, questionnaires administered one year apart gave an interclass correlation coefficient of 0.65 for ranked total sun exposure between two interviews with the coefficient higher for men (0.73) than for women (0.54) although like our study, not statistically significant (200). The use of kappa to measure reliability can be problematic because kappa values will depend on the prevalence of the condition and distribution of the marginals (195). We used a weighted kappa for ordered categories so that partial credit would be given to small error versus large error (195). Additionally, kappa values depend on the number of categories with more categories resulting in lower values (198). Kappa does, however, account for agreement that may occur by chance alone. The use of kappa is more appropriate than the use of

percent agreement. Percent agreement is the simplest method of summarizing agreement for categorical variables and has the advantage of being useful for any number of categories. Percent agreement is artificially increased when the proportion of negative-negative results is high or when the prevalence of the condition is high.

Overall, there was evidence for substantial reproducibility for factors related to assignment into skin cancer risk group and self-reported history of skin lesions, with self-reported sun sensitivity questions being somewhat less reliable. In all comparisons, women were more consistent reporters than men. These results suggest that self-reported measures of skin cancer risk should be reasonably reliable for use in screening subjects into studies. Further studies are required to further identify the characteristics of individuals with poor reliability of self-reported measures.

SUMMARY AND FUTURE DIRECTIONS

Non-melanoma skin cancer is the most common form of cancer in the United States and the most common malignant neoplasm in the White population, afflicting about 20% of the general population at some point in life (1, 2, 4). Exposure to UV radiation is by far the most common cause of NMSC with mechanisms for the oncogenic effect being immunosuppression and DNA changes that lead to clonal expansion of malignant keratinocytes (1). SCC has been shown to be strongly related to estimated total sun exposure (RR=1.53,

95% CI 1.02-2.27) and occupational sun exposure (RR=1.64, 95% CI 1.26-2.13), but only weakly associated with sun burn (43). BCC has shown only a weak association with occupational exposure (RR=1.19, 95% CI 1.07-1.32) but shows a more significant association with non-occupational (intermittent) sun exposure (RR=1.38, 95% CI 1.24-1.54) (43). NMSC will continue to grow as a public health concern because evidence indicates that the incidence of NMSC is estimated to be increasing by 2 to 3 percent per year (2).

Through epidemiological studies multiple risk factors for NMSC have been identified and include: chronic sun exposure, sunburns- especially blistering burns in childhood, cumulative and childhood sun exposure, fair skin, light hair and light eyes, poor ability to tan, exposure to ionizing radiation, chronic ulceration and inflammation, human papillomavirus infection, chemicals (coal-tar, psoralens, UVA, arsenic, cigarette smoking), family history of skin cancer, personal history of skin cancer, impaired immune system, scarred or traumatized skin (ulcerations, burns), actinic keratoses, telangiectasia on the ears, geographic location, older age, male sex, freckling, and Celtic ancestry (1, 2). Many of these risk factors are modifiable but others such as a person's phenotype, genotype and perhaps place of residence are not. In addition people with a history of BCC or SCC are at increased risk for a new skin cancer, including additional basal and squamous cell carcinomas and melanomas. Fifty percent of patients treated for basal or squamous cell carcinoma have another skin cancer within 4 years (1). Recurrent skin cancer often behaves more

aggressively than the original lesion and patients with a previous BCC or SCC have an increased risk for melanoma (3% to 17%) (1).

Epidemiological and laboratory evidence have suggested a role for both the retinoids and NSAIDs as chemopreventive agents for NMSC. One large randomized clinical trial (SKICAP BCC/SCC) evaluated the usefulness of retinol and isotretinoin in preventing the recurrence of NMSC in a population who had at least one prior NMSC. Unfortunately this trial failed to find a reduction in risk for those taking one of the retinoid intervention agents. The study suffered from toxicity issues and participant withdrawal prior to completion of the protocol. NSAIDs as a chemopreventative agent for NMSC have never been studied in a randomized clinical trial, although ample evidence from other types of cancer, in vivo and in vitro studies exist to support the hypothesis that NSAIDs could play a role in prevention. The present studies (Hypothesis 1 and 2) sought to explore the chemopreventative possibilities of these agents further.

The current dose response analysis of the original SKICAP BCC/SCC data confirms that neither retinol nor isotretinoin were able to reduce the risk of NMSC recurrence in a population of people with a previous NMSC. Curiously there seems to be an increase in risk for those in the first quartile of each of the interventions for SCC that has been unexplainable. Although the background literature for the use of retinoids quite strongly supports that NMSC recurrence would be reduced with their use, neither the original intent to treat analysis nor our dose response analysis were able to confirm this.

The analysis of NSAIDs data adds to the small body of literature on this subject and confirms that NSAIDs use may reduce the risk of SCC but not BCC in an at risk population. The present study indicates that those who have reported more recent use and use of a non-ASA NSAIDs are at a reduced risk of developing a new SCC, but no effect was found for BCC.

Time, as it relates to the development of cancer and the preventive effect of potential agents, is a large issue for chemoprevention studies that affects both of these analyses. Unfortunately, good evidence about the time period in the carcinogenesis pathway when these agents or any agents could be effective is lacking. Information on the length of time these agents should be taken before an effect is seen is also lacking. In addition both of these analyses were based on studies with 3-5 year intervention periods, thus both analyses are bounded by this arbitrary time period.

Determining risk based on phenotypic traits continues to be of importance for all studies of NMSC. Assigning risk allows for studies to examine groups of homogenous people and to better interpret results. The literature is clear that those with increasing sun sensitivity, as measured by decreasing ability to tan following repeated exposure to the sun, have a substantial gradient of increasing risk with increasing sensitivity for both BCC and SCC, with the steepest gradient in the most sun sensitive people (43). Because of this, ability to tan or likeliness of burning are variables that are always collected and included in the final analysis of NMSC studies. For the most part, this information is self-reported in

most studies. Determining the reliability of these self reports and also the ability of potential participants to report their history of NMSC and pre-malignant lesions such as AK is an important issue. Our study revealed that there was evidence for substantial reproducibility related to risk group assignment and self-reported history of NMSC, with self-reported sun sensitivity being less reliable. These results suggest that self-reported measures of skin cancer risk are reasonably reliable for use in screening subjects into studies. Additional work needs to be done to assess reliability and validity of risk assessment questionnaires. In addition the development of a reliable and valid standard questionnaire to assess risk would greatly add to the field and also allow for more cross study/population interpretation.

Continuing to explore chemoprevention strategies for NMSC is important because, although NMSC does not result in substantial mortality, NMSC does have the ability to cause substantial morbidity, including disfigurement and loss of function, and treatment is costly (3). These three studies have added useful information to the existing literature on NMSC chemoprevention and have generated additional hypothesis to be explored. More work in the laboratory needs to be done to decipher the mechanisms by which NSAIDs may exert a chemopreventative effect in those at risk for NMSC. Additionally large data bases containing information on NMSC diagnosis and use of NSAIDs should be utilized to further confirm these results. The final phase of this work would be to conduct a randomized clinical trial in a population of at risk people.

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APPENDIX A- Manuscript 1:
Effect of Non-Steroidal Anti-Inflammatory Drugs on Non-Melanoma Skin Cancer
Incidence in the SKICAP-AK Trial

Effect of Non-Steroidal Anti-Inflammatory Drugs on Non-Melanoma Skin Cancer
Incidence in the SKICAP-AK Trial

Clouser, Mary C., MPH, Ph.Dc.,^{1,2} Roe, Denise J., DrPH,^{1,2} Foote, Janet A.,
Ph.D.,^{1,2} Harris, Robin B., Ph.D.,^{1,2}

¹Mel and Enid Zuckerman College of Public Health, ² Arizona Cancer Center,
University of Arizona

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ABSTRACT

Recent studies link the prostaglandin metabolic pathway to skin carcinogenesis expanding possibilities that cyclooxygenase inhibitors may be utilized in non-melanoma skin cancer (NMSC) chemoprevention. Using data from a study of the efficacy of retinol supplementation on incidence of NMSC, we sought to determine the role of non-steroidal anti-inflammatory drugs (NSAIDs) in NMSC development. Cox proportional hazards models describe the relationship between NSAID use and time to first SCC or BCC among participants categorized by use pattern: continuous users (use for length of study duration), new users (use for less than study duration), and non users. For SCC and BCC, there was a statistically significant protective effect for subjects who reported use for less than study duration (HR=0.49, 95% CI 0.28-0.87 & HR=0.43, 95% CI 0.25-0.73, respectively). Categorical examination of NSAIDs (aspirin (ASA) vs. non-ASA NSAIDs) showed significant effects for BCC among those using non-ASA NSAIDs for less than the study duration (HR=0.33, 95% CI 0.13-0.80). For SCC and BCC, NSAID use of shorter duration and potentially more recent, was more protective than continual or longer duration of use. These results are counter to the idea that longer duration of NSAID use is more protective. Additional investigations are needed into the role NSAIDs play in the chemoprevention of NMSC.

INTRODUCTION

Skin cancer is the most common form of cancer in the United States with 50% of Americans living to age 65 having skin cancer at least once (1, 2). Although mortality from non-melanoma skin cancer (NMSC), which includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is relatively low, the high incidence leads to a substantial public health burden, making NMSC an appropriate target for chemoprevention and ongoing research. Non-steroidal anti-inflammatory drugs (NSAIDs) have been useful therapy for treating large numbers of cutaneous pathologies for decades (3). NSAIDs act by inhibiting prostaglandin synthesis via the cyclooxygenase (COX) pathway. The linking of the prostaglandin metabolic pathway to skin carcinogenesis expands the possibility that compounds which inhibit this pathway may be utilized as effective treatments and preventatives for NMSC (3).

Findings from both in-vitro and in-vivo studies continue to provide convincing evidence that NSAIDs can reduce the risk of onset and also multiplicity of NMSC lesions (4). In addition, topical NSAIDs such as diclofenac gel, have been successfully used to treat actinic keratosis (AK), a precursor lesion of SCC (5, 6). Several epidemiological studies have also suggested reductions in NMSC in NSAID users (7, 8). The current analyses use data from a large, previously completed, skin cancer prevention trial to evaluate the relationship between self-reported NSAID use and the occurrence of first NMSC.

METHODS

The 2297 subject SKICAP-AK study was a double blind, randomized, placebo-controlled trial testing the hypothesis that daily supplementation of retinol (25,000 IU) for 5 years reduces the incidence of skin cancer in high risk individuals. A full description of the study design, methods and results were previously published, none of the authors have any conflict of interest to declare (9, 10). Enrollment and consenting of participants began in June 1984 and continued until November 1988 in two primary clinical centers, Phoenix and Tucson, Arizona. Eligibility required at least 10 clinically apparent actinic lesions and minimal (<2) if any NMSC history.

Concomitant medication information was captured at baseline, updated at every clinic visit (twice yearly) and on the self-administered exit interview form. The exit interview form asked the following: "Are you currently taking or using any prescription or over the counter medications? If so, please list the names of the medications along with the reason for taking it, medication daily dose, and length of time (in months) medication was taken." Medications reported on the exit form were coded and organized by category, with each medication assigned a six digit code referencing the category and a four digit code referencing specific medication name. Medication information collected at baseline and at each subsequent clinic visit was never coded or entered into the data base.

Based on reported number of months a medication had been taken at exit, an approximate "start date" for NSAID use was generated. NSAID use was then defined as: (1) Non-users were participants who reported no NSAID use on the

exit questionnaire or reported NSAID use only after their first diagnosis of an NMSC; (2) New users were those participants who reported use of NSAIDs but for a period less than their length of time on study and prior to first diagnosis of NMSC; (3) Continuous users were those who reported use of NSAIDs equal to or longer than their time on study and prior to first diagnosis of NMSC. NSAID use was further defined as use of any type of NSAID (any NSAID use), use of only aspirin (ASA only), and use of only a non-aspirin NSAID (non-ASA only).

A validation study was conducted assessing validity of NSAID use reported at exit. Records from each clinic visit were reviewed to assess medication reporting at baseline and during clinic visits for 68 participants, 39 who reported use and 28 who reported no use. The baseline question on concomitant medications was phrased “It may be that any benefits from the study drug are modified by other medications. Would you, therefore, please list the prescription drugs which you are now taking more than once per week?” and at clinic visits the question was phrased “Benefits from the study drug may be modified by other medications. Would you, therefore, please list the prescription drugs or aspirin which you now take more than once each week?” Of those who reported NSAID use at exit 90 percent (36), reported use during their clinic visits. Of the four participants who reported use at exit but not at any clinic visit two completed the exit survey multiple years after their last clinic visit. Of the 28 participants who reported no NSAID use at exit, 64 percent reported no use at

the clinic visits and ten (36%) reported some use. One of these ten discordant findings was due to data entry error.

All skin cancer end points were monitored after randomization and confirmed by pathology. Participants were examined for skin lesions by a study dermatologist or their own dermatologist at least once a year and underwent a full-body skin examination by a study dermatologist when they exited the study. Participants with suspicious skin lesions were referred for biopsy and treatment and were closely followed by study staff to ensure that biopsies were performed and a diagnosis obtained. In addition endpoints were identified by participant self-report at study follow-up visits, by review of pathology records of dermatologists, pathology laboratories and the southeast Arizona skin cancer registry. Diagnostic pathology slides were obtained on all biopsies, centrally reviewed and confirmed by the study dermatopathologist.

Of the original 2297 participants in the intervention study, 320 did not complete an exit visit form and/or the medication questionnaire and were excluded from the current analysis. In addition subjects who reported a history of NMSC (n=568) prior to randomization and those who had a melanoma diagnosed (n=7) were also excluded, leaving 1,402 subjects in the final analysis data set. The analysis sample was statistically significantly younger (mean age 60.0 (10.3) vs. 61.0 (10.6), $p=0.0023$) than the parent skin cancer prevention trial population and included proportionally more women (32% versus 30%).

A total of 334 first NMSCs among 284 participants were confirmed throughout the study. Ninety-six participants had only SCC diagnosed, 138 had only BCC, and 50 had both SCC and BCC diagnosed during follow-up (first new BCC followed by a first new SCC=21, first new SCC followed by a first new BCC=24, first new SCC and first new BCC diagnosed on the same date=5).

All analyses were performed using intercooled STATA version 9.0 StataCorp, 4905 Lakeway Drive, College Station, Texas. Chi square tests compared basic demographic characteristics between NSAID users and non-NSAID users, ASA only users and non-NSAID users, and non-ASA NSAID users and non-NSAID users. Two sided Fisher's exact tests compared characteristics of the NSAID duration groups (new versus continuous users) and compared characteristics of those with no diagnosis of NMSC, diagnosis of SCC and diagnosis of BCC. Two sample independent t-tests were used to compare means.

To examine the relationship between NSAID use and development of NMSC, hazard ratios and 95% confidence intervals were estimated. Cox proportional hazards models modeled time to the development of first BCC or SCC and adjusted for treatment group, age and gender. Participants who developed both BCC and SCC during the study period were included in both the BCC and the SCC groups. Other potential confounding factors such as clinic site, smoking status, skin reaction to the sun, eye color, hair color, ethnic background of the parents and history of AK diagnosis prior to first SCC/BCC diagnosis, were

evaluated in initial models by comparing the adjusted and unadjusted hazard ratios. The interaction between treatment group and NSAID medication use was evaluated by comparing adjusted and unadjusted models, but was not significant.

RESULTS

For the 1,402 subjects included in these analyses mean time on study was 60.7 months (SD= 11.5, range 31.0-91.9). Selected demographic and behavioral characteristics of subjects using NSAIDs versus non-users are shown in Table 1. There were few differences between users and non-users. NSAID users tended to be older, with 68.2 percent of the NSAID user group being greater than 60 years of age (mean 63.4) compared to 50.1 percent of the non-NSAID users group being greater than 60 years of age (mean 58.8). This difference in age was present regardless of whether subjects were ASA only users or non-ASA NSAID users. Proportionally more women reported using non-ASA NSAIDs compared to men 41.3 percent versus 31.7 percent respectively ($p=0.02$).

Continuous users of NSAIDs tended to be older compared to new users (64.7 versus 62.1 years $p=0.078$). (Table 2) There were no significant differences for duration of use related to gender, clinic, type of NSAID used or the stated reason for taking the medication (data not shown). For new users the median time of use in the ASA only group was 24 months (95% CI, 24-36) and for those taking non-ASA NSAIDs it was 13 months (95% CI, 12-24). For continuous users the median duration of use for the ASA only group was 100 months (95%

CI, 73.7-120) and in the non-ASA NSAID group the median months of use was 120 (95% CI, 85.6-120).

Table 3 compares characteristics of study participants by NMSC outcomes. There were few differences between the NMSC outcomes based on gender, weekly sun exposure in hours, sun sensitivity, or smoking status. Age was the only statistically significant characteristic between outcome groups, with participants greater than 60 being more likely to be diagnosed with NMSC than those under 60 years of age ($p=0.011$).

The relationship between NSAID use and development of first BCC or SCC is shown in Table 4. There was a statistically significant protective effect for BCC among those who reported any NSAID use (HR=0.58, 95% CI 0.39-0.85) as compared to those reporting no NSAID use. When NSAID use was stratified into new users versus continuous users, there was a protective effect only for new users (HR=0.43, 95% CI=0.25-0.73). For continuous users, the relationship was less protective and not statistically significant (HR=0.88, 95% CI=0.52-1.50). Similarly, there was a statistically significant protective effect for development of an SCC only for the new user NSAID group (HR=0.49, 95% CI 0.28-0.87). The continuous user group showed no effect for SCC development (HR=1.11, 95% CI 0.65-1.92).

NSAID use was categorized by ASA use only or use of a non-ASA NSAID. Although the use of ASA was protective for both BCC and SCC, the relationships were not statistically significant (HR=0.64, 95% CI 0.38-1.06, HR=0.71, 95% CI

0.41-1.22, respectively). New use of either ASA only or non-ASA NSAID was likewise protective for both BCC and SCC but not statistically significant (HR=0.58, 95% CI 0.30-1.10, HR=0.51, 95% CI 0.24-1.10). Among continuous users, there was a non-significant protective effect for BCC only (HR=0.74, 95% CI 0.35-1.6 for BCC and HR=1.08, 95% CI 0.52-2.22 for SCC). Use of non-ASA NSAIDs was associated with being protective for BCC only in the new users group (HR=0.33, 95% CI 0.13-0.80).

DISCUSSION

In these analyses of data from a population at high risk for skin cancer, we found a protective effect for NSAID use on the development of first BCC or SCC among participants reporting a shorter duration or more recent use versus those reporting continuous use. The protective effect for initial BCC was most evident for non-ASA NSAID use, whereas protection for development of an initial SCC was not confined to a specific NSAID category. Although typical dose-response relationships suggest that longer duration of NSAID use enhances protection, long-term NSAID use was not significantly protective for either NMSC outcome. In addition NSAID use was expected to be more protective with regards to SCC rather than BCC, but these analyses did not confirm that expectation. However, these results are consistent with the results of a recently published study by Grau et al. (2006).

Many studies have looked at the use of NSAIDs and ASA in various cancers with mixed results. Some of the most convincing studies were from the colon cancer/polyp prevention arena where multiple studies using ASA or NSAIDs for prevention have reported protective effects (11, 12, 12-22). Use of NSAIDs has also been associated with a reduced risk of esophageal, stomach, ovarian and breast cancers and increased risk for lung, kidney and prostate cancers (23, 24)

The effect of NSAID use on skin cancer has been examined in vitro, in multiple animal models, as a topical agent to treat certain skin conditions, and in a few epidemiological studies. There have been no prospective randomized clinical trials reported to date. Grau and colleagues published a study in 2006 looking at the association of self-reported NSAID use with the risk of BCC and SCC using data from the randomized, Skin Cancer Chemoprevention Study of oral β -carotene (7). The three response categories were no use, sporadic use (less or half of questionnaires positive for NSAID use) or frequent use (more than half of the questionnaires positive). Grau and colleagues found that for BCC, NSAID use exhibited a weak protective effect in crude analyses, which attenuated after adjustment. However, use of NSAIDs in the year previous to diagnosis reduced the odds for development of SCC by almost 30% (adjusted OR=0.71, 95% CI 0.48-1.04) (7).

Butler et al. published a nested case control study looking at the risk of AK and SCC in those reporting NSAID use who were part of the randomized,

Queensland, Australia Nambour Skin Cancer Prevention Trial comparing daily sunscreen vs. discretionary use of sunscreen and β -carotene supplements vs. placebo (8). Butler and colleagues found that participants who used NSAIDs eight or more times weekly for more than one year had a lower incidence of SCC (OR=0.07, 95% CI 0.01-0.71) and those who used full dose NSAIDs two or more times per week for more than five years also had a lower SCC incidence (OR=0.20, 95% CI 0.04-0.96) (8).

Chronic inflammation correlates with increased risk of developing cancer in the affected organ and can be caused by UV radiation and other factors (25), (26). Arachidonic acid metabolism, which can be triggered by the effect of ultraviolet radiation on the cell membrane in the case of skin, initiates the inflammatory process and this metabolism along with prostaglandin synthesis and the cyclooxygenase pathway is linked with the promotion and progression of cancers (26). The importance of elevated prostaglandins in tumor promotion has been studied through the use of inhibitors such as NSAIDs, which block prostaglandin production by inhibiting both COX isoforms (COX-1 and COX-2) (25). In the skin, the pathway leading to prostaglandin synthesis liberates oxygen-derived free radicals, which may cause tissue damage during the inflammatory response, and in addition prostaglandins may promote the retention of UV-damaged cells via inhibition of apoptosis (3).

Three classes of COX inhibitors exist; aspirin synthesized from salicylic acid, indomethacin and other NSAIDs, and the first selective COX-2 inhibitors-

the coxibs (e.g., celecoxib and rofecoxib) (27). A study evaluating the expression of COX in human and mouse skin biopsy has shown COX-1 is observed throughout the epidermis whereas COX-2 increases in the more differentiated suprabasilar keratinocytes (28). BCC expresses little if any COX-1 or COX-2 whereas both COX isozymes are strongly expressed in SCC deriving from a more differentiated layer of the epidermis (28). COX-2 expression in human keratinocytes appears to be related to differentiation of these cells both in vivo and in culture (28). Based on the metabolic pathway, NSAIDs are potentially good chemopreventative agents for skin cancer, specifically SCC.

Although the present SKICAP-AK study captured information about duration of NSAID use, the information is self-reported on an exit survey and is only an estimate of the total duration of use. Our small validation study comparing self-report of NSAID use at the clinic visits as compared to the exit survey exposes misclassification in exposure (NSAID use) related to those considered non-users based on their exit but who reported NSAID use at clinic visits. It is likely there are more true NSAID users in our population than what we captured via the exit interview thus causing our estimates to be biased toward the null. A variation in the wording of the medication use question may have prompted different recall. Our analysis sample was younger and included more women than the original SKICAP-AK population, both younger age and being female are related to a reduced risk of NMSC possibly leading to the lack of risk seen for SCC.

Similar to both the Grau and Butler studies, the present analyses required decisions about how to categorize NSAID use (7, 8). The literature currently lacks any consistent definition of what constitutes chronic use. There is also a lack of evidence suggesting the amount of time one may need to use NSAIDs in order to see a protective effect, along with the effect that timing of cumulative or intense UV insult may have on NSAID-derived protection. Variability in NSAID dosing and dosage relative to other physiological factors, such as body fat and skin photo-sensitivity and thickness, add complexities that could not be addressed in the current study.

Other biological factors which may affect the COX pathway may need further investigation at the basic science level. Our findings suggest that the shorter duration or more recent use appears more protective for development of first NMSC. Tolerization may be one mechanism playing a role in this finding. Chronic use of NSAIDs may lead to receptor over expression and alternate signaling pathway activation.

We observed some potential variation in the magnitude of the protective effect by type of NSAID. Statistically significantly protective effects were found primarily for non-ASA NSAID use and in particular for BCC development. However, the collected data and the small sample size did not allow evaluation of more specific types of NSAIDs. Each type of non-ASA NSAID provides different levels of COX-1 and COX-2 inhibition and some NSAIDs may be better skin

carcinogenesis chemopreventers than others. This remains yet another area of investigation.

These analyses have several strengths. This cohort was well monitored with regular in-person study visits and exams by a study dermatologist on an annual basis. Chart reviews were completed to ensure acquisition of all skin-related biopsy or treatment information. Diagnosis of NMSC was verified through acquisition of the specimen slide and central pathology review.

These results are counter to the idea that longer duration of NSAID use is more protective, an idea that permeates the literature on NSAIDs and chemoprevention. They are also counter to the idea that we would expect to see the major effect in SCC because of the pathways by which NSAIDs do their chemoprevention work and the pathway by which SCC develops. This may indicate that NSAIDs may have effects in other ways than currently known and that NSAIDs block different signaling pathways in carcinogenesis process for BCC development versus SCC. It is clear that more research at the basic science level remains to be done as well as more controlled prospective studies in order to understand the role of NSAIDs in the chemoprevention of NMSC.

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Table 1: Characteristics of Study Participants at Baseline by NSAID Use
(n=1402)

Characteristic	No NSAID Use	Any NSAID Use	P value*	ASA only	p value*	Non-ASA NSAID Only	p value*
<i>n</i> (%)	1081 (77.1%)	321 (22.9%)		170 (53.0%)		151 (47.0%)	
<i>Age</i> (%)			<0.0001		<0.0001		0.009
≤60	524 (49.9%)	112 (31.8%)		50 (26.0%)		62 (38.8%)	
>60	526 (50.1%)	240 (68.2%)		142 (74.9%)		98 (61.3%)	
<i>Age (mean ±SD)</i>	58.8 (10.6)	63.4 (8.4)	<0.0001	64.4 (7.5)	<0.0001	62.1 (9.1)	0.0003
<i>Sex</i>			0.815		0.064		0.017
Female	333 (31.7%)	114 (32.4%)		48 (25.0%)		66 (41.3%)	
Male	717 (68.3%)	238 (67.6%)		144 (75.0%)		94 (58.8%)	
<i>Clinic</i>			0.383		0.750		0.299
Tucson	671 (63.9%)	234 (66.5%)		125 (65.1%)		109 (68.1%)	
Phoenix	379 (36.1%)	118 (33.5%)		67 (34.9%)		51 (31.9%)	
<i>Smoking Status**</i>			0.736		0.858		0.734
Never	424 (40.5%)	146 (41.5%)		79 (41.1%)		67 (41.9%)	
Former	504 (48.1%)	172 (48.9%)		98 (51.0%)		74 (46.3%)	
Current	120 (11.5%)	34 (9.7%)		14 (7.3%)		19 (11.9%)	
<i>Treatment</i>			0.499		0.639		0.578
Active	517 (49.2%)	166 (47.2%)		91 (47.4%)		75 (46.9%)	
Placebo	533 (50.8%)	186 (52.8%)		101 (52.6%)		85 (53.1%)	

* Chi square comparison group is no NSAID use

** Two subjects lack baseline information on smoking status

Table 2: Comparison Between Length of NSAID Use and Population Characteristics

Characteristic	New users: use for less than study time	Continuous users: use for \geq to time on study	p-value*
<i>Age (%)</i>			0.048
≤ 60	80 (38.3)	30 (26.8)	
> 60	129 (61.7)	82 (73.2)	
<i>Age (mean \pmSD)</i>	62.06 (8.64)	64.70 (7.98)	
<i>Sex</i>			0.806
Female	70 (33.5)	39 (34.8)	
Male	139 (66.5)	73 (65.2)	
<i>Clinic</i>			0.806
Tucson	138 (66.0)	72 (64.3)	
Phoenix	71 (34.0)	40 (35.7)	
<i>NSAID</i>			0.413
ASA	107 (51.2%)	63 (56.3%)	
<i>Months of use (median, 95% CI)</i>	24 (24, 36)	100 (73.7, 120)	
Non-ASA NSAID	102 (48.8%)	49 (43.8%)	
<i>Months of use (median, 95% CI)</i>	13 (12,24)	120 (85.6, 120)	

*Fishers exact test except for continuous age (two sample independent t-test)

Table 3: Characteristics of Sample Participants with Diagnosis of SCC and/or BCC (n=1402)

Characteristic	No NMSC Diagnosis	BCC	SCC	SCC/BCC	p-value
<i>Age (%)</i>					0.011
≤60	529 (47.3%)	57 (41.3%)	30 (31.3%)	20 (40.0%)	
>60	589 (52.7%)	81 (58.7%)	66 (68.8%)	30 (60.0%)	
<i>Sex</i>					0.133
Female	373 (33.4%)	36 (26.1%)	24 (25.0%)	14 (28%)	
Male	745 (66.6%)	102 (73.9%)	72 (75.0%)	36 (72.0%)	
<i>Weekly sun exposure (h)</i>					0.818
0-10	463 (41.4%)	63 (45.7%)	40 (41.7%)	21 (42.0%)	
>10	655 (58.6%)	75 (54.4%)	56 (58.3%)	29 (58.0%)	
<i>Skin reaction to sun*</i>					0.295
Always burn-tan little/usually burn- tan minimally	482 (43.2%)	56 (40.6%)	49 (51.0%)	27 (54.0%)	
Burn moderately-tan average/burn minimally- tan easily	544 (48.8%)	74 (53.6%)	40 (41.7%)	22 (44.0%)	
Rarely burn-tan easily/never burn-tan easily	90 (8.1%)	8 (5.8%)	7 (7.3%)	1 (2.0%)	
<i>Smoking Status</i>					0.252
Never	459 (41.1%)	62 (44.9%)	36 (37.5%)	15 (30.0%)	
Former	531 (47.5%)	68 (49.3%)	48 (50.0%)	29 (58.0%)	
Current	128 (11.5%)	8 (5.8%)	12 (12.5%)	6 (12.0%)	

* 2 subjects had missing information for skin reaction to the sun

Table 4: NSAID Use and Development of First Basal Cell Carcinoma (BCC) or Squamous Cell Carcinoma (SCC)

	BCC*		SCC*	
	Hazard Ratio**	95% CI	Hazard Ratio**	95% CI
<i>All NSAIDs</i>				
Any Reported Use	0.575	0.388-0.852	0.697	0.460-1.06
New Users	0.428	0.252-0.729	0.488	0.275-0.867
Continuous Users	0.879	0.515-1.50	1.11	0.647-1.92
<i>ASA Only</i>				
Any Reported Use	0.637	0.384-1.06	0.711	0.413-1.22
New Users	0.579	0.304-1.10	0.512	0.238-1.10
Continuous Users	0.742	0.346-1.59	1.08	0.524-2.22
<i>Non- ASA NSAIDs Only</i>				
Any Reported Use	0.599	0.338-1.06	0.797	0.446-1.42
New Users	0.327	0.134-0.798	0.533	0.234-1.22
Continuous Users	1.26	0.614-2.59	1.39	0.643-3.02

*Participants with a diagnosis of both BCC and SCC are included in both groups for this analysis

**All Cox proportional hazards models adjusted for treatment, age & gender, Non-users are the referent category.

APPENDIX B - Manuscript 2:

Dose Response of Retinol and Isotretinoin in the Prevention of Non-Melanoma
Skin Cancer Recurrence

Dose Response of Retinol and Isotretinoin in the Prevention of Non-Melanoma
Skin Cancer Recurrence (Version #12)

Clouser, Mary C., MPH, Ph.Dc.,^{1,2} Roe, Denise J., DrPH,^{1,2} Foote, Janet A.,
Ph.D.,^{1,2} Harris, Robin B., Ph.D.,^{1,2}

¹Mel and Enid Zuckerman College of Public Health, ² Arizona Cancer Center,
University of Arizona

Requests for reprints can be sent to:

Mary Clouser, M.P.H

Arizona Cancer Center

1430 E. Ft. Lowell

Suite 301

Tucson, AZ 85719

mclouser@u.arizona.edu

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ABSTRACT

The retinoids have been extensively studied in animal models, epidemiological and therapeutic studies due to their ability to treat and prevent skin malignancy. Using data from a randomized, double blind, study of the efficacy of retinol or isotretinoin versus placebo on the recurrence of non-melanoma skin cancer (NMSC) in high risk subjects, a reanalysis of the original intent to treat analysis was performed in a dose response format. Total mg/kg dose of isotretinoin or retinol was calculated for each study participant. Cox proportional hazards models describe the relationship between dose quartiles of isotretinoin and retinol use and time to first occurrence of squamous cell carcinoma (SCC) or basal cell carcinoma (BCC) in crude and adjusted models. Neither the isotretinoin nor retinol models showed any significance at any quartile for reduction in first BCC or SCC occurrence. Crude and adjusted retinol models show a statistically significant increase in risk of developing an SCC in the first dose quartile, while only the crude model shows a statistically significant increase in risk in the first quartile of the isotretinoin model. For retinol and SCC hazard ratios for the first quartile were as follows; HR= 2.92, 95% CI 1.67-5.10 crude, HR= 1.95, 95% CI 1.00-3.80 adjusted. For isotretinoin and SCC hazard ratios for the first quartile were as follows; HR=2.38, 95% CI 1.35-4.19 crude, HR= 1.69, 95% CI 0.87-3.31 adjusted. Test for trend was not significant in any of the models. These analyses confirm the results of the original intent to treat

analyses and in addition raise an interesting question related to the potential for increased risk for patients in the first quartile of retinol dose.

INTRODUCTION

Fifty percent of Americans who live to be age 65 will have skin cancer at least once, making it the most common form of cancer in the United States(1, 2). Life time exposure to the sun's ultraviolet rays is the most important environmental factor involved in the development of skin cancer, with susceptibility to sunburn likely the most important genetic factor (3). Non-melanoma skin cancer (NMSC), which includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is an important target for chemoprevention and ongoing research because of its high incidence. Along with lung, bladder, and breast cancer, skin cancer has been successfully treated with retinoids including vitamin A and various synthetic derivatives (4).

The retinoids exert a variety of effects and are vital for embryogenesis, reproduction, vision, glycoprotein production, regulation of inflammation, growth, and differentiation of normal and neoplastic cells in vertebrates, and tend to accumulate preferentially in the skin (5-9). The potential chemopreventative properties of retinoids have been studied extensively in various animal models. Epidemiologic studies and clinical trials have demonstrated the activity of vitamin A compounds in the treatment or prevention of skin malignancies (10-19).

Actinic keratoses were the first skin lesions to be successfully treated with topically-applied all-trans-retinoic acid (5, 20).

The SKICAP-BCC/SCC study, a large Phase III study conducted between 1985 and 1990, examined the effect of retinol and isotretinoin on the recurrence of non-melanoma skin cancer among participants with a verified skin cancer history (21). Based on an intent to treat analysis, the study found no difference between those who received the placebo compared to those who received the isotretinoin or retinol in the time to first new occurrence of BCC or SCC (21). Over 95% of the study participants reported taking at least half of the total number of capsules and over 80% taking at least 75% (21). However, capsule count adherence was lower for the retinoid groups as compared to the placebo group; 76% and 82%, respectively took at least 3 quarters of their capsules (22). Participants in the retinol groups experienced a 1% higher rate of clinical adverse symptoms, mostly elevations in serum cholesterol or liver enzymes, as compared to the placebo group (22). This higher rate of adverse symptoms lead to 35% of those in the retinol groups discontinuing use of their study medication prior to completion of the study; by the end of 3 years, only 50% of the randomized participants were still in follow-up (21, 22).

This current project examines a dose-response approach to determine if the total dose of retinol or isotretinoin affected incidence of new SCC or BCC among this population at high risk for new skin cancers. Because attrition rates were stated to be high, especially in the retinoid groups and because the

SKICAP-SCC/BCC study investigators believe that “due to the less than anticipated duration of participation for participants in the trial the effect of retinoids in higher risk subjects was not truly evaluable” this dose response analysis is being done to understand if there was a reduction in BCC/SCC recurrence in participants who were able to stay on study and complete the intervention (22).

METHODS

A full description of the study design and methods of the SKICAP-BCC/SCC study have been previously published (21, 23). Eligible participants were those who had: (1) at least four biopsy-proven skin cancers, (2) one NMSC within 12 months of consent, (3) no diagnosis of melanoma or internal cancer within the past year, (4) were between 21 and 85 years of age, if female, not of childbearing potential, and (5) willing to attend visits at the study clinic, visiting the study dermatologist or personal dermatologist yearly for at least the next three years.

Between January 1985 and June 1990, 719 people in the Tucson, Phoenix and Yuma, Arizona areas and the San Diego, California area consented to the study. Five hundred twenty-five were found to be eligible for randomization and successfully completed a run-in period by achieving a 75% adherence level to the daily capsule intake regimen (21). Participants were randomly assigned to receive either 25,000 IU of oral retinol (n=173), isotretinoin

(5mg for those under 145lbs and 10mg for those equal to or greater than 145lbs) (n=178) or placebo (n=174), daily for three years (21).

Estimation of Total Dose Received

For the current dose response analysis, the total number of pills taken by each participant enrolled in the treatment arms was calculated based on physical pill count adherence percentages maintained in the electronic data base.

Compliance greater than 100% was set to equal 100%. The total number of pills consumed was then converted to total dose per kilogram for each participant.

Supplemental Vitamin A intake information (yes or no) was collected at baseline and monitored throughout the study. Very few study participants reported any supplemental Vitamin A intake (9.3% at baseline (supplemental intake greater than 5000 IU daily was an exclusionary criteria) and a comparison of supplemental vitamin A intake at baseline among the three treatment groups revealed no statistical difference (p=0.49). Therefore no attempt was made to add supplemental Vitamin A use into total dose per kilogram.

Assessment of End Points

All skin cancer end points were monitored after randomization and confirmed by pathology. Participants were examined for skin lesions by a study dermatologist or their own dermatologist at least once a year, and underwent a full-body skin examination by a study dermatologist at study end. Participants with suspicious skin lesions were referred for biopsy and treatment, and were

closely followed by study staff to ensure that biopsies were performed and a diagnosis obtained. In addition, endpoints were identified by participant self-report at study follow-up visits, by review of pathology records of dermatologists, pathology laboratories and the Southeast Arizona skin cancer registry.

Diagnostic pathology slides were obtained on all biopsies, centrally reviewed and confirmed by the study dermatopathologist. Participants were encouraged to continue the study regimen even if a new BCC or SCC was diagnosed.

This analysis mimics the original intent to treat analysis using a dose response approach. Quartiles of total intervention use were generated for the retinol and isotretinoin group. Two primary differences are inherent in the current dose response approach analysis. The original intent to treat analysis adjusted for *a priori* selected variables including self-reported large mole and freckle count (described as those moles greater in circumference than a pencil eraser). Along with missing counts for a significant proportion of the study population, the mole and freckle count in itself does not appear to contribute substantially more than information already included between the skin reaction to sun and typical hours of sun exposure. Secondly, lesions diagnosed as in-situ were not included in the number of NMSC contributing to the skin cancer history in the original analysis; the current dose response approach includes SCC-in situ, Bowen's and keratoacanthomas as contributing to both the NMSC history and new cutaneous cancer occurrences.

Other Variables

In initial analyses multiple baseline variables commonly associated with risk for NMSC were included as potential confounders. Total reported usual weekly sun exposure was a combination of self-reported total weekday hours and weekend hours of sun exposure dichotomized to 0-10 hours or greater than 10 hours. The variables skin reaction to the sun, eye color, hair color, mother's ethnic origin and father's ethnic origin were categorized according to known phenotypic traits that would make an individual at higher or lower risk of NMSC. The variable skin reaction to the sun was split into four categories: always/usually burn (tan little/usually burn-tan minimally), burn moderately (tan average), burn minimally (tan easily and above average and rarely/never burn (tan easily). The variable smoking status was split into three categories, never smoker, former smoker, and current smoker). History of NMSC was considered separately for both BCC and SCC and dichotomized into those with a history of less than 10 or a history of greater than or equal to 10 prior BCC or SCC. In addition months on study was assessed as both an effect modifier and a confounder because total dose taken is inextricably tied to how long a participant was active in the study.

Analysis

All analyses were performed using intercooled STATA version 9.0 (StataCorp, 4905 Lakeway Drive, College Station, Texas). Chi square tests compared basic demographic characteristics between the quartiles of use for

each of the interventions and one way analysis of variance was used to compare means. T-tests were used to compare both the mean dose of retinol and isotretinoin in the first quartile to the placebo group. A Kruskal-Wallis test was used to compare median time on study for those taking retinol by quartiles.

To examine the relationships between quartiles of total retinol dose received versus placebo and total isotretinoin dose received versus placebo on the development of NMSC, hazard ratios and 95% confidence intervals were estimated. Cox proportional hazards models estimated time to the development of BCC or SCC occurrence. Participants who developed both a BCC and SCC occurrence during the study period were included in both the BCC and the SCC analysis. Test for trend was accomplished by estimating Cox proportional hazards models without using the categorical dose variable. Potential confounding factors were evaluated in initial models by comparing the adjusted and unadjusted hazard ratios. Factors were included in the final models only if the adjusted hazards ratio changed by greater than 10%. Months on study was evaluated as a potential effect modifier by creating an interaction term including months on study and dose. Likelihood ratio tests were used to assess the significance of this interaction term.

The examination included three modeling approaches: a crude model, a model adjusted for age, gender and months on study, and a model adjusted for age, gender, skin reaction to the sun, total time in sun, months on study and history of NMSC. In addition, three alternative modeling approaches were

performed in an attempt to understand the increased risk associated with the first dose quartile of retinol and SCC. These models were as follows: 1) A model was constructed where all subjects who had not been on study at least six months were removed from the data set; 2) All events that occurred in the first six months were removed from the data set but all subjects were kept; and 3) Retinol dose was converted to mg ($1\mu\text{g}$ retinol=3.33 IU vitamin A activity) and retinol and isotretinoin dose were added together, calculated into mg/kg and analyzed as one treatment group.

RESULTS

Of the 525 participants in the study, 102 participants had no diagnosis of either BCC or SCC after randomization. Two-hundred had at least one SCC diagnosed after randomization, and 132 had at least one diagnosis of BCC after randomization. One-hundred-seventy participants were diagnosed with both a BCC and SCC after randomization. Median time on study was 30.4 months for all participants (30.4 months for those on retinol, 29.7 months for isotretinoin and 31.3 for placebo).

The baseline characteristics did not differ by retinol dose quartiles with the exception of age (Table 1). Those in the highest quartile of dose were younger with a mean age of 61 years ($p=0.0009$) as compared to participants in the other dose quartiles (68, 69 or 67 years) or placebo (65 years). Table 2, which indicates the distribution of baseline characteristics for participants by quartiles of

isotretinoin dose, reveals no differences between the dose groups with the exception of gender ($p=0.011$). The two groups with the highest intake of the isotretinoin were 80% and 84% male, while the two lowest intake groups were 53% and 66% male compared to the placebo group that was 72% male.

Table 3 examines the relationship between retinol dose level, including placebo group participants and time to first new occurrence of BCC or SCC. For SCC, the crude and partially adjusted models show a statistically significant increase in risk of developing an SCC for the first dose quartile with the fully adjusted model just meeting statistical significance at the $p=0.05$ level. Test for trend was not significant. In the crude model those in the first quartile of retinol dose have a HR of 2.92 (95% CI 1.57-5.10) and this increased HR also remains in the adjusted models, 2.06 (95% CI 1.06-4.80) and 1.95 (95% CI 1.00-3.80) respectively. None of the three models showed any significance at any level for BCC first occurrence.

The relationships between the synthetic retinoid isotretinoin dose level and occurrence of BCC or SCC (Table 4) revealed a different outcome. Only the crude model for SCC showed a statistically significant increased HR in the first dose quartile, 2.38 (95% CI 1.35-4.19). For both the partially and fully adjusted model, there was no statistically significant increase in risk for participants in the first dose quartile, 1.17 (95% CI .724-1.88) and 1.69 (95% CI .866-3.31), respectively. Test for trend was not significant in any of the models. Again there were no significant results for BCC occurrence.

In an attempt to identify potential alternate explanations for the increased risk of SCC in the first retinol quartile, additional comparisons were made. The mean dose of retinol in the first quartile (48,481.1 mg/kg) indicated that the patients received a dose statistically significantly greater than those in the placebo group ($p < 0.0001$). Not unexpectedly, median time on study for those in the first quartile was less than for those in the other three quartiles. The median time on study for those taking retinol in quartile one was 6.6 months, quartile two was 25.1 months, quartile three 38.8 months and quartile four 53.4 months ($p = 0.0001$). In addition, alternate analysis approaches involving the removal of all participants not on study for at least six months, removal of all NMSC diagnosis occurring in the first six months and conversion of the intervention doses in order to create a total dose and one intervention group. None of these additional approaches significantly changed the models.

Off study reasons were investigated by quartile of dose received. For both retinol and isotretinoin the major reason participants left the study in the lowest quartile were for toxicity consistent with the interventions (retinol=46.5%, isotretinoin=52.0%) and unwillingness to continue (retinol=30.3%, isotretinoin=31.9%). For quartile two the majority of subjects completed the study (retinol=62.8%, isotretinoin=53.3%) and a smaller percentage were unwilling to continue (retinol=18.6%, isotretinoin=24.4%). For quartiles three and four the majority completed the study, with fewer numbers unwilling to continue.

DISCUSSION

These analyses evaluating dose response confirm the findings of the original intent to treat analysis. The daily regimen of 25,000 IU of retinol or 5 or 10 mg of isotretinoin were not effective at preventing new occurrences of SCC or BCC among a population with histories of multiple cutaneous cancers. In the current analysis all models, crude and adjusted, evaluating SCC occurrence and actual retinol dose level received showed that there was a statistically significant increase in risk of recurrence for those in the first dose quartile, even after adjustment for potential confounders such as age, gender, skin reaction to sun, total time in sun, months on study and history of NMSC. Test for trend was not significant either for protection or for increased risk.

In the SKICAP-BCC/SCC study no protective effect for development of SCC or BCC was found for either isotretinoin or retinol using the intent to treat analyses. The original study reported fairly good compliance for those who remained on study but also indicated that the attrition rates were high in all groups. During conduct of the SKICAP-BCC/SCC study, new toxicity information about one of the intervention agents surfaced (21). This information was conveyed to all participants because of the double blinded nature of the trial design. This strong message of potential side effects given to each individual participant by the study interviewers may have increased the attrition rate over what would have been expected. The study population became sensitized to slight deviations from their normal health and could have ascribed it to their study

medication and stopped the study. This increased attrition diminished the ability of the trial to detect a difference between the placebo and treatment groups and thus, the trial was not fully evaluable(MOON).

An additional limitation in longer studies such as SKICAP is in separating the effects of normal aging and increased prevalence of chronic disease with potential adverse effects consistent with intervention. By recruiting a population with histories of multiple cutaneous cancers results, the mean age of the study group was slightly above the typical retirement age of 65 years. Substantial changes in lifestyle and perceived health can accompany the changes associated with this stage regardless of participation in intervention trials.

The current standard for analysis of clinical trial data is intent to treat analysis (also known as use-effectiveness). Intent to treat analysis seeks to answer the question “Is it better to adopt a policy of treatment A if possible, with deviations if necessary, or a policy of treatment B if possible, with deviations if necessary, for patients with a particular medical condition?” (24, 25). The intent to treat analysis requires inclusion of all randomized patients in the analysis, in their originally randomized assignment groups, regardless of whether they remained on protocol for the duration of the study (26).

Arguments for use of intent to treat are many. Intent to treat analysis, in the presence of non compliance, reduces differences among treatments making it more difficult to demonstrate that one is better than the other, retains the original sample size and power, controls for bias, prevents a researcher from

obscuring the facts in favor of their own theories, and maintains a very conservative analysis (24, 27). Because the randomization process helps control for potential confounders, measured and unknown, it is believed that only the original assignment retains this guarantee (24, 25, 28). If subjects who deviate from the protocol because of toxicity, age, disease severity, etc. are omitted, then groups may not be comparable and the biases that were originally controlled for through randomization may affect the outcome of the analyses. In addition, the argument has been made that it is not reasonable to generalize study results to only compliant participants with similar disorders, because the majority of people are noncompliant under normal circumstances and do not adhere to a proscribed regimen (24, 28).

There are also arguments against the exclusive use of intent to treat analysis for clinical trial data. Sheiner et al. argue that intent to treat analyses do not provide valid significance levels, estimates, or interval estimates either for use-effectiveness in regular medical practice or for the effect of the actually administered therapy (method effectiveness) (25). These researchers argue that method-effectiveness maybe be more relevant to medical decision making than use-effectiveness and that trials should be designed and analyzed to provide both results. Understanding potential benefit given full compliance and the expected benefit averaged over rates of compliance in a particular trial are considered to provide important information, especially for clinicians (25). The intent to treat estimate only provides an average prognosis of a mixture of

compliers and of people who may not have taken the drug at all or who left the study prematurely. This average prognosis may not apply to any individual patient. Substantial deviations from the protocol through either loss to follow-up or drop out can dilute the data and diminish the ability of a trial to detect a difference between effects of treatment (25, 28).

Intent to treat analysis reveals the answer to the question “Is a treatment effective?” while an explanatory analysis seeks to answer the efficacy question “Can this treatment work?”. The secondary analysis of these data using a dose response approach seeks to answer the question “Can this treatment work?” because it examines the study from a compliance point of view, and takes into consideration the total dose of intervention consumed by each individual participant.

Overall reported toxicity of level two or greater in the SKICAP BCC/SCC study was mild, and the isotretinoin-treated group reported more side effects than either the placebo or retinol group. The majority of toxicities reported were in the mucocutaneous category (21). However, by year 2 (28 months) of the study (visit 5), only 63% of the original 175 subjects remained on study in the isotretinoin treatment group, 68% of the 173 remained on study in the retinol group, and 72% of the 173 in the placebo group remained on study.

One of the major drawbacks of the retinoids is the toxicity associated with the physiologic accumulation of excessive amounts. The first and classical reported cases of acute hypervitaminosis A involve Eskimos and Arctic explores

ingesting polar bear or seal liver meat, and then developing severe headaches, drowsiness, irritability, nausea, and vomiting, erythema and desquamation of the face, trunk, palms and soles of the feet with symptoms resolving in 7 to 10 days (29). Absorption differences and variability in the risk of accumulation and toxic effects can differ depending on the retinoid being used. Synthetic forms such as isotretinoin are thought to distribute more uniformly in the body and are thought to be less likely to cause the severe adverse effects related to the accumulation of excessive levels of retinol in the liver. Unfortunately, the various synthetic retinoids are associated with additional adverse effects, rather than providing the answer to eliminate toxicity related to high retinoid intake. Side effects of the synthetic retinoids include mucocutaneous drying and chapping leading to cheilitis, facial dermatitis, conjunctivitis, dryness of the nasal mucosa with minor nosebleeds, dry mouth with thirst, xerosis, hair loss, palmoplantar desquamation, stratum corneum fragility or easy peeling due to minor frictional trauma and scratching, paronychia and nail abnormalities (30).

Our dose response analysis evaluated participants by original group assignment but based on total dose per kilogram body weight; therefore, the characteristics of the groups based on the original randomization remain intact. Additionally we looked at the characteristics of subjects in each treatment group by dose quartile, and there were few differences by quartile. Those in the highest quartile of retinol appeared to be younger, possibly indicating that those participants who were younger were able to stay on study longer or more able to

tolerate the intervention. However, many of the potential adverse effects of the retinoid dosing such as skin and mucous membrane dryness and hair loss are also common occurrences with aging. In the retinoid intervention study with its older age population it was not possible to discern some of the potential intervention related adverse effects from age related changes. For isotretinoin there were differences in quartile by gender indicating that men may have stayed on study longer.

Models with potential confounders such as skin reaction to the sun, total time in the sun, months on study, history of NMSC, age and gender were assessed in our dose response analyses. Based on the fully adjusted models these analyses indicate that there may be an increase in risk of recurrent SCC for those in the lowest quartile of retinol. In addition three alternative analyses were done in an attempt to better assess the increased risk in the first quartile for retinol and SCC. Unfortunately, none of the three alternative analyses of the data set provided an outcome significantly different from the original dose response analysis.

In order to try and explain why participants in the lowest quartile of total retinol were at increased risk for developing SCC, we compared time on study, mean dose, and off study reasons, by quartile of dose. Those in the lower quartile of dose were on study for less time than those in the upper quartiles, however this is to be expected as the total amount of intervention a participant took would be heavily dependent on the amount of time they were on study. In

addition, mean dose between the placebo group and the first quartile was significantly different so participants in the first quartile did receive drug. The reasons why participants in the first quartile went off study were quite different from those in the other quartiles. First quartile participants most frequently reported their reason for going off study as toxicity consistent with the intervention, and in the other three quartiles participants went off because they were exiting or unwilling to continue (small percentage). Median time on study for those with and without a BCC or SCC occurrence during the study was compared by quartile of dose and showed no evidence that those who developed an NMSC had a shorter time on study versus those who did not.

The present project examined the efficacy of retinoid interventions in preventing the development of new NMSC among a population with a history of multiple and recent cutaneous cancers. Side effects were more prevalent and greater attrition was associated with the isotretinoin. A review of 13 serum retinol studies, both prospective and retrospective, and a large case control study showed that low vitamin A levels existed in the cancer populations and potentially played a role for incidence, basically suggesting that the lower the serum vitamin A level, the greater the cancer risk (31, 32). However other studies have suggested that low serum retinol may be a metabolic consequence of cancer rather than a precursor of cancer (33).

Dietary intake studies of the association between retinol have shown inconsistent results with BCC and SCC (34). Of the 11 dietary studies reviewed

only one hospital based case control study by Wei et al. showed an inverse association between the use of vitamin A supplements in the last 5 years and the development of BCC (OR=0.20, 95% CI, 0.06-0.62) (18).

Oral retinoid therapy seems to prevent or delay development of cutaneous carcinomas for patients with some cutaneous diseases with a high risk of malignancy, such as xeroderma pigmentosum, Mibelli's prokeratosis, Gorlin's disease, Ferguson-Smith disease and solar keratosis (35). In addition, several small studies that treated patients with other cutaneous diseases who also already had BCC with high dose retinoids, oral or topical, reported that the tumors responded well to the treatment and, in many cases, the treatment reduced the number of existing tumors or prevented onset of new tumors (13, 15, 17, 36-39). Thus there is substantial literature indicating that retinoids may have the ability to prevent BCC.

A nested cohort study compared patients' own tumor experience while using and not using retinoids for patients participating in a Psoralen + UVA treatment (PUVA) follow-up study who reported at least 1 year of substantial retinoid use between 1985 and 2000. They found that retinoid use was associated with a 30% reduction in SCC ($p=0.02$) (19). The incidence of SCC significantly decreased during years of substantial retinoid use (Incidence rate ratio=0.79, 95% CI 0.65-0.95), but there was no association between BCC incidence and oral retinoid use (19). In a study of 981 subjects who had two or more previously confirmed BCCs randomized to 10 mg isotretinoin or placebo,

there was no significant difference in the cumulative percentage of patients with an occurrence of BCC at a new site or the annual rate of BCC formation after 36 months. Subjects reported significant adverse systemic effects (11). The combined results of these two studies indicate no effect of retinoids on BCC development but significant reduction on cutaneous SCC development associated with retinoid intervention. In addition, studies done with the retinoid acitretin and etretinate in renal transplant recipients have shown that these agents are effective in reducing NMSC and AK incidence in this high risk population (40-43).

There has been little recent research on retinoids and skin cancer prevention. Recent studies of head and neck squamous cell cancer (HNSCC) have been null. In a randomized, 8 year trial (3 years on intervention and 4 years of follow-up) in 1,190 patients with a history of stage I or II HNSCC, low dose isotretinoin was found to be no more effective in reducing the rate of second primary tumors than placebo (HR=1.06, 95% CI 0.83-1.35) or increasing the rate of survival (HR=1.03, 95% CI 0.81-1.32) (44). In addition Bonelli et al. found no advantage for patients previously treated for Stage II and IV HNSCC who took isotretinoin as compared to those in the control group, 5 year actuarial survival 58.9% for those in treatment versus 57.2% in the control group (p=0.94) (45).

These analyses confirm the results of the original intent to treat analyses and in addition raise an interesting question related to patients in the first quartile of dose for retinol and increased risk. Multiple attempts were made to

understand if this increase in risk could be explained by different characteristics of the study population to no avail. History provides that other vitamin compounds that have been investigated by chemoprevention studies were found to increase the risk for cancer rather than protect from cancer, however there is nothing in the current literature to suggest this is the case with retinols.

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Neck Chemoprevention Study Group. *Oncology Reports* 2004;11:1297-305.

Table 1: Baseline Characteristics by Quartiles of Dose for Retinol (n=347)

Variable	Quartiles of Total Dose					p-value
	Placebo (0)	≤1347.7 mg/kg	1347.7-2580.5 mg/kg	2580.6-3994.6 mg/kg	>3994.6 mg/kg	
Age (years)	64.8 ± 10.5	67.7 ± 8.0	68.7 ± 7.06	67.1 ± 8.86	60.9 ± 10.38	0.0009
Male gender	126 (72.4)	32 (72.4)	33 (76.7)	34 (77.3)	28 (65.1)	0.710
Skin reaction to sun						0.607
Always or usually burn tan little or minimal	70 (40.2)	20 (46.5)	16 (37.2)	20 (45.5)	14 (32.6)	
Burn moderately/tan Average	68 (39.1)	18 (41.9)	15 (34.9)	16 (36.4)	16 (37.2)	
Burn minimally/tan easily	15 (8.6)	1 (2.3)	6 (14.0)	1 (2.3)	6 (14.0)	
Rarely/never burn tan Easily	21 (12.1)	4 (9.3)	6 (14.0)	7 (15.9)	7 (16.3)	
Total hours in sun	14.4 ± 12.4	15.3 ± 11.6	16.1 ± 11.7	12.6 ± 9.7	13.4 ± 11.1	0.646
History of NMSC						
SCC						0.471
<10	155 (89.1)	39 (90.7)	42 (97.7)	39 (88.6)	40 (93.0)	
≥10	19 (10.9)	4 (9.3)	1 (2.3)	5 (11.4)	3 (7.0)	
BCC						0.271
<10	82 (47.1)	23 (53.5)	25 (58.1)	18 (40.9)	16 (37.2)	
≥10	92 (52.9)	20 (46.5)	18 (41.9)	26 (59.1)	27 (62.8)	

Table 2: Baseline Characteristics by Quartiles of Dose for Isotretinoin (n=178)

Variable	Quartiles of Total Dose					p-value
	Placebo (0)	≤56.36 mg/kg	56.36-124.0 mg/kg	125.0-223.9 mg/kg	>223.9 mg/kg	
Age (years)	64.8 ± 10.5	67.9±7.7	64.6 ± 9.9	66.4 ± 10.1	61.5 ±11.3	0.438
Male gender	126 (72.4)	29 (65.9)	24 (53.3)	36 (80.0)	37 (84.1)	0.011
Skin reaction to sun						0.737
Always or usually burn tan little or minimal	70 (40.2)	16 (36.4)	20 (44.4)	18 (40.0)	20 (45.5)	
Burn moderately/tan Average	68 (39.1)	18 (40.9)	18 (40.0)	21 (46.7)	15 (34.1)	
Burn minimally/tan easily	15 (8.6)	3 (6.8)	3 (6.7)	3 (6.7)	7 (15.9)	
Rarely/never burn tan Easily	21 (12.1)	7 (15.9)	4 (8.9)	3 (6.7)	2 (4.6)	
Total hours in sun	14.4 ± 12.4	12.3 ± 9.0	14.0 ± 11.1	11.8 ± 8.7	15.3 ± 15.7	0.544
History of NMSC						
SCC						0.498
<10	155 (89.1)	40 (90.9)	40(88.9)	44 (97.8)	40 (90.9)	
≥10	19 (10.9)	4 (9.1)	5 (11.1)	1 (2.2)	4 (9.1)	
BCC						0.300
<10	82 (47.1)	22 (50.0)	24 (53.3)	29 (64.4)	20 (45.5)	
≥10	92 (52.9)	22 (50.0)	21 (46.7)	16 (35.6)	24 (46.6)	

Table 3: Relationship of Retinol Dose Level to First Recurrence of BCC or SCC

Model	BCC			SCC		
	HR	p-value	95% CI	HR	p-value	95% CI
Crude						
Units/Kg dose						
0 (placebo)	-			-		
≤1347.7	1.38	0.137	.903-2.09	2.92	<0.000	1.67-5.10
1347.7-2580.5	.996	0.985	.665-1.49	1.26	0.454	.684-2.33
2580.6-3994.6	1.31	0.144	.912-1.88	1.53	0.090	.940-2.48
>3994.6	.931	0.710	.639-1.36	.724	0.260	.414-1.27
Test for trend		p=0.793			p=0.783	
Adjusted (1)						
Units/Kg dose						
0 (placebo)	-					
≤1347.7	1.17	0.528	.724-1.88	2.06	0.033	1.06-4.00
1347.7-2580.5	.916	0.677	.605-1.39	1.03	0.935	.548-1.92
2580.6-3994.6	1.21	0.303	.839-1.76	1.30	0.301	.792-2.13
>3994.6	1.06	0.777	.706-1.59	0.934	0.820	.517-1.69
Test for trend		p=0.547			p=0.650	
Adjusted (2)						
Units/Kg dose						
0 (placebo)	-					
≤1347.7	1.18	0.501	.728-1.92	1.95	0.049	1.00-3.80
1347.7-2580.5	1.00	0.987	.659-1.53	1.29	0.436	.681-2.43
2580.6-3994.6	1.33	0.141	.910-1.94	1.27	0.350	.769-2.10
>3994.6	.853	0.466	.557-1.31	1.03	0.925	.562-1.88
Test for trend		p=0.853			p=0.419	

1. Adjusted for Age, gender and months on study
2. Adjusted for Age, gender, skin reaction to sun, total time in sun, months on study and history of NMSC

Table 4: Relationship of Isotretinoin Dose Level to First Recurrence of BCC or SCC

Model	BCC			SCC		
	HR	p-value	95% CI	HR	p-value	95% CI
Crude						
Mg/Kg dose						
0 (placebo)	-			-		
≤56.36	1.49	0.032	1.03-2.16	2.38	0.003	1.35-4.19
56.36-124.0	1.02	0.907	.697-1.50	1.33	0.31	.762-2.33
125.0-223.9	.884	0.556	.586-1.33	1.39	0.02	.840-2.29
>223.9	1.01	0.956	.704-1.45	0.998	0.10	.584-1.68
Test for trend		p=0.697			p=0.631	
Adjusted (1)						
Mg/Kg dose						
0 (placebo)	-					
≤56.36	1.41	0.119	.916-2.17	1.68	0.116	.879-3.22
56.36-124.0	1.02	0.907	.692-1.51	1.50	0.170	.840-2.68
125.0-223.9	.841	0.414	.556-1.27	1.33	0.269	.802-2.20
>223.9	1.03	0.891	.704-1.50	1.08	0.753	.638-1.86
Test for trend p=		p=0.797			p=0.650	
Adjusted (2)						
Mg/Kg dose						
0 (placebo)	-					
≤56.36	1.03	0.889	.653-1.64	1.69	0.123	.866-3.31
56.36-124.0	.905	0.620	.609-1.34	1.48	0.190	.823-2.64
125.0-223.9	.841	0.419	.551-1.28	1.53	0.100	.921-2.57
>223.9	1.23	0.297	.836-1.80	1.27	0.385	.738-2.19
Test for trend p=		p=0.789			p=0.108	

1. Adjusted for Age, gender and months on study
2. Adjusted for Age, gender, skin reaction to sun, total time in sun, months on study and history of NMSC

APPENDIX C – Manuscript 3:
Risk Group, Skin Lesion History and Sun Sensitivity Reliability in Squamous Cell
Skin Cancer Progression

Risk Group, Skin Lesion History and Sun Sensitivity Reliability in Squamous Cell
Skin Cancer Progression

Clouser, Mary C.,^{1,2} Harris, Robin B.,^{1,2} Roe, Denise J.,^{1,2} Saboda, Kathylynn,²
Ranger-Moore, James,^{1,2} Duckett, Laura,² Alberts, David S.^{1,2}

¹Mel and Enid Zuckerman College of Public Health, ²University of Arizona
Cancer Center

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ABSTRACT

In studies of skin cancer, participants are often classified into risk groups based on self-reported history of sun exposure or skin characteristics. We sought to determine the reliability of self-reported skin characteristics among participants of a study to evaluate markers for non-melanoma skin cancer (NMSC). Multiple questionnaires and screening protocols, were administered over a 3-month period to individuals from three risk groups: existing sun damage on forearms but no visible actinic keratoses (Pre-AKs) (n=91), visible AKs (AK) (n=38), and history of resected squamous cell skin cancer in the last 12 months (SCC) (n=35). We assessed consistency of risk group assignment between telephone screen and study dermatologist assignment, self-reported sun sensitivity (Telephone Recruitment Form vs. participant completed profile), and self-reported history of NMSC skin lesions (Telephone Recruitment Form vs. health history). There was substantial agreement between probable risk group and final assignment ($k=0.76$, 95% CI=0.65-0.85) and agreement did not differ by gender. Agreement for self-reported sun sensitivity was moderate ($\kappa_{\text{weighted}}=0.46$, 95% CI=0.36-0.56) with higher agreement for women. For self-reported NMSC lesion history between two interviews, 24 days apart, kappa estimates ranged from 0.66 to 0.78 and were higher for women than men. Overall, there was evidence for substantial reproducibility related to risk group

assignment and self-reported history of NMSC, with self-reported sun sensitivity being less reliable. In all comparisons, women had higher kappa values than men. These results suggest that self-reported measures of skin cancer risk are reasonably reliable for use in screening subjects into studies.

INTRODUCTION

Skin cancer is the most common form of cancer in the United States (1). Skin cancer afflicts about 20% of the general population at some point in life, and 50% percent of Americans who live to be age 65 will have skin cancer at least once (2, 3). Skin cancer, including melanoma, has been mainly associated with particular skin phenotypes (fair complexion, tendency to sunburn, freckles) and sun exposure (4).

Participant self-report is heavily relied upon in epidemiological studies; however reliability of this information may vary. In cancer prevention studies, self-report is frequently used to classify participants into risk groups for future disease and to identify potential risk factors. Self-report is useful because it reduces time and length of recruitment. It is important to determine the reliability of items included in questionnaires because this variability will affect the validity of measurements and comparability between studies.

In the current analysis, we sought to determine how reliably participants were being placed in risk groups by comparing trained staff interviewers who did initial telephone screening and probable risk group determination by final dermatologist risk assignment. Secondly, we sought to determine level of consistency at two different time points for participant perception of their sun sensitivity. Lastly, we examined the consistency of participant self-report of their NMSC and actinic keratosis (AK) history during the initial telephone screening interview and later reported via a self-administered health history form.

MATERIALS AND METHODS

Study Design

These data resulted from the Skin Biomarkers Study conducted by the University of Arizona Cancer Center over an approximately three month period. This study was designed to assess the reproducibility of various surrogate endpoint biomarkers within the skin carcinogenesis pathway, specifically the variability of polyamine levels, p53 expression and PCNA expression. Subjects were recruited from university and community dermatology clinics, advertisements and a skin cancer registry. Eligible subjects were males and females of at least 18 years of age who were willing to use skin protector factor (SPF) 50 sunscreen applied daily. Subjects included three different probable risk groups: sun damage on forearms with no visible AK's which is called the Pre-AK group, visible AKs which is called the AK group and history of resected squamous cell carcinoma in the last 12 months (the SCC group).

The Biomarkers Study assessed 851 people via telephone for eligibility. Of those, 199 appeared to be eligible, agreed to participate and consented at the eligibility clinic visit. At some point after consent, 29 were found to be ineligible. Of those who remained eligible, 91 were assigned to the Pre-AK group, 38 to the AK group and 35 to the SCC group (Figure 1). Of the 164 subjects assigned to probable risk groups, 143 completed the three-month study and are the focus of these analyses. Information on the design and some results of the study have been previously published (5, 6).

Questionnaires/Assessments

Throughout the three month study, subjects completed various questionnaires to provide information about history of skin cancers, sun damage to skin and sun sensitivity. Figure 1 shows the study flow and when various forms were collected. The “Telephone Recruitment Form” was used to assess a potential participant’s initial eligibility as well as gather basic information about past diagnosis of skin conditions, current medications, sun exposure, and sun damage. The interviewer used this information to assign a potential participant into one of the previously described risk groups.

At the eligibility visit, the participant was assessed by the study dermatologist to confirm eligibility and to assign a final risk group. Participants also returned a completed self-administered “Health History Form”. This form asked about medical conditions and included a dermatology history section with questions about past diagnoses of AK, skin cancer, and skin biopsy results. A self-administered “Participant Profile Form “ was returned at the time of Visit 1, approximately 43 days after the initial telephone screen. Phenotypic characteristics, demographics, sun exposure, use of sunscreen, history of sun burn, occupational and environmental exposures, residential history, medical exposures and smoking history were included on this profile.

Statistical Analysis

Chi square tests were used to compare basic demographic characteristics between the three final risk groups, ANOVA and Bonferroni multiple comparisons were used to look at potential differences in mean age between groups.

To test the reliability between independent groups, Cohen's Kappa was utilized. For comparison between the multiple levels of self-reported sun sensitivity, Weighted Kappa was estimated. This statistic took advantage of the ordered categories, so that partial credit was given to small error versus large error (7). Because there was no clear-cut "gold" standard for any of the interviews or questionnaires, equal weight was applied to both sets or readings (7). A Kappa statistic less than zero would suggest poor agreement, 0 to .20 slight, .21 to .40 fair, .41 to .60 moderate, .61 to .80 substantial, .81 to 1.00 almost perfect (8, 9). Confidence intervals were calculated for the kappa statistic using the STATA command "kapci". STATA utilizes an analytical method for simple two by two comparisons and a bootstrap method in the case of dichotomous variables. When the bootstrap method was utilized STAT was asked to perform 1000 repetitions.

RESULTS

Table 1 shows the baseline demographic characteristics, phenotypic characteristics, skin characteristics, and sun protective habits of participants by assigned risk group for the 143 participants who completed the study and are

included in our analysis. There were few differences between assigned risk groups, with the exception of gender. There were more men in both the AK (75.8%) and SCC (62.5%) groups than in the Pre-AK group (33.3%). In addition those in the SCC group tended to be older, mean age 65.8 years compared to 58.1 years in the Pre-AK group. Similarly there were few differences for phenotypic characteristics, skin characteristics and sun protective habits with the exception of “ever use of sunlamps”. Self-reported usage was very low in the AK group (3.0%) compared to the other groups.

Table 2 shows the agreement between risk group assignment by staff telephone interviewer and study dermatologist. Agreement between risk group assignment was substantial ($k= 0.76$, 95% CI=0.65-0.85) and there were no differences in agreement by gender of the participant. The telephone interviewers misclassified 12, (17.4%) of true Pre-AK subjects as AK. Only 5 (15.6%) of the true AK subjects were misclassified, 4 as Pre-AK and 1 as SCC. A total of 3 (9.7%) SCC subjects were misclassified and all three were placed in the AK group instead of the SCC group.

Questions on the interviewer “Telephone Recruitment Form” and the self-administered “Participant Profile” asked subjects to describe what happens to their skin after a specified amount of sun exposure. Eligible responses were from one of 5 categories related to burning and tanning. Table 3 shows the response to both questionnaire administrations and gives more specific detail to wording differences between questionnaires. There was an average of 43 days

between the administration of the two instruments. Using weighted kappa, agreement was moderate (κ weighted=0.46, 95% CI=0.36-0.56), with higher agreement for women (κ weighted =0.53, versus 0.36).

Table 4 shows agreement between self-reported history of NMSC at the two questionnaire administrations 24 days apart. The kappa value for AK history was $k=0.66$ 95% CI=0.54-0.78, for SCC $k=0.78$, 95% CI=0.65-0.91 and for BCC $k=0.75$, 95% CI=0.55-0.94, all considered to be substantial agreement and all significantly different than zero. Kappa values differed by gender but the differences were not significant as there is overlap in the confidence intervals. In addition, although not shown, individuals were more likely to report a diagnosis of NMSC at the telephone recruitment than on the self-reported health history.

DISCUSSION

In epidemiologic research, as in any scientific endeavor, the interpretability of observed results depends to a considerable extent on the accuracy of the measurements (10). In most situations, "truth" is not known and, in order to judge the quality of measurement, one must settle for an assessment of agreement between multiple imperfect sources of information or between multiple measurements using a single imperfect source of information (10).

In this study, the objectives of the analyses were three-fold. We sought to determine how reliably participants were placed into risk groups by trained telephone interviewers compared with a study dermatologist's assessment.

Secondly, the consistency between self-reported sun sensitivity was assessed. Lastly, we examined the consistency of participant self-reported history of skin lesions, specifically NMSC and AK. We noted extremely good agreement ($k=0.76$, 95% CI=0.65-0.85) between the classification of potential study participants into risk groups by trained telephone interviewers and the final assignment by a study dermatologist. During the recruitment phase of a clinical study, large numbers of people are often screened to find the few who qualify. Recruitment and screening is a time consuming process and study costs increase dramatically if study dermatologist time is necessary for initial screening. In addition, if the study seeks to recruit specific numbers into each risk group, participant's risk group must be immediately and accurately identified.

Despite the level of good agreement, misclassification did exist, and not surprisingly, this misclassification centered on assignment of risk groups Pre-AK and AK. The telephone interviewers misclassified 17.4% of true Pre-AK subjects as AK. Similarly 15.6% of the true AK subjects were misclassified, with 4 people (1.3%) classified by the screener as Pre-AK and 1 (3.1%) as an SCC. Among the final SCC group 3 SCC subjects (9.7%) were misclassified and all three had been placed in the AK group instead of the SCC group by the screener. The screeners placed subjects into risk groups based on information they collected during the interview while the dermatologists made group assignments based on skin examinations. Since the telephone interviewers based their decisions on participant report, it would appear that subjects were more likely to report having

AKs when they did not actually have any. This could be due to lack of knowledge pertaining to identification of an AK, or the difficulty of an untrained person with sun damaged skin to differentiate an AK from other sun damage. It may also be true that potential participants over exaggerated their skin damage on the telephone because they had a strong desire to be eligible and participate in the study.

As risk group classification increased in seriousness, misclassification decreased. There was more misclassification in the Pre-AK risk group and much less in the SCC risk group. If only the telephone interviewer was used to classify subjects into risk groups, there would be more true Pre-AK subjects in the AK group. This could then make it more difficult to distinguish between groups during analysis of biomarkers. For example, if there was a specific biomarker associated with development of SCC, this type of misclassification could decrease the likelihood of detecting any gradient between the disease groups.

In our study, agreement was not as strong for self-reported sun sensitivity measures (κ weighted=0.46, 95% CI=0.36-0.56). One caveat needs to be highlighted. The sun sensitivity questions being compared on the two forms were not worded in precisely the same manner. The question on the interviewer administered "Telephone Recruitment Form" and the self-administered "Participant Profile" differed slightly. The "Telephone Recruitment Form" was more focused toward assessment of whether an individual's untanned skin burns

in the sun, and the self-reported “Participant Profile” focused on descriptions of tanning in addition to burning.

The concept of sun-reactive skin typing was created in 1975 to classify persons with white skin in order to select the correct initial doses of ultraviolet A (UVA) (in joules per cubic centimeter) for the treatment of psoriasis-oral methoxsalen photochemotherapy (PUVA)(11). It was decided that a brief personal interview regarding the history of the person’s sunburn and suntan experience was one approach to estimate the skin tolerance to ultraviolet radiation (UVR) exposure and the Fitzpatrick skin typing system was created (11). The “Fitzpatrick skin typing system” has been used by the US Food and Drug Administration in its guidelines for sunscreen products for over-the-counter human use (11).

Self-reported sun sensitivity is used to assess skin type and, therefore, risk for skin cancer. Only a few studies looking at the reliability of these measures are available in the literature and report better reliability than our study. Reliability, assessed by comparing answers to the same question at different time points, is utilized because the measures do not have a “gold standard”. In the multi-centre South European case-control study, a sub-sample of participants were re-interviewed and reaction to sun exposure was assessed on a four level scale(4). Weighted kappa for skin reaction to sun exposure was 0.61 (95% CI=0.53-0.70) which is slightly higher than the five level weighted kappa from our current study ($k=0.46$, 95% CI=0.36-0.56). (Recall that the value of kappa is

affected by the number of categories) In a case-control study of melanoma that included test-retest reliability of self-reported exposure to sun sensitivity, there was good consistency with kappa values for ability to tan and tendency to burn of 0.66 and 0.62 respectively (12).

In a case-control study nested within the Nurse's Health Study cohort, Weinstock et al. reported that test-retest reliability of tanning questions was high in the prevalent case group (Spearman's $r=0.78$) and control group (Spearman's $r=0.76$), but lower in the incident case group (Spearman's $r=0.59$)(13). Their study had a similar caveat in that the questions were not worded identically between the two questionnaires. Weinstock et al. also found that, among women diagnosed with melanoma after the first questionnaire and before the second, there was a substantial shift toward reporting a reduced ability to tan (14).

This highlights an important issue for development of study questionnaires. The issue of burnability and tannability are separate issues to subjects and need to be considered separately. In a study by Rampen et al. neither tannability nor burnability were linked very closely to the minimal erythemal dose (MED) which would be the "gold standard" of sun sensitivity (15). Rampen et al. investigated burning and tanning histories in 790 white students, 18 to 30 years old, with a self-administered questionnaire to classify them into skin types based on the Fitzpatrick scheme (burning tendency after one hour of sun exposure in early summer and the tanning ability after regular sun exposure during summer were recorded as follows: 0, none; 1, mild; 2, moderate; and 3,

severe/intense) (15). MED was measured in a subgroup of this population. There was no statistically significant correlation with the self-reported burning tendency and the MED. Skin typing on the basis of self-reported burning tendency and tanning ability may be subjective because subjects tended to over-record no burning and under record no tanning. The correlation with biologic complexion factors like hair and eye color and freckling tendency was somewhat better for self-reported tanning than for the burning propensity (15).

The authors concluded that self-reported burning-tanning histories do not provide a valid means of skin typing, when compared with the MED (15). It may be that a better way to characterize sun sensitivity would be through proxy measures such as hair and eye color and freckling tendency which appear to be more reliably reported by subjects. Weinstock et al. found test-retest reliability of hair color assessment by questionnaire was high with the Spearman correlation coefficient between 0.76 and 0.87. Sun sensitivity may be subject to recall bias when assessed by ability to tan, but not when assessed by hair color (14). There is a need for further studies to look at the issue of skin type classification more closely.

Based on Weinstock et al.'s results, we might have expected that the SCC risk group would have been more reliable reporters of sun sensitivity or that there would be a gradient of response with the Pre-AK group being the less reliable reporters then the AK or SCC groups. However, we found that all risk groups were equally as reliable when reporting sun sensitivity (data not shown).

Results from previous studies are consistent with our findings on agreement for self-reported history of NMSC. We found more serious skin conditions had higher agreement (for AK history $k=0.66$, 95% CI=0.54-0.78 for SCC $k=0.78$, 95% CI=0.65-0.91 and for BCC $k=0.75$, 95% CI=0.55-0.94). In a study by Ming et al., self-reported history of skin cancer was compared with the gold standard of chart documentation. Patients were found to recall their cancer history quite well, with correct identification highest for melanoma (95% of cases) and lowest for basal cell carcinoma (84% of cases) (16). In a study by Bergmann et al., assessing agreement of self-reported medical history using an in-person interview versus a self-administered questionnaire, kappa values of 0.83-0.88 were found for cancer reporting. Lower values were found for less severe or more transient disease, with the disease being reported at the interview but not on the questionnaire (17). Our study also found that NMSC diagnosis was more likely to be reported to the phone interviewer than on the self-administered "Health History Form".

The results of our study suggest that women may be more reliable reporters than men, however, the literature does not always support this finding. In an Australian study of ocular melanoma, that gathered information on sun exposure in the first four decades of a person's life, questionnaires administered one year apart gave an interclass correlation coefficient of 0.65 for ranked total sun exposure between two interviews with the coefficient higher for men (0.73) than for women (0.54) although like our study not statistically significant (18).

The use of kappa to measure reliability can be problematic because kappa values will depend on the prevalence of the condition and distribution of the marginals (7). We used a weighted kappa for ordered categories so that partial credit would be given to small error versus large error (7). Additionally, kappa values depend on the number of categories with more categories resulting in lower values (19). Kappa does, however, account for agreement that may occur by chance alone. The use of kappa is more appropriate than the use of percent agreement. Percent agreement is the simplest method of summarizing agreement for categorical variables and has the advantage of being useful for any number of categories. Percent agreement is artificially increased when the proportion of negative-negative results is high or when the prevalence of the condition is high.

CONCLUSIONS

Overall, there was evidence for substantial reproducibility for factors related to assignment into skin cancer risk group and self-reported history of skin lesions, with self-reported sun sensitivity questions being somewhat less reliable. In all comparisons, women were more consistent reporters than men. These results suggest that self-reported measures of skin cancer risk should be reasonably reliable for use in screening subjects into studies. Further studies are required to further identify the characteristics of individuals with poor reliability of self-reported measures.

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Figure 1: Biomarkers Study Schema

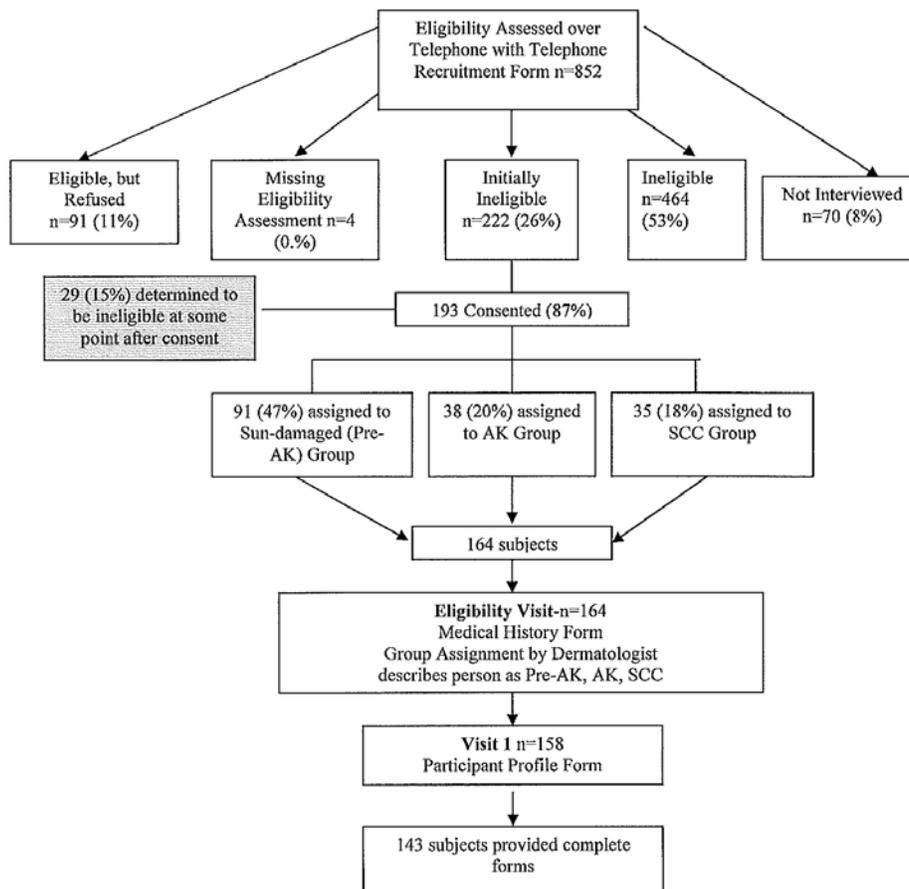


Table 1: Demographic Characteristics, Phenotypic Characteristics, Skin Tanning Characteristics, and Sun Protective Habits of Participants by Assigned Risk Group

	Risk Group Assignment§				p-value**
	Pre-AK n=78	AK n=33	SCC n=32	Total n= 143	
<i>Age (mean years)</i>	58.1	61.0	65.8	60.5	0.068
<40-49	19 (24.4%)	5 (15.2%)	1 (3.1%)	25 (17.5%)	
50-59	18 (23.1%)	6 (18.2%)	6 (18.8%)	30(21.0%)	
60-69	30 (38.5%)	16 (48.5%)	14 (43.8%)	60 (42.0%)	
>70	11 (14.1%)	6 (18.2%)	11 (34.4%)	28 (19.6%)	
<i>Male</i>	26 (33.3%)	25 (75.8%)	20 (62.5%)	71 (49.7%)	<0.001
<i>Ethnicity</i>					0.790
White	75 (96.2%)	32 (97.0%)	30 (93.8%)	137(95.8%)	
Other	3 (3.8%)	1 (3.0%)	2 (6.3%)	6 (4.2%)	
<i>Skin Tanning Characteristics</i>					0.207
Always burns, no tan	11 (14.1%)	7 (21.2%)	5 (15.6%)	23 (16.1%)	
Always burns, tans Minimally	16 (20.5%)	11 (33.3%)	9 (28.1%)	36 (25.2%)	
Burns moderately	28 (35.9%)	7 (21.2%)	13 (40.6%)	48 (33.6%)	
Burns minimally	16 (20.5%)	8 (24.2%)	2 (21.9%)	26 (18.2%)	
Rarely Burns	7 (9.0%)	0 (0.0%)	3 (9.4%)	10 (7.0%)	
Experienced painful sun burn (yes)	57 (73.1%)	28 (84.9%)	21 (65.6%)	106 (74.1%)	0.174
<i>Exposure</i>					0.10
Hours per week in sun during past month (M-F 9AM-4PM) (mean ±SD)	7.41 ±14.6	14 ±18.2	8.66 ±12.1	9.2 ± 15.1	
Hours per week in sun during past month (Sat-Sun 9AM-4PM) (mean ±SD)	4.41 ±6.64	6.45 ±9.98	4.84 ±7.72	5.0 ± 7.8	
Ever use of sun lamps (yes)	16 (20.5%)	1 (3.0%)	7 (21.9%)	24 (16.8%)	0.054
<i>Use of sunscreen in past year</i>					0.649
Never	10 (12.8%)	1 (3.0%)	4 (12.5%)	15 (10.5%)	
Rarely used	13 (16.7%)	4 (12.1%)	6 (18.8%)	23 (16.1%)	
Sometimes	25 (32.1%)	12 (36.4%)	9 (28.1%)	46 (32.2%)	
Usually	21 (26.9%)	9 (27.3%)	6 (18.8%)	36 (25.2%)	
Always*	9 (11.5%)*	7 (21.2%)	7 (21.9%)	23 (16.1%)	

*One person stated that they rarely used sunscreen but always used it on their face

**p value for chi square test for difference between risk groups or analysis of variance for hours per week
§ Pre-AK=sun damage on forearms, no visible AK's, AK= visible AKs, SCC= history of resected squamous cell carcinoma (SCC) in the last 12 months

Table 2: Comparison of Risk Group Assignment by Telephone Eligibility Screeners and Dermatologists

Group Assigned by Dermatologists	Probable Group Assigned at Telephone Eligibility Screening			
	Pre-AK	AK	SCC	Total
Pre-AK	57	12	0	69
AK	4	28	1	32
SCC	0	3	28	31
Total	61	42	29	133*

Kappa=0.76

Percent Agreement=85.0%

95% CI (0.65-0.85)

*There were 10 subjects for which the telephone interviewer made no determination as to the probable group.

Table 3: Comparison of Participant Reported Skin Response to Sun on “Telephone Recruitment Form” and Self-reported “Participant Profile”

Telephone Eligibility

	Always burn easily with blistering & peeling	Usually burn, no blistering & some peeling	Burn moderately, some degree of tanning or freckling	Burn minimally, tan easily	Rarely or never burn, tan easily	Total
Participant Profile						
Always burn, never tan, extremely sun sensitive	11	8	4	0	0	23
Always burn, tan minimally, very sun sensitive	6	14	13	2	1	36
Burn moderately, tan gradually & uniformly to light brown, sun sensitive	3	13	26	6	0	48
Burn minimally, tan well to moderate brown, minimal sun sensitivity	0	3	9	13	1	26
Rarely burn, tan well to dark brown, minimal sun sensitivity & never burn, deeply pigmented, sun insensitive	0	0	1	7	2	10
Total	20	38	53	28	4	143

Kappa=0.46

Percent Agreement=84%

95% CI (0.36-0.56)

Table 4: Comparison of Self-report of Diagnosis of Skin Cancer Lesions at Two Time Points

Health History Questionnaire			
Telephone Recruitment Questionnaire	Kappa	% Agreement	95% CI
<i>History of Actinic Keratosis</i>	0.659	83%	0.54-0.78
By Gender			
Male	0.514	76%	0.32-0.71
Female	0.804	90%	0.67-0.94
By Group			
Pre-AK	0.651	83%	0.48-0.82
AK	0.526	82%	0.22-0.83
SCC	0.460	84%	0.87-0.85
<i>History of Squamous Cell Carcinoma*</i>	0.781	93%	0.65-0.91
By Gender			
Male	0.701	88%	0.51-0.89
Female	0.891	97%	0.74-1.00
<i>History of Basal Cell Carcinoma*</i>	0.745	96%	0.55-0.94
By Gender			
Male	0.682	94%	0.39-0.97
Female	0.817	97%	0.57-1.00

*Info from biopsy table on the Health History form

APPENDIX D - Tables and Figures Related to NSAID Analysis

APPENDIX E - Tables and Figures Related to Biomarkers Reliability Analysis

Figure 1: Biomarkers Study Schema

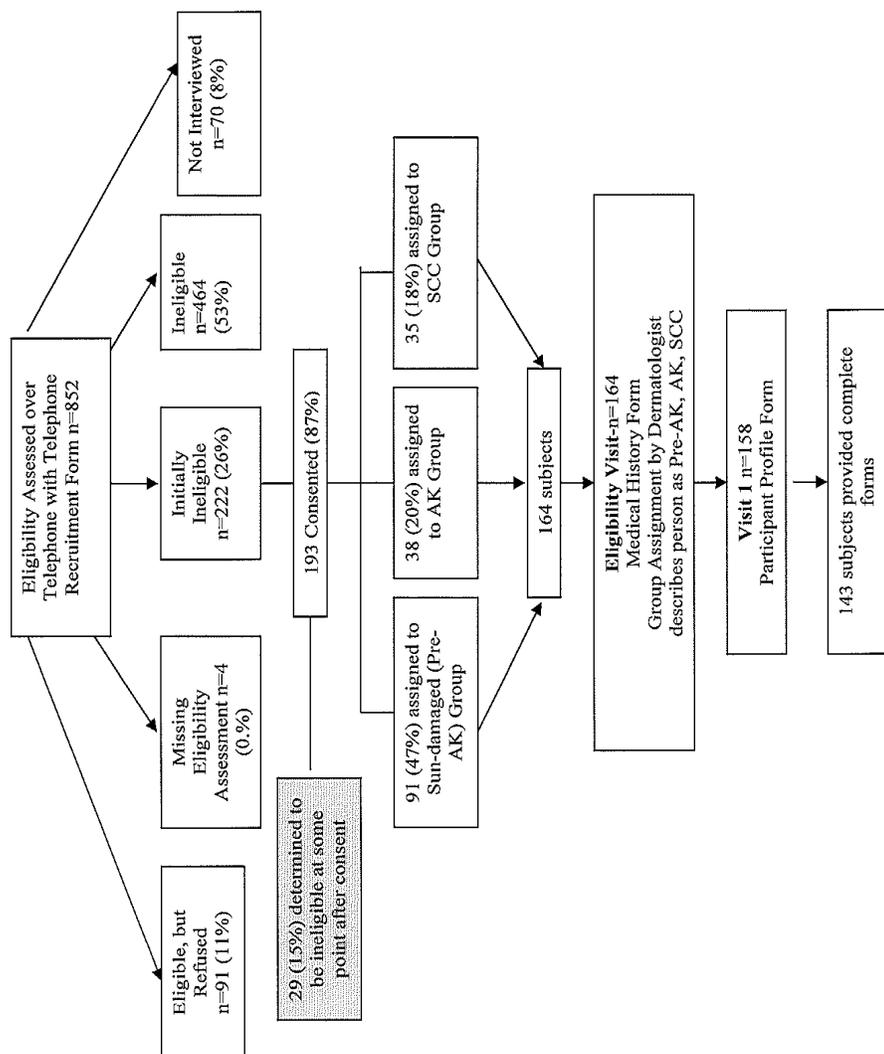


Table 1: Biomarkers Study Visits

Pre-Visit	Eligibility Visit	Visit 1 1 Wk. after Screening	Visit 2 2 Wks. After V1	Visit 3 1 Wk after V2	Visit 4 3 Wks. After V3	Visit 5	Visit 6	Visit 7
Telephone Screening	Dermatological Assessment and Screening	Initial Visit	Baseline Biopsies		Month 1	Month 2	Final Biopsies	
Eligibility Screening Mail: Medical History Appt. Letter Consent Form	Sign Consent Dr. completes: Mole & Freckle Form Dermatological Assessment of Sun Damage Form Send Home: Appt. Reminder Participant Profile Form AFFQ Health Point Survey	Blood Draw Complete: Vitals, Ht, Wt., Master Accrual Provide Calendars Dispense Sunscreen	Biopsies Complete: Photos, Exam & Biopsies Adherence Follow-up Biopsy Map Review Meds.	Complete: Adherence Follow-up Review Meds.	Complete: Adherence Follow-up Review Meds.	Complete: Adherence Follow-up Food Records	Biopsies Blood Draw Complete: Photos, Exam & Biopsy Record Form Vitals Dermatologic al Assessment of Sun Damage Form Adherence Follow-up Review Meds.	Study Completion: Evaluation Form

Table 1: Duplicate and Triplicate NSAIDs Use, Reason Code, Daily Dose and Length of Time Used (months)

<u>Patient ID</u>	<u>Medication Code</u>	<u>Reason for Taking</u>	<u>Daily Dose</u>	<u>Time (months)</u>
wilr24b	13001010003	8	60009	36
wilr24b	4002010023	5	30009	60
bauf09	13001010003	8	80009	60
bauf09	13001010023	82	10009	60
camb33	13001010003	8	60009	4
camb33	13001010023	8	55009	60
dunk10	13001010003	8	40009	84
dunk10	13001010023	24	4009	6
glep22	4002010023	5	11209	108
glep22	13001010059	47	20009	156
harc34	13001010003	8	16	1
harc34	13001010023	21	16	12
mure22	13001010003	47	114.209	24
mure22	13001010023	47	51604	120
quev15	13001010097	8	150009	1
quev15	13001010023	8	65009	36
quip20	13001010023	99	32509	48
quip20	13001010003	8	60009	30
weia08	13001010003	8	20009	48
weia08	13001010023	8	32509	240
knig22	13001010023	47	32509	120
knig22	13001010130	31	10009	60
leew31	13001010015	47	37509	36
leew31	13001010023	47	97509	36
macw23	13001010023	47	5005	48
macw23	13001010043	47	30009	48
meeb07	13001010130	8	-9	360
meeb07	4002010023	5	16009	420
merl34	13001010306	8	7509	36
merl34	13001010023	8	1505	120
morb15a	13001010015	8	50009	24
morb15a	4002010023	82	32509	36
muma08	13001010003	8	160009	144
muma08	13001010023	8	520009	144
shic10	13001010306	8	15009	1
shic10	13001010130	8	7509	3
symh08	13001010107	8	16	24
symh08	13001010023	8	32516	60
whil20	13001010023	5	504	20
whil20	13001010107	8	2009	60
divj16	4002010023	5	32509	18
divj16	13001010141	47	7509	3
hile16	13001010023	6	32509	24
hile16	13001010003	47	40009	12
elbf06	13001010003	8	120009	36
elbf06	13001010059	23	40009	12
elbf06	13001010023	23	504	24
flan32	13001010023	21	65009	36
flan32	13001010003	47	2009	36
flan32	13001010023	47	100009	36
shaj11	13001010003	8	20009	2

Table 1: Duplicate and Triplicate NSAIDs Use, Reason Code, Daily Dose and Length of Time Used (months)-Continued

<u>Patient ID</u>	<u>Medication Code</u>	<u>Reason for Taking</u>	<u>Daily Dose</u>	<u>Time (months)</u>
shaj11	13001010015	8	50009	48
shaj11	4002010023	6	13509	4.5

Table 2: Reason Codes for All NSAID Use

Reason Code	Frequency	Percent	Cumulative Percent
-9	22	3.99	3.99
5	79	14.34	18.33
6	67	12.16	30.49
8	201	36.48	66.97
13	9	1.63	68.60
14	2	0.36	68.97
21	16	2.90	71.87
23	13	2.36	74.23
24	4	0.73	74.95
25	1	0.18	75.14
31	2	0.36	75.50
42	3	0.54	76.04
47	80	14.52	90.56
53	2	0.36	90.93
61	11	2.00	92.92
80	11	2.00	94.92
82	23	4.17	99.09
99	5	0.91	100.00
Total	551	100.00	

-9=no reason given, missing

5=heart condition (angina/arrhythmia)

6=anti-coagulation/blood thinner

8=osteoarthritis

13=musculoskeletal conditions (bursitis, sprains, strains, spasms, tendonitis)

14=circulatory problems (phlebitis)

21=headache/migraine

23=high blood pressure-hypertension

24=high cholesterol

25=high triglycerides

31=neuritis

42=rheumatoid arthritis

47=pain

53=osteoporosis

61=inflammatory conditions

80=prevent stroke

82=prevention/Dr. Rx/health

99=other

Table 3: Dose of NSAID Use and Units of Use

Daily Dose/Units of Use	Frequency	Percent	Cumulative Percent
-9	11	2.00	2.00
0.2513	1	0.18	2.18
0.509	1	0.18	2.36
0.513	8	1.45	3.81
04	2	0.36	4.17
1.2504	2	0.36	4.54
1.504	1	0.18	4.72
100009	9	1.63	6.35
10009	8	1.45	7.80
1004	2	0.36	8.17
1009	3	0.54	8.71
1016	2	0.36	9.07
10709	1	0.18	9.26
11209	1	0.18	9.44
113	17	3.09	12.52
114.209	1	0.18	12.70
116	1	0.18	12.89
120009	7	1.27	14.16
12509	2	0.36	14.52
13	21	3.81	18.33
130009	7	1.27	19.60
1309	1	0.18	19.78
13509	1	0.18	19.96
13909	1	0.18	20.15
150009	7	1.27	21.42
15009	14	2.54	23.96
1505	1	0.18	24.14
16	15	2.72	26.86
160009	7	1.27	28.13
16009	1	0.18	28.31
162.509	8	1.45	29.76
16209	2	0.36	30.13
180009	6	1.09	31.22
195009	1	0.18	31.40
200009	1	0.18	31.58
20009	14	2.54	34.12
2004	1	0.18	34.30
2009	20	3.63	37.93
213	5	0.91	38.84
22509	1	0.18	39.02
23509	1	0.18	39.20
240009	7	1.27	40.47
24009	1	0.18	40.65
250009	3	0.54	41.20
25009	8	1.45	42.65
2509	3	0.54	43.19
260009	1	0.18	43.38
27009	1	0.18	43.56
28009	1	0.18	43.74
292509	1	0.18	43.92
300009	6	1.09	45.01

Table 3: Dose of NSAID Use and Units of Use-Continued

Daily Dose/Units of Use	Frequency	Percent	Cumulative Percent
30009	10	1.81	46.82
3000916	1	0.18	47.01
3004	1	0.18	47.19
3109	1	0.18	47.37
315009	2	0.36	47.73
320009	2	0.36	48.09
32509	92	16.70	64.79
32516	1	0.18	64.97
35009	2	0.36	65.34
37509	7	1.27	66.61
390009	2	0.36	66.97
40009	27	4.90	71.87
4004	1	0.18	72.05
4009	3	0.54	72.60
409	1	0.18	72.78
4209	1	0.18	72.96
45009	1	0.18	73.14
46.4309	1	0.18	73.32
50009	18	3.27	76.59
5005	1	0.18	76.77
5009	6	1.09	77.86
504	26	4.72	82.58
505	2	0.36	82.94
509	3	0.54	83.48
516	1	0.18	83.67
51604	1	0.18	83.85
520009	1	0.18	84.03
54	1	0.18	84.21
55009	3	0.54	84.75
5512	1	0.18	84.94
60009	12	2.18	87.11
60016	1	0.18	87.30
609	1	0.18	87.48
65009	17	3.09	90.56
75009	10	1.81	92.38
7509	16	2.90	95.28
80009	13	2.36	97.64
8009	1	0.18	97.82
8109	5	0.91	98.73
8509	1	0.18	98.91
9	1	0.18	99.09
90009	1	0.18	99.27
97509	3	0.54	99.82
one tablet	1	0.18	100.00
Total	551	100.00	

09= mg

12= tablespoons

04= grains (5 grains is equal to 325mg)

05= grams

16= as needed

13= tablets or capsules

Table 4: Summary of All NSAID Use (months)

Variable	n	Mean	Std. Dev.	Min	Max
months	544	58.35213	79.90328	.06	600

Table 5: All NSAID Use (months)

Months	Frequency	Percent	Cumulative Percent
.06	1	0.18	0.18
.5	4	0.73	0.91
1	23	4.17	5.08
1.5	1	0.18	5.26
2	18	3.27	8.53
2.5	1	0.18	8.71
3	14	2.54	11.25
4	10	1.81	13.07
4.5	1	0.18	13.25
5	6	1.09	14.34
6	18	3.27	17.60
7	2	0.36	17.97
8	5	0.91	18.87
9	3	0.54	19.42
10	6	1.09	20.51
11	1	0.18	20.69
12	44	7.99	28.68
13	3	0.54	29.22
14	4	0.73	29.95
15	1	0.18	30.13
16	1	0.18	30.31
17	1	0.18	30.49
18	14	2.54	33.03
20	2	0.36	33.39
22	2	0.36	33.76
24	48	8.71	42.47
25	1	0.18	42.65
27	2	0.36	43.01
28	1	0.18	43.19
30	7	1.27	44.46
36	63	11.43	55.90
42	1	0.18	56.08
48	34	6.17	62.25
50	1	0.18	62.43
52	1	0.18	62.61
54	2	0.36	62.98
55	2	0.36	63.34
58	1	0.18	63.52
60	70	12.70	76.23
66	1	0.18	76.41
70	1	0.18	76.59
72	13	2.36	78.95
75	1	0.18	79.13
78	2	0.36	79.49
80	1	0.18	79.67
84	9	1.63	81.31
96	7	1.27	82.58
100	1	0.18	82.76
108	3	0.54	83.30
120	28	5.08	88.38
132	1	0.18	88.57

Table 5: All NSAID Use (months)-Continued

Months	Frequency	Percent	Cumulative Percent
136	1	0.18	88.75
144	13	2.36	91.11
156	2	0.36	91.47
168	2	0.36	91.83
179	1	0.18	92.01
180	8	1.45	93.47
192	1	0.18	93.65
204	2	0.36	94.01
228	1	0.18	94.19
240	13	2.36	96.55
264	1	0.18	96.73
324	1	0.18	96.91
360	2	0.36	97.28
384	1	0.18	97.46
420	2	0.36	97.82
540	1	0.18	98.00
560	2	0.36	98.37
564	1	0.18	98.55
600	1	0.18	98.73
.	7	1.27	100.00
Total	551	100.00	

Figure 1: Histogram- Months of NSAID Use

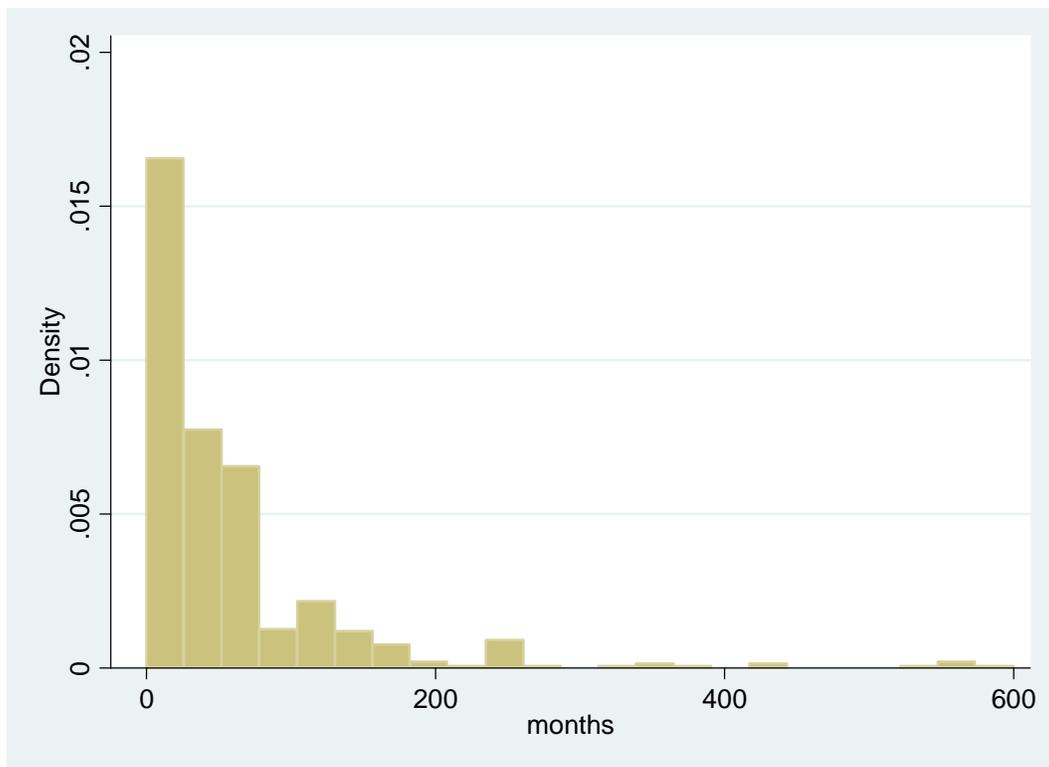


Table 6: List of Codes for Medication Falling Into Anti-Coagulant Category
(Aspirin etc. 4002010023/13001010023)

Medication Code	Frequency	Percent	Cumulative Percent
13001010003	86	15.61	15.61
13001010015	39	7.08	22.69
13001010023	126	22.87	45.55
13001010043	2	0.36	45.92
13001010059	33	5.99	51.91
13001010089	6	1.09	52.99
13001010097	10	1.81	54.81
13001010107	25	4.54	59.35
13001010130	14	2.54	61.89
13001010141	3	0.54	62.43
13001010181	2	0.36	62.79
13001010262	2	0.36	63.16
13001010276	7	1.27	64.43
13001010306	27	4.90	69.33
13001010312	2	0.36	69.69
4002010023	167	30.31	100.00
Total	551	100.00	

13001010003= advil, ibuprofen, medipren, motrin, nuprin

13001010015= anaprox, naprosyn, naproxen

13001010023= acetylsalicylic acid, alka seltzer plus, asa, ascription, aspirin, bufferin, cama, ecotrin, equagesic,excedrin, fiorinal, norgesic, percodan, zorprin

13001010043= butazolidin, phenylbutazone

13001010059= clinoril, sulindac

13001010089= diflunisal, dolobid

13001010097= disalcid, salsalate

13001010107= feldene, piroxicam

13001010130= indocin, indomethacin

13001010141= ketoprofen

13001010181= fenoprofen, nalfon

13001010262= meclofenamate, meclomen

13001010276= tolectin, tolmentin

1300101306= diclofenac, voltaren

1300101312= hydroxychloroquine, plaquenil

4002010023= acetylsalicylic acid, asa, ascription, aspirin, bufferin, cama, ecotrin

Table 7: Reasons for Taking NSAIDs in the Aspirin Category

Reason Code	Frequency	Percent	Cumulative Percent
-9	19	6.48	6.48
5	78	26.62	33.11
6	67	22.87	55.97
8	46	15.70	71.67
14	2	0.68	72.35
21	7	2.39	74.74
23	10	3.41	78.16
24	4	1.37	79.52
25	1	0.34	79.86
47	23	7.85	87.71
53	1	0.34	88.05
80	11	3.75	91.81
82	21	7.17	98.98
99	3	1.02	100.00
Total	293	100.00	

-9=no reason given, missing
5=heart condition (angina/arrhythmia)
6=anti-coagulation/blood thinner
8=osteoarthritis
14=circulatory problems (phlebitis)
21=headache/migraine
23=high blood pressure-hypertension
24=high cholesterol
25=high triglycerides
47=pain
53=osteoporosis
80=prevent stroke
82=prevention/Dr. Rx/health
99=other

Table 8: Summary of the Length of Time People Reported Taking NSAIDs in the Aspirin Category

Variable	n	Mean	Std. Dev.	Min	Max
Months	288	62.65799	82.2019	1	600

Table 9: Tabulation of Length of Time in Months for People Who Reported Use in the Aspirin Category

Length of Use (months)	Frequency	Percent	Cumulative Percent
-9	5	1.71	1.71
1	6	2.05	3.75
2	6	2.05	5.80
3	4	1.37	7.17
4	5	1.71	8.87
4.5	1	0.34	9.22
5	3	1.02	10.24
6	7	2.39	12.63
7	1	0.34	12.97
8	2	0.68	13.65
9	2	0.68	14.33
10	3	1.02	15.36
11	1	0.34	15.70
12	22	7.51	23.21
13	2	0.68	23.89
14	3	1.02	24.91
15	1	0.34	25.26
16	1	0.34	25.60
17	1	0.34	25.94
18	7	2.39	28.33
20	2	0.68	29.01
22	2	0.68	29.69
24	32	10.92	40.61
25	1	0.34	40.96
27	1	0.34	41.30
30	3	1.02	42.32
36	38	12.97	55.29
42	1	0.34	55.63
48	15	5.12	60.75
52	1	0.34	61.09
55	1	0.34	61.43
58	1	0.34	61.77
60	41	13.99	75.77
70	1	0.34	76.11
72	8	2.73	78.84
75	1	0.34	79.18
78	1	0.34	79.52
80	1	0.34	79.86
84	3	1.02	80.89
96	5	1.71	82.59
100	1	0.34	82.94
108	1	0.34	83.28
120	15	5.12	88.40
144	10	3.41	91.81
156	1	0.34	92.15
168	2	0.68	92.83
180	4	1.37	94.20
228	1	0.34	94.54
240	8	2.73	97.27

Table 9: Tabulation of Length of Time in Months for People Who Reported Use in the Aspirin Category-Continued

Length of Use (months)	Frequency	Percent	Cumulative Percent
264	1	0.34	97.61
324	1	0.34	97.95
420	2	0.68	98.63
504	1	0.34	98.98
540	1	0.34	99.32
560	1	0.34	99.66
600	1	0.34	100.00
Total	293	100.00	

Table 10: Daily Reported Doses for the Aspirin Category of NSAIDs

Daily Dose	Frequency	Percent	Cumulative Percent
-9	1	0.34	0.34
0.2513	1	0.34	0.68
0.509	1	0.34	1.02
0.513	8	2.73	3.75
04	2	0.68	4.44
1.2504	2	0.68	5.12
1.504	1	0.34	5.46
100009	1	0.34	5.80
10009	1	0.34	6.14
1004	2	0.68	6.83
1009	2	0.68	7.51
1016	1	0.34	7.85
10709	1	0.34	8.19
11209	1	0.34	8.53
113	17	5.80	14.33
116	1	0.34	14.68
12509	2	0.68	15.36
13	11	3.75	19.11
130009	6	2.05	21.16
1309	1	0.34	21.50
13509	1	0.34	21.84
13909	1	0.34	22.18
150009	3	1.02	23.21
15009	1	0.34	23.55
1505	1	0.34	23.89
16	6	2.05	25.94
160009	1	0.34	26.28
16009	1	0.34	26.62
162.509	8	2.73	29.35
16209	2	0.68	30.03
195009	1	0.34	30.38
200009	1	0.34	30.72
2004	1	0.34	31.06
213	3	1.02	32.08
22509	1	0.34	32.42
23509	1	0.34	32.76
250009	2	0.68	33.45
25009	6	2.05	35.49
2509	1	0.34	35.84
260009	1	0.34	36.18
28009	1	0.34	36.52
292509	1	0.34	36.86
300009	3	1.02	37.88
30009	2	0.68	38.57
3000916	1	0.34	38.91
3004	1	0.34	39.25
3109	1	0.34	39.59
315009	2	0.68	40.27
32509	91	31.06	71.33
32516	1	0.34	71.67
35009	2	0.68	72.35
37509	1	0.34	72.70

Table 10: Daily Reported Doses for the Aspirin Category of NSAIDs-Continued

Daily Dose	Frequency	Percent	Cumulative Percent
390009	2	0.68	73.38
4004	1	0.34	73.72
4009	1	0.34	74.06
4209	1	0.34	74.40
46.4309	1	0.34	74.74
50009	4	1.37	76.11
5005	1	0.34	76.45
5009	1	0.34	76.79
504	26	8.87	85.67
505	2	0.68	86.35
509	3	1.02	87.37
516	1	0.34	87.71
51604	1	0.34	88.05
520009	1	0.34	88.40
54	1	0.34	88.74
55009	1	0.34	89.08
5512	1	0.34	89.42
65009	17	5.80	95.22
75009	1	0.34	95.56
7509	1	0.34	95.90
80009	1	0.34	96.25
8009	1	0.34	96.59
8109	5	1.71	98.29
8509	1	0.34	98.63
90009	1	0.34	98.98
97509	2	0.68	99.66
one tablet	1	0.34	100.00
Total	293	100.00	

The last two digits indicate the medication units

09= mg

12= tablespoons

04= grains (5 grains is equal to 325mg)

05= grams

16= as needed

13= tablets or capsules

Table 11: Eye Color

Eye Color	Frequency	Percent	Cumulative Percent
-9	2	0.09	0.09
1	1,203	52.37	52.46
2	391	17.02	69.48
3	183	7.97	77.45
4	284	12.36	89.81
5	233	10.14	99.96
6	1	0.04	100.00
Total	2,297	100.00	

-9=missing

1=blue

2=green

3=grey

4=light brown

5=dark brown

6=black

Table 12: Hair Color

Hair Color	Frequency	Percent	Cumulative Percent
-9	2	0.09	0.09
1	421	18.33	18.42
2	879	38.27	56.68
3	598	26.03	82.72
4	132	5.75	88.46
5	265	11.54	100.00
Total	2,297	100.00	

-9=missing

1=blonde

2=light brown

3=dark brown

4=black

5=red

Table 13: Ethnicity of Mother

Mother's Ethnicity	Frequency	Percent	Cumulative Percent
0	31	1.35	1.35
1	32	1.39	2.74
2	1	0.04	2.79
3	3	0.13	2.92
4	205	8.92	11.84
5	23	1.00	12.84
6	1,800	78.36	91.21
7	95	4.14	95.34
8	107	4.66	100.00
Total	2,297	100.00	

0=?

1=American Indian or Alaskan Native

2=Asian or Pacific Islander

3=Black

4=Eastern European

5=Hispanic

6=Northern European

7=Southern European

8=Other

Table 14: Ethnicity of Father

Father's Ethnicity	Frequency	Percent	Cumulative Percent
0	28	1.22	1.22
1	26	1.13	2.35
3	2	0.09	2.44
4	197	8.58	11.01
5	16	0.70	11.71
6	1,817	79.10	90.81
7	108	4.70	95.52
8	103	4.48	100.00
Total	2,297	100.00	

0=?

1=American Indian or Alaskan Native

3=Black

4=Eastern European

5=Hispanic

6=Northern European

7=Southern European

8=Other

Table 15: Disposition of Questioned Subjects Left in Analysis Data

Pt. ID	Medication Code	Daily Dose	# of Months	Reason Taken	Why Left In/Alteration
mure22	13001010023	5 grains	120	Neck & joint pain	length of reported use is long and reason for taking makes it likely a chronic use
milh30	04002010023	10grains	144	Triglycerides	length of reported use is long and reason for taking although states triglycerides it is likely for cardiovascular reasons and this would indicate a chronic use
hute23	13001010023	2 to 8 as needed	60	Joint and muscle pain	length of reported use is long and reason for taking makes it likely a chronic use. In addition as needed my refer to the number of pills.
ledw18	13001010003	As needed	36	Back pain	length of reported use is long and reason for taking makes it likely a chronic use.
Legh18	13001010023	1 as needed	144	Arthritis	length of reported use is long and reason for taking makes it likely a chronic use.
Meri07	13001010003	PRN	6	Arthritis	reason for taking makes it likely for chronic use.
Mors28	04002011002 3	PRN	600	Headaches	length of reported use is long
Catc21	13001010097	Only as needed	4	Arthritis	reason for taking makes it likely for chronic use
Garh21	13001010023	1 daily as	60	Arthritis	length of reported use is long and

		needed				
Harc34	13001010003	2-4 daily as needed	1	Arthritis		reason for taking makes it likely a chronic use. length of reported use is long and reason for taking makes it likely a chronic use. In addition as needed my refer to the number of pills.
Hasd38	1300101003	2 or 3 as needed	12	Headaches		length of reported use is long and reason for taking makes it likely a chronic use. In addition as needed may refer to the number of pills.
Hilb16	13001010023	10 grains PRN	120	Arthritis		length of reported use is long and reason for taking makes it likely a chronic use.
Davs24	13001010023	5 grains PRN	24	Pain		length of reported use is long and reason for taking makes it likely a chronic use
Symh08	13001010023	325 as needed	60	Arthritis		length of reported use is long and reason for taking makes it likely a chronic use.
Symh08	13002000109	PRN	24	Arthritis		length of reported use is long and reason for taking makes it likely a chronic use.

Table 16: Disposition of Questioned Subjects Removed From Analysis Data

Pt. ID	Medication Code	Daily Dose	# of Months	Reason Taken	Decision
Dalw19	1300101000 3	600mg rare only as needed	48	Heel spurs	Removed from data because the subject clearly states only as needed as part of the response and it is likely heel spurs may require less chronic dosing

APPENDIX F - SKICAP Questionnaires

Page 2

PATIENT CHARACTERISTICSHeight _____ Feet _____ Inches
Weight _____ PoundsEye color: 1. blue 2. green 3. gray 4. light brown
 5. dark brown 6. black

Hair color as young adult:

 1. blond 2. light brown 3. dark brown
 4. black 5. red

What is the predominant ethnic origin of your parents?

	Mother	Father
1. American Indian or Alaskan Native	_____	_____
2. Asian or Pacific Islander	_____	_____
3. Black	_____	_____
4. Eastern European	_____	_____
5. Hispanic	_____	_____
6. Northern European	_____	_____
7. Southern European	_____	_____
8. Other (please specify)	_____	_____

Skin Reaction to Sun

Based on the first 30 to 45 minutes of exposure to the summer sun, do you:

1. always burn easily and severely (painful burn); tan little or none and peel.
2. usually burn easily and severely (painful burn); tan minimally or lightly, also peel.
3. burn moderately and tan about average.
4. burn minimally and tan easily and above average with each exposure
5. rarely burn, tan easily and substantially.
6. never burn and tan easily and substantially.

Education:

- | | |
|---|--|
| <input type="checkbox"/> 1. 8th grade or less | <input type="checkbox"/> 5. Some college |
| <input type="checkbox"/> 2. Some high school | <input type="checkbox"/> 6. College graduate |
| <input type="checkbox"/> 3. High school grad | <input type="checkbox"/> 7. Graduate School |
| <input type="checkbox"/> 4. Vocational/Trade School | |

Marital Status:

- | | |
|-------------------------------------|--|
| <input type="checkbox"/> 1. Single | <input type="checkbox"/> 3. Widowed |
| <input type="checkbox"/> 2. Married | <input type="checkbox"/> 4. Divorced/Separated |

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Please print in what state (or country, if not in the USA) you lived for at least one year in each decade of your life. List the state in which you lived the longest followed by, at most, two other states.

Birth to 9 yrs _____
 10 to 19 _____
 20 to 29 _____
 30 to 39 _____
 40 to 49 _____
 50 to 59 _____
 60 to 69 _____
 70 to 79 _____
 80 to 89 _____

Amount of time spent in the sun BETWEEN 9 A.M. AND 4 P.M.

During the average week (Monday-Friday), how many hours have you spent in the sun? _____ Hrs.

During the average weekend (Saturday-Sunday), how many hours have you spent in the sun? _____ Hrs.

Do you use sunlamps? Yes No

Do you use sunscreen?* Yes No

* A sunscreen is a substance used to protect the skin from exposure to the sun. Sunscreens are more than suntan oils or lotions. They can be identified by a sun-protection factor, e.g., SPF 15, printed on the container.

If you use sunscreens:

• What is the SPF of your current sunscreen? _____

• How regularly do you use it?

1. Always when in the sun

2. More than half the time

3. Less than half the time

How many years have you used sunscreens? _____

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SUNBURN INFORMATION:

1. Have you ever had a severe sunburn where extreme pain and swelling occurred with blistering of skin and/or where loss of work resulted?

Yes No

2. If yes, please list the following:

Date of severe sunburn	Location(s) on body	Blistering? Yes or No	Loss of Work? Yes or No
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

HEALTH HABITS

How many hours of sleep do you usually get at night?

(1) 6 hours or less (2) 7 hrs. (3) 8 hrs. (4) 9 hrs. or more

How often do you drink wine, beer or liquor?

1. Never 2. Less than once a week 3. Once or twice a week 4. More than twice a week

1. Wine	_____	_____	_____	_____
2. Beer	_____	_____	_____	_____
3. Liquor	_____	_____	_____	_____

When you drink wine, beer or liquor, how many drinks do you usually have at one sitting?

1. One or two drinks 2. Three or four drinks 3. Five or more drinks

Wine	_____	_____	_____
Beer	_____	_____	_____
Liquor	_____	_____	_____

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Here is a list of leisure time activities. How often do you do any of these things?

1. OFTEN 2. SOMETIMES 3. NEVER

1. Active sports (tennis, racquetball, etc.)	_____	_____	_____
2. Swimming or taking long walks	_____	_____	_____
3. Hunting or fishing	_____	_____	_____
4. Taking weekend automobile trips	_____	_____	_____
5. Working in the garden	_____	_____	_____
6. Doing physical exercises	_____	_____	_____
7. Golf, bowling	_____	_____	_____
8. Something else (What?) _____	_____	_____	_____

SMOKING

*1. ^{never} Do you now or have you ever smoked cigarettes (at least one a day for one year's time)? *Never 4yr*

Yes No

2. Do or did you inhale?

1. Never 2. Slightly 3. Moderately 4. Deeply

SMOKING HISTORY

4 Cur
Current Smokers

4 Form
Ex-Smokers

SMOKING HISTORY	Current Smokers	Ex-Smokers
Number smoked per day		
Age at which you began smoking		
Age at which you quit smoking		
Total years smoking filtered cigarettes		
Total years smoking unfiltered cigarettes		

HEALTH HISTORY

Have you ever been treated by a doctor for any of the following conditions?

Yes	No	
_____	_____	1. Heart attack
_____	_____	2. Angina
_____	_____	3. High blood pressure
_____	_____	4. Stroke
_____	_____	5. Diabetes
_____	_____	6. Tuberculosis
_____	_____	7. Chronic bronchitis or emphysema
_____	_____	8. Rectal or colon polyps
_____	_____	9. Chronic colitis, inflammatory bowel disease
_____	_____	10. Osteoporosis
_____	_____	11. Liver disorder
_____	_____	12. High cholesterol
_____	_____	13. Anemia

In the past five years have you had:

Yes	No	
_____	_____	1. Bleeding or sore gums
_____	_____	2. Bruise easily
_____	_____	3. Unusually poor visual adjustment to the dark
_____	_____	4. Fractured hip
_____	_____	5. Constipation or hemorrhoids
_____	_____	6. Frequent or chronic fever

In the past five years, how many times have you been hospitalized? _____

It may be that any benefits from the study drug are modified by other medications. Would you, therefore, please list the prescription drugs which you are now taking more than once per week? (PLEASE PRINT)

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MEDICAL RADIATION EXPOSURES

Have you ever had any of the following?

- 1) X-ray treatments for acne, ringworm, enlarged tonsils, adenoids, thymus
 Yes No #treatments _____ Ages _____ or Year _____
- 2) Treatment with radium, cobalt or other radioactive isotopes
 Yes No #treatments _____ Ages _____ or Year _____
- 3) Upper GI series (x-ray of stomach after drinking barium)
 Yes No #times _____ Ages _____ or Year _____
- 4) Lower GI series (Barium enema)
 Yes No #times _____ Ages _____ or Year _____
- 5) Kidney x-rays
 Yes No #times _____ Ages _____ or Year _____
- 6) Chest x-rays
 Yes No #times _____ Ages _____ or Year _____
- 7) Fluoroscopy of lung
 Yes No #times _____ Ages _____ or Year _____
- 8) Liver Scan
 Yes No #times _____ Ages _____ or Year _____
- 9) Bone x-rays
 Yes No #times _____ Ages _____ or Year _____
- 10) CT scan
 Yes No #times _____ Ages _____ or Year _____

FAMILY HISTORY

Were either of your natural parents ever told by a doctor that they had:

	<u>FATHER</u>		<u>Don't Know</u>	<u>MOTHER</u>		<u>Don't Know</u>
	<u>Yes</u>	<u>No</u>		<u>Yes</u>	<u>No</u>	
A. Skin cancer	_____	_____	_____	_____	_____	_____
B. Any type of cancer: specify _____	_____	_____	_____	_____	_____	_____
C. Age of parent at diagnosis _____	_____	_____	_____	_____	_____	_____
D. Liver Disease	_____	_____	_____	_____	_____	_____
E. Increased blood lipids or fat (triglycerides, cholesterol)	_____	_____	_____	_____	_____	_____

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FAMILY HISTORY (continued)Have any other close relatives had cancer? Yes NoIs your father still alive? Yes No How old is he? _____

If not, how old was he when he died? _____ Cause of death: _____

Is your mother still alive? Yes No How old is she? _____

If not, how old was she when she died? _____ Cause of death: _____

Females: Have your mother, sister(s) or daughter(s) had breast cancer?

 Yes No If yes, who?

<input type="checkbox"/>	Mother	Age at diagnosis	_____
<input type="checkbox"/>	Sister	Age at diagnosis	_____
<input type="checkbox"/>	Daughter	Age at diagnosis	_____

How many sisters do you have? _____

How many daughters do you have? _____

OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES

Are you currently:

- | | |
|-------------|--------------|
| 1. Employed | 4. Homemaker |
| 2. Retired | 5. Other |
| 3. Disabled | |

What has been your usual occupation or job - the one you have worked at the longest?

Job/occupation _____

Number of years employed in this occupation _____

Latest position or job title _____

Business, field or industry _____

In your work or daily life, are (were) you regularly exposed to any of the following? If "yes", indicate the number of years exposed.

EXPOSED TO:	Check One		1st year Exposed	No. of Years Exposed
	Yes	No		
1. Asbestos				
2. Chemicals/Acids/Solvents				
3. Coal or Stone Dusts				

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OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES (continued)

EXPOSED TO:	Check One		1st Year Exposed	No. of Years Exposed
	Yes	No		
4.Coal Tar/Pitch Asphalt				
5.Diesel Engine Exhaust				
6.Dyes				
7.Formaldehyde				
8.Gasoline Exhaust				
9.Pesticides/Herbicides				
10.Textile Fibers/Dust				
11.Wood Dust				
12.X-rays/Radioactive Material				

Have you had a medical problem as a result of this? Yes No
 (If yes, please specify _____)

Was this treated by a physician? Yes No

PSYCHOLOGICAL AND SOCIAL FACTORS

How many people can you call on for help and/or to discuss private matters?

None 1 or 2 3 to 5 6 to 9 10 or more

How many of these people do you see at least once a month?

None 1 or 2 3 to 5 6 to 9 10 or more

Do you belong to any of these kinds of groups?

1. A social or recreation group yes no
2. A labor union, commercial group, or professional association yes no
3. A church group yes no

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PSYCHOLOGICAL AND SOCIAL FACTORS (continued)

4. A group concerned with children
(such as a PTA or Boys Scouts, etc.) yes no
5. A group concerned with community
improvement, charity or service yes no
6. Other group _____
(Describe: _____) yes no

On the average, how often have you done each of these things during the past 12 months?

	(1)	(2)	(3)	(4)	(5)
	Never in past year	1-5 times in past year	Once a month or once every 2 months	Once every 2-3 weeks	At least once a week
1. Go to movies	1.				
2. Go to sports events	2.				
3. Go to concerts, plays, etc.	3.				
4. Go to fairs, museums, etc.	4.				
5. Go to meetings/voluntary groups	5.				
6. Go to church/synagogue	6.				

DIETARY INTAKE

During the past year, have you taken any vitamins or minerals?

1. Yes, sometimes 2. Yes, every day 3. No

If you are, would you please list which vitamins and the dose of each?

	No. of Years	No./Week	Dose/size
1. Multiple vitamins	_____	_____	_____
Brand name - _____			
2. Vitamin A	_____	_____	_____
3. Vitamin C	_____	_____	_____
4. Vitamin E	_____	_____	_____
5. Other _____	_____	_____	_____
6. Other _____ (may include: _____ calcium; _____ yeast; _____ selenium; _____ zinc; _____ iron; _____ beta-carotene; _____ cod liver oil, etc.)	_____	_____	_____

Have you ever had an adverse reaction to vitamins? Yes No

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It is important that we stay in touch with you during this study. The following names and addresses will help us locate you if we lose contact. Thank you.

Your Regular Physician (other than your dermatologist)

Name: _____

Address: _____

City/State/Zip: _____

Telephone: _____

Spouse

Name: _____

Daytime Telephone: _____

Relative or Friend

Name: _____

Address: _____

City/State/Zip: _____

Relationship: _____ Telephone: _____

Thank you for filling out this form. We appreciate your interest very much. We are very pleased that you have chosen to participate because without your support and cooperation this study could not exist. We hope the medication will lower your chances of cancer.

Thomas E. Moon, Ph.D. and
Norman Levine, M.D.
University of Arizona
Cancer Center

FOR OFFICE USE ONLY		
FORM	DATE	INITIALS
On Study/enter	_____	_____
QUAC	_____	_____

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SKICAP-AK and SKICAP BCC/SCC Study Follow-up
Form

Visit: 2 F _____

SKIN CANCER PREVENTION PROGRAM
PARTICIPANT'S FOLLOW-UP FORM

Please answer all questions by checking the appropriate box or printing in the information requested. All information will be regarded as STRICTLY CONFIDENTIAL and will be used for statistical purposes only.

Thank you for your continued cooperation in this study.

PARTICIPANT IDENTIFICATION

Participant Name: _____ / _____ / _____
(First) (Initial) (Last)

TODAY'S DATE: _____
mo/ day/ yr

DERMATOLOGICAL PROGRESS

Are you currently being followed by a:

- Study dermatologist
 Private dermatologist
 Both

Name of current private dermatologist: _____

Date last seen: _____

Date you expect to be seen next: _____

Since your last visit with us have you had a biopsy done? Yes No

Since your last visit with us have you used Retin-A? Yes No

If yes, how many months? _____

Face Yes No

Arms Yes No

Other Yes No Describe: _____

Since your last visit, have you used Efudex, Fluroplex? Yes No

If yes,

Face Yes No

Arms Yes No

Other Yes No Describe: _____

(continued next page)

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PARTICIPANT CHARACTERISTICS

Average number of hours between 9 a.m. and 4 p.m. per week spent outside in the sun in the past 30 days:

Total hours per week: ___ (M-F) Total hours on weekend: ___ (Sat-Sun)

Do you currently smoke cigarettes? Yes No

If yes, on the average, how many packs per day do you smoke? _____

Do you inhale the cigarette smoke? Yes No

Are the cigarettes you smoke? Filtered? Unfiltered?

Since your last visit, have you observed or experienced any of the following:

	Date of Disorder		Was Medication Prescribed?	
	Yes	No	Yes	No
Skin Disorder	___	___	___	___
High Cholesterol	___	___	___	___
Nausea/vomiting	___	___	___	___
Heart attack or Stroke	___	___	___	___
Cancer-other than skin	___	___	___	___
Other major illness	___	___	___	___

Please specify _____

Have you seen another physician since your last visit? Yes No
If yes, state reason (please print).

Since your last visit, have there been any changes in your medication intake? (This includes prescription drugs or over-the-counter [i.e., aspirin] which you now take more than once a week.) Yes No

If yes, please list the medication(s). (please print)

_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion

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Since your last visit, have you made any changes in your vitamin intake?
 yes No. If yes, please list.

	<u>No./Week</u>	<u>Dose/Size</u>				
1. Multiple vitamin	_____	_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
List Brand Names	_____					

2. Vitamin A	_____	_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
3. Vitamin C	_____	_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
4. Vitamin E	_____	_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion
5. Other	_____	_____	<input type="checkbox"/>	Addition	<input type="checkbox"/>	Deletion

Do you now use sunscreen? Yes No
 (Note: A sunscreen is more than oil or lotion and can be identified by a sun protection factor [e.g., SPF 15, etc.] printed on the container.)

What is the SPF of your current sunscreen? _____

How regularly do you use sunscreen? _____

1. Always when in the sun: _____
2. More than half the time: _____
3. Less than half the time: _____
4. Never: _____

With what frequency have you taken your capsules in the past three months?

Daily _____

Missed 1-2 per week _____

Missed 3+ per week _____

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4. Currently, after repeated and prolonged exposure to the sun, would your skin be:

1. Not tanned, may freckle
 2. Mildly tanned due to a tendency to peel
 3. Moderately tanned
 4. Very tanned

5. Do you have a noticeable year-around tan? Is there a difference in color between the skin on your face, neck or arms and the skin covered by your shirt/blouse?

1. YES
 2. NO

Average number of hours BETWEEN 9 A.M. AND 4 P.M. per week spent outside in the sun in the past 30 days:

6. During the average week (Monday-Friday), how many hours have you spent in the sun? _____Hrs.

7. During the average weekend (Saturday-Sunday), how many hours have you spent in the sun? _____Hrs.

8. Have you used a sunlamp since starting the study? Yes No

If YES: how many times since starting the study? _____

9. Do you use sunscreen? Yes No
 (NOTE: A sunscreen is more than oil or lotion and can be identified by a sun protection factor [e.g., SPF 15, etc.] printed on the container).

a) What is the SPF of your current sunscreen? _____

b) How regularly do you use sunscreen?

1. Always when in the sun _____
 2. More than half the time _____
 3. Less than half the time _____
 4. Never: _____

10. Do you use a sunscreen during the entire year? Yes No

If NO, which season(s) do you use a sunscreen:

- Yes No Spring (Mar., Apr., May)
 Yes No Summer (June, July, Aug.)
 Yes No Fall (Sept., Oct., Nov.)
 Yes No Winter (Dec., Jan., Feb.)

11. Have you ever used Retin-A? Yes No IF NO, go to #13.

If YES, how many years have you used Retin-A? _____

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12. How often have you used Retin-A?

1. 5-7 times a week
 2. 3-4 times a week
 3. 1-2 times a week
 4. less than once a week

What area of your body have you used Retin-A?

- Face Yes No
 Arms Yes No
 Other Yes No Describe: _____

13. Have you ever used Efudex or Fluroplex? Yes No

- If YES, Face Yes No How many treatments have you had since starting the study? _____
 Arms Yes No How many treatments have you had since starting the study? _____
 Other Yes No Describe: _____
 How many treatments have you had since starting the study? _____

14. What is your current marital status:

1. Single 3. Widowed
 2. Married 4. Divorced/Separated

15. Compared with those your age and sex, how would you rate your current level of physical activity?

1. MUCH MORE PHYSICALLY ACTIVE THAN AVERAGE
 2. MORE PHYSICALLY ACTIVE THAN AVERAGE
 3. ABOUT AVERAGE
 4. LESS PHYSICALLY ACTIVE THAN AVERAGE
 5. MUCH LESS PHYSICALLY ACTIVE THAN AVERAGE

16. Are you currently:

- employed 1. temporarily laid off 4.
 housewife 2. unemployed 5.
 retired 3.

If CURRENTLY EMPLOYED:

What is your job/occupation? _____
 How many hours a week do you work? _____

17. Have you ever worked on a farm? Yes No

If YES, have you ever worked with pesticides or herbicides containing arsenic?

1. I have worked with pesticides or herbicides containing arsenic.
 2. I have worked with pesticides or herbicides but I do not know if they contained arsenic.
 3. I did not work with pesticides or herbicides.
 4. I worked with pesticides or herbicides but they did not contain arsenic.

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What was the first year of exposure to pesticides or herbicides? _____

TOTAL years of exposure? _____

18. Have you ever been exposed to any compounds containing arsenic (other than pesticides or herbicides)?

Yes No

If YES, what compounds? _____

What was the first year of exposure? _____

TOTAL years of exposure? _____

SMOKING

19. Do you currently smoke cigarettes?

Yes No

If YES, on the average how many packs per day do you smoke? ____

Do you inhale the cigarette smoke? Yes No

Are the cigarettes you smoke Filtered? Unfiltered?

20. Have you ever smoked: a pipe 1 Yes 2 No

cigars 1 Yes 2 No

If YES: Do you currently smoke: a pipe 1 Yes 2 No

cigars 1 Yes 2 No

21. Currently, how often do you drink wine, beer or liquor?

1. Never 2. Less than once a week 3. Once or twice a week 4. More than twice a week

1. Wine	_____	_____	_____	_____
2. Beer	_____	_____	_____	_____
3. Liquor	_____	_____	_____	_____

When you drink wine, beer or liquor, how many drinks do you usually have at one sitting?

1. One or two drinks 2. Three or four drinks 3. Five or more drinks

Wine	_____	_____	_____
Beer	_____	_____	_____
Liquor	_____	_____	_____

HEALTH HISTORY

22. Since you started with us in the study, have you ever been treated by a doctor for any of the following conditions?

<u>Yes</u>	<u>No</u>		<u>Yes</u>	<u>No</u>
_____	_____	1. Heart attack	_____	_____
_____	_____	2. Angina	_____	_____
_____	_____	3. High blood pressure	_____	_____
_____	_____	4. Stroke	_____	_____
_____	_____	5. Diabetes	_____	_____
_____	_____	6. Chronic bronchitis or emphysema	_____	_____
_____	_____	7. Rectal or colon polyps	_____	_____
_____	_____	8. Chronic colitis, inflammatory bowel disease	_____	_____
_____	_____	9. Osteoporosis	_____	_____
_____	_____	10. Any other serious condition (excluding cancer) Please specify.	_____	_____

If YES: Did you have the condition before starting the study?

23. Have you had cancer (other than non-melanoma skin cancer) since starting the study?

[]1. Yes []2. No

If YES: What type of cancer(s): (a) _____ (b) _____

At which hospital were you diagnosed or did you receive treatment?
Who was your treating physician?

1. _____

2. _____

26. What is the name and address of your primary care physician?

NAME: _____

ADDRESS: _____

27. Have you been seen only by the Study dermatologist?

Yes No

If **NO**:

28. What is the name of your current dermatologist?

NAME: _____

ADDRESS: _____

29. Please list the name(s) of any other doctor(s) who have examined you for your skin condition(s) since you started the Study (including HMOs, general practitioner, plastic surgeon, or former dermatologist).

NAME: _____	Office Use Only	NAME: _____	Office Use Only
CITY & STATE: _____		CITY & STATE: _____	
DATE LAST SEEN: _____		DATE LAST SEEN: _____	
NAME: _____	Office Use Only	NAME: _____	Office Use Only
CITY & STATE: _____		CITY & STATE: _____	
DATE LAST SEEN: _____		DATE LAST SEEN: _____	

30. Now that you have completed your participation in the SKIN CANCER PREVENTION STUDY, we would like to get your feedback on several aspects of the study and your experiences with taking the study pills. By collecting this information, we hope to be able to improve our procedures to make our future studies as rewarding for the participants as possible. Please respond below by indicating your level of agreement with the statements as follows:

STRONGLY AGREE = SA, AGREE = A, NEITHER AGREE NOR DISAGREE = N,
DISAGREE = D, OR STRONGLY DISAGREE = SD

Please circle the response that best represents how you feel:

	1	2	3	4	5
1. It was convenient to take the study pills every day.	SA	A	N	D	SD
2. I did not mind the taste of the study pills.	SA	A	N	D	SD
3. The study staff respected my ideas or concerns about taking the study pills.	SA	A	N	D	SD
4. The pills caused no unpleasant side effects.	SA	A	N	D	SD
5. It was difficult to remember to take the pills every day.	SA	A	N	D	SD

	1	2	3	4	5
6. Others in my household did not like my being on this study.	SA	A	N	D	SD
7. I got the feeling the research staff was supportive of my efforts to keep myself free of skin cancer.	SA	A	N	D	SD
8. I was too busy to take the pills every day.	SA	A	N	D	SD
9. It was not difficult filling out any of the forms I was given on this study.	SA	A	N	D	SD
10. I did not mind going to the study clinic for my regular visits.	SA	A	N	D	SD
11. I like receiving the results of my lab tests for free.	SA	A	N	D	SD
12. The research staff did not provide encouragement to me in my efforts to take the study pills.	SA	A	N	D	SD
13. My family was supportive of my efforts to be in this study.	SA	A	N	D	SD
14. Participating in this study was too difficult for me.	SA	A	N	D	SD

31. These next few questions are meant to determine how satisfied you were with various aspects of the study. Please indicate your level of satisfaction with each item as follows:

VERY SATISFIED = VS, SATISFIED = S, NEITHER SATISFIED NOR DISSATISFIED = N, DISSATISFIED = D, VERY DISSATISFIED = VD

Once again, circle the response that best describes how you feel.

How satisfied were you with:

	1	2	3	4	5
1. The instructions that were given to you on how to take the study pills every day.	VS	S	N	D	VD
2. The availability of the research study staff.	VS	S	N	D	VD
3. The clinic's parking facilities were:	VS	S	N	D	VD
4. The amount of information you were given while on study.	VS	S	N	D	VD

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32. Do you think you have been/were taking the Vitamin A or the placebo?

1. Vitamin A
 2. Placebo
 3. I have no feeling either way

What makes you think this? _____

In the future we hope to conduct further studies similar to the Skin Cancer Prevention Study and would like your comments so that we may improve our future studies.

33. What features did you LIKE about the Skin Cancer Prevention Study?

34. What features did you NOT LIKE about the Skin Cancer Prevention Study?

35. The results of the study will be available in 1993. Do you want us to send you the results when they become available?

1. YES
 2. NO

Thank you for filling out this form. We appreciate your participation in the Skin Cancer Prevention Study very much. Without your support and cooperation this study could not have been accomplished.

Thomas E. Moon, Ph.D. and
 Norman Levine, M.D.
 University of Arizona
 Cancer Center

FOR OFFICE USE ONLY		
FORM	DATE	INITIALS
On Study/enter	_____	_____
QUAC	_____	_____

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10. Do you use a sunscreen during the entire year? Yes No

If NO, which season(s) do you use a sunscreen:

- | | | | | | |
|--------------------------|-----|--------------------------|----|--------|---------------------|
| <input type="checkbox"/> | Yes | <input type="checkbox"/> | No | Spring | (Mar., Apr., May) |
| <input type="checkbox"/> | Yes | <input type="checkbox"/> | No | Summer | (June, July, Aug.) |
| <input type="checkbox"/> | Yes | <input type="checkbox"/> | No | Fall | (Sept., Oct., Nov.) |
| <input type="checkbox"/> | Yes | <input type="checkbox"/> | No | Winter | (Dec., Jan., Feb.) |

11. Have you ever used Retin-A? Yes No IF NO, go to #13.

If YES, how many years have you used Retin-A? _____

12. How often have you used Retin-A?

- | | | |
|--------------------------|----|-----------------------|
| <input type="checkbox"/> | 1. | 5-7 times a week |
| <input type="checkbox"/> | 2. | 3-4 times a week |
| <input type="checkbox"/> | 3. | 1-2 times a week |
| <input type="checkbox"/> | 4. | less than once a week |

What area of your body have you used Retin-A?

- | | | | | |
|-------|--------------------------|-----|--------------------------|----|
| Face | <input type="checkbox"/> | Yes | <input type="checkbox"/> | No |
| Arms | <input type="checkbox"/> | Yes | <input type="checkbox"/> | No |
| Other | <input type="checkbox"/> | Yes | <input type="checkbox"/> | No |

Describe: _____

13. Have you ever used Efudex or Fluoroplex? Yes No

If YES, Face Yes No How many treatments have you had since starting the study? _____

Arms Yes No How many treatments have you had since starting the study? _____

Other Yes No Describe: _____
How many treatments have you had since starting the study? _____

17. Have you ever worked on a farm? Yes No

If YES, have you ever worked with pesticides or herbicides containing arsenic?

- | | | |
|--------------------------|----|---|
| <input type="checkbox"/> | 1. | I have worked with pesticides or herbicides containing arsenic. |
| <input type="checkbox"/> | 2. | I have worked with pesticides or herbicides but I <u>do not know</u> if they contained arsenic. |
| <input type="checkbox"/> | 3. | I did not work with pesticides or herbicides. |
| <input type="checkbox"/> | 4. | I worked with pesticides or herbicides but they <u>did not</u> contain arsenic. |

What was the first year of exposure to pesticides or herbicides? _____

TOTAL years of exposure? _____

18. Have you ever been exposed to any compounds containing arsenic (other than pesticides or herbicides)?

Yes No

If YES, what compounds? _____

What was the first year of exposure? _____

TOTAL years of exposure? _____

29. Please list the name(s) of any other doctor(s) who have examined you for your skin condition(s) since you started the Study (including HMOs, general practitioner, plastic surgeon, or former dermatologist).

NAME: _____	Office Use Only	NAME: _____	Office Use Only
CITY & STATE: _____		CITY & STATE: _____	
DATE LAST SEEN: _____		DATE LAST SEEN: _____	
NAME: _____	Office Use Only	NAME: _____	Office Use Only
CITY & STATE: _____		CITY & STATE: _____	
DATE LAST SEEN: _____		DATE LAST SEEN: _____	

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DISAGREE = D, OR STRONGLY DISAGREE = SD

Please circle the response that best represents how you feel:

	1	2	3	4	5
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2. I did not mind the taste of the study pills.	SA	A	N	D	SD
3. The study staff respected my ideas or concerns about taking the study pills.	SA	A	N	D	SD
4. The pills caused no unpleasant side effects.	SA	A	N	D	SD
5. It was difficult to remember to take the pills every day.	SA	A	N	D	SD

	1	2	3	4	5
6. Others in my household did not like my being on this study.	SA	A	N	D	SD
7. I got the feeling the research staff was supportive of my efforts to keep myself free of skin cancer.	SA	A	N	D	SD
8. I was too busy to take the pills every day.	SA	A	N	D	SD
9. It was not difficult filling out any of the forms I was given on this study.	SA	A	N	D	SD
10. I did not mind going to the study clinic for my regular visits.	SA	A	N	D	SD
11. I like receiving the results of my lab tests for free.	SA	A	N	D	SD
12. The research staff did not provide encouragement to me in my efforts to take the study pills.	SA	A	N	D	SD
13. My family was supportive of my efforts to be in this study.	SA	A	N	D	SD
14. Participating in this study was too difficult for me.	SA	A	N	D	SD

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Once again, circle the response that best describes how you feel.

How satisfied were you with:

	1	2	3	4	5
1. The instructions that were given to you on how to take the study pills every day.	VS	S	N	D	VD
2. The availability of the research study staff.	VS	S	N	D	VD
3. The clinic's parking facilities were:	VS	S	N	D	VD
4. The amount of information you were given while on study.	VS	S	N	D	VD