Viruses in Skin Cancer (VIRUSCAN): Study Design and Baseline Characteristics of a Prospective Clinic-Based Cohort Study

Rossybelle P. Amorrortu[1], Neil A. Fenske[2], Basil S. Cherpelis[2], Laxmi Vijayan[1], Yayi Zhao[1], Juliana Balliu[1], Jane L. Messina[2,3,4], Vernon K. Sondak[4], Anna R. Giuliano[5], Tim Waterboer[6], Michael Pawlita[6], Tarik Gheit[7], Massimo Tommasino[7], and Dana E. Rollison[1,5]

ABSTRACT

Background: Accumulating evidence suggests that cutaneous viral infections are risk factors for the development of keratinocyte carcinomas. The Viruses in Skin Cancer (VIRUSCAN) Study, a prospective cohort study, was established in 2014 to investigate the risk of keratinocyte carcinoma associated with cutaneous human papillomavirus and polyomavirus infection and the possible interaction with ultraviolet radiation exposure (UVR).

Methods/Results: VIRUSCAN incorporates repeated measures of viral infection using multiple markers of infection and quantitative measures of UVR using a spectrophotometer. Participants were recruited between July 14, 2014 and August 31, 2017 at the University of South Florida Dermatology Clinic in Tampa, FL. After excluding 124 individuals with prevalent keratinocyte carcinomas at baseline, 1,179 participants (53.2% women, 46.8% men, all ages 60 years and older) were followed for up to 4 years with routine skin exams occurring every 6 to 12 months. Here, we present the VIRUSCAN Study design, methods, and baseline characteristics, including demographics, sun exposure behavior, quantitative UVR exposure measurements, and cutaneous viral prevalence, for the full study cohort.

Conclusions: The VIRUSCAN Study will provide critical temporal evidence needed to assess the causality of the role cutaneous viral infections play in the development of keratinocyte carcinomas, as well as the potential interaction between cutaneous viral infections and UVR exposure.

Impact: Study findings will be valuable in future development of novel keratinocyte carcinoma prevention strategies.

Introduction

Keratinocyte carcinoma, comprised of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is the most frequently occurring cancer in the United States (1). Keratinocyte carcinomas incidence increased by 35% between 2006 and 2012, and an estimated 5.4 million keratinocyte carcinoma cases were diagnosed in 2012. Despite its low metastatic potential and mortality rates, keratinocyte carcinoma results in over 2,000 deaths annually in the United States (2). Furthermore, individuals are frequently diagnosed with multiple keratinocyte carcinomas, requiring multiple surgeries throughout their lifetimes and resulting in significant patient morbidity and substantial economic burden at the national level (3, 4). Established host risk factors for keratinocyte carcinoma include older age, male sex, and sun-sensitive skin likely to freckle and burn (5, 6). Ultraviolet radiation (UVR) is an environmental risk factor for KC, with intermittent and childhood UVR associated with increased risk of BCC (7, 8) and chronic sun exposure associated with SCC (7). Immunologic deficiencies and chronic immunosuppression are also linked to the development of keratinocyte carcinomas, particularly SCC (9, 10). The continued increase in keratinocyte carcinoma incidence, despite general awareness of the harms of sun exposure (1), underscores the need to identify additional modifiable risk factors that may lead to the development of novel prevention strategies.

Several epidemiologic studies have observed keratinocyte carcinomas to be associated with markers of cutaneous viral infections, including cutaneous human papillomaviruses (cuHPV; refs. 11–14) and Merkel cell polyomavirus (MCV), a cutaneous human papillomavirus (HPyV; ref. 15). CuHPV infections are particularly common in immunodeficient (16–18) and immunosuppressed individuals (19–21), but are also observed in immunocompetent populations, with cuHPV infection occurring early in life: 55%–70% cuHPV DNA prevalence has been reported for infants and children up to 4 years old with similar prevalence rates observed among their parents (22, 23). CuHPV seroprevalence increases with age, with rates of 32%, 58%, and 60% observed in children ages 1–14, young adults ages 15–34, and older adults ages >34, respectively (24). CuHPV DNA prevalence has also been shown to increase with age (25, 26). While there are no established risk factors for cuHPV infection, previous studies have reported associations between cuHPV prevalence and UVR exposure (27, 28), light skin phenotypes (29, 30), male gender (29, 31, 32), and smoking (29, 30). Similar to cuPHV, HPyV infections also occur in early childhood (33), with MCV seroprevalence rates increasing with age: 40%, 85%, and 90% in ages 1–4, 15–19, 50–79, respectively (34). While seroprevalence rates suggest most adults have been exposed to cuHPV and MCV at some

[1]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, Florida. [2]Department of Dermatology and Cutaneous Surgery, University of South Florida College of Medicine, Tampa, Florida. [3]Department of Anatomic Pathology, Moffitt Cancer Center, Tampa, Florida. [4]Department of Cutaneous Oncology, Moffitt Cancer Center, Tampa, Florida. [5]Center for Immunization and Infection Research in Cancer, Moffitt Cancer Center, Tampa, Florida. [6]Infections and Cancer Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany. [7]Infections and Cancer Biology Group, International Agency for Research on Cancer—World Health Organization, Lyon, France.

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Corresponding Author: Dana E. Rollison, Moffitt Cancer Center, 12902 Magnolia Drive, SRB 1ST 21206, Tampa, FL 33612. Phone: 813-745-6530; Fax: 813-745-8332; E-mail: dana.rollison@moffitt.org

point in their lifetime, and presumably cleared, DNA-based markers of infection indicate that infections can be newly acquired in adulthood (27, 35).

Findings between cuHPV infection and keratinocyte carcinomas have been most consistently observed for cuHPV types in genus β ("β-HPV") and cutaneous SCC (36). Experimental studies have identified multiple signaling pathways disrupted by β-HPV oncoproteins, with both *in vitro* (37–40) and mouse models (41–43) demonstrating the cooperation between β-HPV infection and UVR in SCC development. In humans, cuHPV infections are more likely to be detected in tumors that occur in sun-exposed areas of the body (28). A few epidemiologic studies have sought to investigate the interaction between UVR exposure and β-HPV infection by examining SCC risk in subgroups of β-HPV seroreactivity and skin phenotype (11, 32) or past self-reported UVR exposure (44). While positive interactions were observed, these studies were limited by subjective UVR measurements and reliance on single time point assessments of both UVR exposure and β-HPV seroreactivity. Furthermore, no studies have investigated the potential interaction between UVR and infection with cuHPV types in other genera.

The Viruses in Skin Cancer (VIRUSCAN) Study is a prospective cohort study being conducted at the Moffitt Cancer Center and the University of South Florida Dermatology Clinic, in Tampa, FL, to investigate the risk of keratinocyte carcinoma associated with cuHPV/HPyV infections. The primary study aims are to estimate baseline prevalence of type-specific cuHPV/HPyV infections using multiple biospecimens, determine the risk of incident keratinocyte carcinomas associated with baseline infections, and evaluate UVR as a potential cofactor in virus-associated keratinocyte carcinomas risk. Furthermore, longitudinally banked biospecimens will be used to estimate the risk of incident keratinocyte carcinomas associated with incident and persistent cuHPV/HPyV infection and investigate the concordance between virus types present prior to keratinocyte carcinomas diagnosis, and viral DNA detected in subsequent incident tumors. The VIRUSCAN Study addresses several research needs, as it is one of the few prospective studies to incorporate repeated measures of cuHPV/HPyV infection, multiple biomarkers of cuHPV/HPyV infection, and quantitative measurements of UVR exposure. Here we describe the VIRUSCAN Study design, methods, and baseline characteristics.

Materials and Methods

Participants and eligibility criteria

Participants were recruited from the University of South Florida Dermatology Clinic, a high volume dermatology clinic in which patients undergo routine total body skin exams for the early detection of skin cancers. University of South Florida Dermatology Clinic patients undergoing total body skin examination are often self-referred, including those who seek treatment of a dermatologic condition and are advised to undergo total body skin examination while they are in the clinic and those who attend the clinic specifically for skin cancer screening.

University of South Florida Dermatology Clinic patients aged 60 or older were eligible to enroll in the VIRUSCAN Study. The recruitment age was restricted to this age group to optimize the number of incident cancers detected during follow-up. Patients with a history of either BCC or SCC were eligible, but patients with a history of both BCC and SCC were excluded to ensure all study participants were naïve to at least one type of keratinocyte carcinoma at baseline. To maximize the generalizability of study findings to broad populations of individuals at

risk for keratinocyte carcinomas, no additional exclusion criteria were applied. As such, organ transplant recipients were included but comprised only 1% of the VIRUSCAN Study population and will be excluded from a sensitivity analysis to assess whether associations observed differ from those observed in the full cohort. Eligible patients scheduled to undergo a total body skin examination were identified via daily medical chart reviews and approached for study participation during their clinic visit. The study was approved by the University of South Florida Institutional Review Board, and all participants provided written informed consent.

During the design phase of the VIRUSCAN Study, enrollment was projected to be 1,500 based on historical clinic records. Using this fixed sample size, previously published incidence rates of keratinocyte carcinoma (45), and assuming an alpha error rate of 0.05, power of 80%, and false discovery rate of <10%, the calculated minimal detectable hazard ratios (MDHR) for the association between cuHPV/HPyV infection at baseline and SCC ranged from 1.89 to 2.82 for 40% to 10% of the cohort testing virus-positive at baseline, respectively, and 1.64 to 2.23 for keratinocyte carcinoma overall. Over 3 years of enrollment, 2,929 patients were approached, of whom 1,303 (44.5%) consented. While the number of enrolled participants was less than projected, the observed SCC and keratinocyte carcinoma incidence rates are trending higher than projected, therefore the final analyses will have more than adequate statistical power to detect the associations of interest.

While there were no differences in the age of participants (mean = 69.1 years, SD = 6.4) versus nonparticipants (mean = 69.9 years, SD = 7.5 P = 0.06), females were more likely to enroll in the study than men (52.2% female vs. 47.8% men, P = 0.03). Of the 1,303 participants enrolled, 124 (9.5%) underwent biopsies as a result of the baseline total body skin examination that were subsequently determined to be malignant after pathology review. These participants with prevalent keratinocyte carcinoma at baseline (83 SCC, 78 BCC, and 1 basosquamous carcinoma) were excluded from follow-up visits. The remaining 1,179 participants were followed for up to 4 years, depending on the year of enrollment in the study. Follow-up visits coincided with participants' routine total body skin examination appointments at the University of South Florida Dermatology Clinic, the frequency of which varied, depending on the participant's skin cancer risk profile, including skin type, family history of skin cancer, and sun exposure history. Typically, participants returned to the clinic every 6–12 months for a total body skin examination. For example, among the 421 participants enrolled in the first year of the study, 87.9% returned to the clinic at least once during the subsequent three years of follow-up, with 69.4% returning at least twice and 53.9% returning three or more times.

Data collection

Table 1 summarizes data and biospecimen collection for VIRUSCAN Study participants at baseline and during follow-up. Variables were broadly classified as patient demographics, skin cancer risk factors, medical history, cutaneous viral infection status, tumor characteristics, and measures of UV exposure.

Questionnaire

Participants completed an electronic questionnaire at baseline that included information on demographics, sun-protective behaviors, skin cancer risk factors, and medical history (**Table 1**). Participants unable to complete the questionnaire during the visit were emailed a link to complete the questionnaire online. Nonresponders were sent up to five e-mail reminders followed by a final phone call reminder. The overall questionnaire response rate was 97.5%, with no differences observed

Table 1. Variables collected in the VIRUSCAN Study, Tampa, FL, 2014–2018.

Source	Variable details	Visit
Study questionnaire		B
Demographics	Gender, age, race, ethnicity, education level, marital status, place of birth	
Sun protection	Use of sunscreen, long sleeves, pants, hats, sunglasses, and sun avoidance	
Sun exposures	Occupational sun exposure, history of sunburn, skin reaction to the sun, use of sun lamps or UV nail dryers	
Phenotypic factors	Eye color, hair color, untanned skin color, number of moles on arm/body	
Skin cancer screening	Number of skin exams per year	
Skin conditions	History of skin biopsies, eczema, psoriasis, acne, vitiligo, actinic keratosis, keratoacanthoma, warts, other skin conditions, treatment for skin conditions	
Cancer history	Melanoma, basal cell carcinoma, squamous cell carcinoma, other skin cancers or other cancers	
Other medical conditions	Organ transplant, Crohn's disease, type 1 diabetes, type 2 diabetes, heart disease, epidermodysplasia verruciformis, lupus, multiple sclerosis, Sezary syndrome, rheumatoid arthritis, osteoarthritis, xeroderma pigmentosum, asthma/bronchitis/emphysema, gout	
Smoking and alcohol	Smoking and drinking habits	
Steroid medication	Use of oral steroids or steroid creams	
Clinical findings of total body skin examinations	Identification of premalignant (actinic keratosis) and malignant lesions [tumor histology, anatomic site (head, neck, torso, arms, legs)]	B and FU
Biospecimens		
Blood	HPV/HPyV serum antibodies	B
Eyebrow hair	HPV/HPyV viral DNA	B and FU
Skin swabs	HPV/HPyV viral DNA	B and FU
Tumor tissue	HPV/HPyV viral DNA, solar elastosis, papillomatosis, hypergranulosis, and crateriform architecture (SCC only)	B and FU
Spectrophotometer	Inner upper arm, forearm, and forehead spectrophotometer reading	B and FU
Sun exposure questionnaire	(subsample $n = 159$)	FU
Sun exposure (past week)	Time outdoors, frequency of sunbathing and spending time in the sun sitting or lying	
Sun protection (past week)	Use of sunscreen, clothing, hat and sun avoidance	

Abbreviations: B, baseline visit; FU, follow-up visit.

between the 1,150 participants who completed the questionnaire and the 29 who did not with respect to age ($P = 0.11$) or gender ($P = 0.58$).

Clinical findings of total body skin exams

The outcomes of the total body skin examination conducted at baseline and throughout follow-up were recorded in the study database, including negative findings, instances of actinic keratosis treated with liquid nitrogen (standard practice at the University of South Florida Dermatology Clinic), and biopsies of suspicious lesions. Medical records were reviewed throughout the follow-up period to document histopathologic results corresponding to any skin biopsies conducted on participants. Characteristics of those lesions determined to be malignant were recorded in the study database, including tumor size, histology and anatomic location (head, neck, torso, arms, and legs).

UVR exposure assessment

To model the time-dependent dynamic of recent UVR exposure and cutaneous viral infection acquisition and persistence, as well as the interaction between recent UVR exposure and infection associated with subsequent keratinocyte carcinoma development, an objective measure of UVR was needed—one that could be obtained over multiple time points in a manner least burdensome to study participants. As such, a spectrophotometer was used to quantify skin pigmentation at baseline and throughout study follow-up as a proxy for UVR exposure, similar to previous epidemiologic studies (46, 47). Skin pigmentation readings were obtained with the Konica Minolta CM-600D spectrophotometer using the specular component included mode with Spectra Magic NX Lite USB Ver. 2.5 software (48). This instrument measures color on three different axes: lightness on a scale of 0 (black) to 100 (white), axis a, indicating color within the red through green range, and axis b, measuring color within the yellow through blue range, with increasing values on a- and b-axes, indicating saturation of color (49). The instrument was calibrated using a white tile every morning per manufacturer guidelines. Each reading was obtained three times, and the average reading was recorded. The interuser reliability of the spectrophotometer readings across two VIRUSCAN Study coordinators was determined to be 0.91.

Natural skin tone was assessed using spectrophotometer readings of the sun-unexposed underside of the upper arm (i.e. the axilla), with higher readings indicating lighter natural skin tone. As shown in **Fig. 1**, spectrophotometer readings significantly decreased with increasing category of self-reported skin tone (50). The degree of recent tanning in response to recent UVR exposure was measured by calculating the difference between the color readings ($\Delta E \times ab$) on an area of sun-exposed skin (top of the forearm or forehead) and the axilla. These anatomic sites were chosen to correspond with the areas where viral infection measurements were obtained and will be used to model the potential interaction between UVR exposure and cutaneous viral infections. The difference between the sun-exposed pigmentation readings (forearm and forehead) and the sun-unexposed pigmentation readings (underarm) was calculated and used as an objective measure of UVR exposure in response to more recent tanning (48). However, as the exact timing of UVR exposure relative to the spectrophotometer readings is unclear, we administered a questionnaire asking about sun exposures over the past week among a subsample of VIRUSCAN participants ($n = 159$) to validate the spectrophotometer readings as a measure of more recent UVR exposure. The questionnaire included sun exposure and sun protection scales from a questionnaire
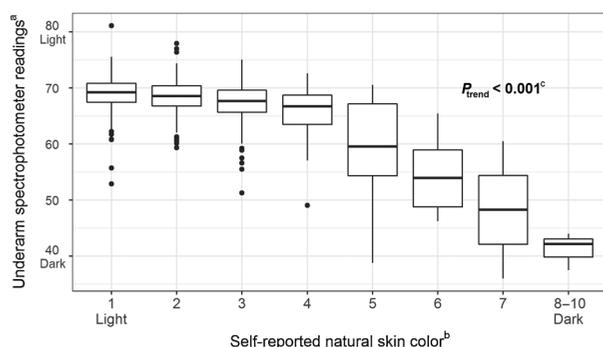
Figure 1.
Association between underarm spectrophotometer reading and self-reported natural skin color in the VIRUSCAN Study, Tampa, FL, 2014–2017. A strong positive correlation was observed between the baseline underarm spectrophotometer readings and self-reported natural skin color. [a]Lower values of the underarm spectrophotometer readings represent darker skin pigmentation. [b]Self-reported natural skin color was assessed with the New Immigrant Survey Color Scale, which included images of hands in a variety of skin shades categorized as ranging from 0 to 10, with 0 representing the lightest skin and 10 representing the darkest skin. [c]The Jonckheere–Terpstra test was used to determine if a trend existed between underarm spectrophotometer readings and self-reported natural skin color.

previously validated against dosimeter-based measures of outdoor time in the past week (51).

Biospecimens

Several biospecimens were obtained for the assessment of cutaneous viral infections. Peripheral blood samples were collected at baseline, processed and stored in aliquots of serum, plasma, and mononuclear cells, with serum samples earmarked for the analysis of antibodies to cuHPV and HPyV. Plucked eyebrow hairs and normal forearm skin swabs (52) were collected at baseline and throughout follow-up for the measurement of cuHPV and HPyV DNA. For those 240 study participants who developed keratinocyte carcinoma throughout the follow-up period, formalin-fixed paraffin-embedded tumor tissue sections and tumor tissue slides were obtained from the USF Pathology Department for the measurement of cuHPV/HPyV viral DNA and histopathologic grading, respectively. The study pathologist reviewed each hematoxylin and eosin–stained slide and graded the degree of solar elastosis present (0–3) in the adjacent normal tissues as a measure of cumulative UVR exposure (53, 54). For SCC, the pathologist described the presence of tumor architectural features possibly associated with cuHPV infection, including papillomatosis, hypergranulosis, and crateriform architecture.

Assessment of past and current cutaneous viral infections

Serum samples were analyzed for antibodies to the L1 capsid protein corresponding to 17 β-HPV types and 7 γ-HPV types, as well as antibodies to both the VP1 capsid protein and T-antigens corresponding to 4 cuHPyV types (Supplementary Table S1). Selected types were those shown to be associated with keratinocyte carcinoma in the literature, in addition to the most prevalent types observed in tumor tissues obtained from our previous case–control study (14, 55). Multiplex serology assays were conducted at the German Cancer Research Center in Heidelberg, Germany, using methods described previously (56, 57).

Baseline and follow-up eyebrow hair, skin swab and tumor samples were shipped to the Infections and Cancer Biology

Group at the International Agency for Research on Cancer for DNA extraction and genotyping. Multiplex/Luminex PCR-assays were used for the measurement of viral DNA corresponding to 46-β HPV types, 52 γ-HPV types, and 5 polyomavirus types (Supplementary Table S1) using methods described previously (52). Analysis of the tumor samples as well as the eyebrow hair and skin swab samples collected throughout follow-up is ongoing. Repeated measures of cutaneous viral infections in the eyebrow hairs and skin swabs will be used to model incident and persistent infections and their association with subsequent keratinocyte carcinoma risk.

Statistical analysis of baseline characteristics

Baseline questionnaire data were largely complete, as <5% of data were missing for each questionnaire item. Given the low rate of missing data, data for select variables were imputed among the 1,150 participants who completed the questionnaire to minimize bias introduced by list-wise deletion of incomplete data. The questionnaire data were complete for age, gender, and ethnicity. However, other variables were imputed, including education level, marital status and place of birth, sun-protective behaviors, and skin cancer risk factors. No medical history-related variables were imputed. The imputation was performed using the multivariate imputation by chained equations algorithm within R, version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria), which imputes data on a variable basis (58). The algorithm specifies an imputation model for each target variable that takes into account all other variables with the assumption that data are missing at random (58). A different type of model was assigned to each variable based on the variables' intrinsic distribution, for example, continuous (predictive mean match), binary (logistic regression), and unordered categorical (polytomous logistic regression) or ordered categorical (proportional odds model). Five imputed datasets were generated and used to calculate pooled χ^2 P values for all analyses here that used the imputed baseline questionnaire data.

To better understand how representative the VIRUSCAN Study population is of the general population, demographics, skin cancer risk factors, and sun-protective behaviors of study participants were compared with the general population, using publicly available data from the 2015 National Health Interview Survey (NHIS; refs. 59, 60). The NHIS is a health survey of the noninstitutionalized U.S. population conducted annually by the Centers for Disease Control and Prevention (60). The NHIS dataset was restricted to adults ≥60 living in the Southern United States for the current analysis, because this age group and geographic region most closely relate to the VIRUSCAN Study population.

The correlation between the spectrophotometer readings obtained at the forearm and forehead were described using Spearman rank correlation. Associations between the spectrophotometer readings and tertiles of self-reported recent sun exposure and protection scales were examined using logistic regression, adjusted for age and gender. The baseline prevalence of cuHPV/HPyV infection was defined as the proportion of patients whose sample was positive for viral DNA (in the skin swabs and eyebrow hairs) or serum antibodies. Viral prevalence rates were calculated for all beta types tested and by species, type-specific prevalence rates are presented for the eyebrow hair and skin swab samples.

Results

Descriptive profile and generalizability of the cohort

Baseline demographics, skin cancer risk factors, and sun-protective behaviors of the VIRUSCAN Study participants are presented

Table 2. Demographic characteristics and skin cancer risk factors and protective behaviors in the VIRUSCAN study, Tampa, FL, 2014–2017 and the southern U.S. population.

Demographic and skin cancer risk factors	VIRUSCAN (N = 1,179) n (%)	Southern NHIS 2015[c] (N = 25,149,699) n (%)		P[d]
Age in years				
Mean (SD)	69.0 (6.3)	70.2 (7.6)		<0.001
Gender				
Female	627 (53.2)	14,073,280 (56.0)		0.055
Male	552 (46.8)	11,076,419 (44.0)		
Race				
White	1,132 (96.1)	20,421,079 (81.2)		<0.001
Others	46 (3.9)	4,728,620 (18.8)		
Ethnicity				
Non-Hispanic	1,115 (94.6)	22,748,364 (90.5)		<0.001
Hispanic or Latino	64 (5.4)	2,401,335 (9.5)		
Sunburn in the past 12 months				
No	1,061 (92.5)	20,241,154 (86.1)		<0.001
Yes	86 (7.5)	3,273,041 (13.9)		
History of any cancer				
No	565 (49.1)	19,269,413 (76.8)		<0.001
Yes	585 (50.9)	5,807,869 (23.2)		
History of skin cancer at study enrollment				
No known skin cancer	687 (59.7)	5,264,232 (90.7)		<0.001
Any type of skin cancer	463 (40.3)	536,843 (9.3)		
Smoking status				
Never smoked	586 (51.0)	13,307,427 (53.1)		<0.001[e]
Former smoker	524 (45.6)	9,180,053 (36.6)		
Current smoker	38 (3.3)	2,563,205 (10.2)		
Reaction to 1-hour sun exposure				
No change	48 (4.2)	3,430,354 (17.9)		<0.001[e]
Tans without sunburn	234 (20.4)	4,556,119 (23.8)		
Mild sunburn with tan[a]	488 (42.6)	4,898,328 (25.6)		
Sunburn without blisters	315 (27.5)	4,198,736 (21.9)		
Blistering sunburn	60 (5.2)	2,052,810 (10.7)		
Ever used a sun lamp or tanning bed				
No	905 (78.8)	1,943,216 (97.2)		<0.001[e]
Yes	243 (21.2)	55,992 (2.8)		
Sunscreen use[b]				
Always	109 (10.5)	4,295,888 (21.2)		<0.001[f]
Often	265 (25.5)	2,155,292 (10.7)		
Sometimes	316 (30.4)	3,180,984 (15.7)		
Rarely	221 (21.3)	2,022,689 (10.0)		
Never	127 (12.2)	8,566,555 (42.4)		
Wore a hat[b]				
Always	245 (23.6)	3,617,098 (17.9)		<0.001[f]
Often	282 (27.1)	1,974,467 (9.8)		
Sometimes	255 (24.5)	2,965,504 (14.7)		
Rarely	141 (13.6)	1,949,292 (9.7)		
Never	116 (11.2)	9,650,956 (47.9)		
Stayed in shade[b]				
Always	109 (10.5)	4,303,200 (21.3)		<0.001[f]
Often	464 (44.7)	6,050,002 (29.9)		
Sometimes	356 (34.3)	6,119,738 (30.2)		
Rarely	81 (7.8)	2,011,929 (9.9)		
Never	27 (2.6)	1,762,869 (8.7)		
Wore protective clothes[b]		Wore long sleeves	Wore long pants	
Always	83 (8.0)	2,660,356 (13.2)	5,605,566 (27.8)	
Often	248 (23.8)	1,537,657 (7.6)	2,545,902 (12.6)	
Sometimes	339 (32.6)	3,404,590 (16.9)	3,666,794 (18.2)	
Rarely	262 (25.2)	2,506,741 (12.4)	1,893,874 (9.4)	
Never	109 (10.5)	10,027,229 (49.8)	6,475,233 (32.1)	

Abbreviations: NHIS, National Health Interview Survey; VIRUSCAN, Viruses in Skin Cancer.

[a]The corresponding category for the NHIS data is as follows: burn mildly with some or no tanning.

[b]The sun exposure time frame differed between the VIRUSCAN questionnaire (if outside in sun for 15 minutes or more) and the NHIS questionnaire (if outside in sun for 1 hour or more) for the sun protection variables.

[c]The NHIS dataset was restricted to adults ages ≥60 years and weighted using the "sample weight" variable.

[d]P values were calculated using χ^2 tests without correction for continuity and confirmed by conditional exact tests.

[e]The following variables were tested using a pooled in symbol test from 5 imputed datasets, including smoke status, reaction to 1 hour sun exposure, and ever use a sun lamp or tanning bed.

[f]The following variables were tested using the Cochran–Armitage trend test: sunscreen use, wore a hat, and stayed in shade.
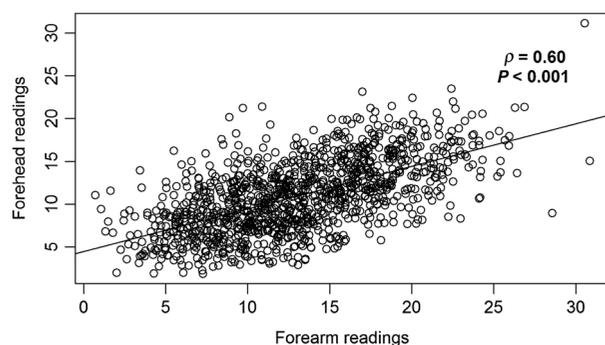
Figure 2.
Correlation between forearm and forehead spectrophotometer readings in the VIRUSCAN Study, Tampa, FL, 2014–2017. A strong positive correlation was observed between the baseline forearm and forehead spectrophotometer readings. ρ and P values were calculated using Spearman correlation.

in **Table 2**, compared with those of the general Southern United States population as measured by the NHIS population. VIRUSCAN Study participants were more likely to have sun-sensitive skin than the NHIS population. For example, 4.2% of VIRUSCAN participants reported no change to their skin after 1 hour sun exposure as compared with 17.9% of the NHIS population (**Table 2**). The VIRUSCAN Study participants were also more likely to report skin cancer risk factors such as use of tanning beds, a history of skin cancer and former smoking. Interestingly, 66.4% and 75.2% of VIRUSCAN Study participants reported use (always/often/sometimes) of sunscreen

Table 3. Correlation between forearm spectrophotometer readings and past-week measures of sun exposure and protection among a subsample of VIRUSCAN study participants ($n = 159$), Tampa, FL, 2014–2017.

| Questionnaire measurement of exposure/protection | Forearm spectrophotometer readings[a] | | |
	Low (≤11.55) n (%)	High (>11.55) n (%)	OR (95% CI)[b]
Exposure scale			
T1	45 (56.2)	34 (43.0)	1.00 (Reference)
T2	22 (27.5)	21 (26.6)	1.03 (0.45–2.32)
T3	13 (16.2)	24 (30.4)	2.48 (1.01–6.33)
			P_{trend}[c] = 0.06
Protection scale			
T1	29 (36.2)	30 (38.0)	1.00 (Reference)
T2	26 (32.5)	26 (32.9)	0.97 (0.42–2.24)
T3	25 (31.2)	23 (29.1)	0.76 (0.32–1.75)
			P_{trend} = 0.54
Ratio of exposure/protection scales			
T1	37 (46.8)	23 (29.1)	1.00 (Reference)
T2	26 (32.9)	24 (30.4)	1.14 (0.49–2.61)
T3	16 (20.3)	32 (40.5)	2.89 (1.24–6.95)
			P_{trend} = 0.01

[a]Forearm spectrophotometer readings were dichotomized based on the median value.
[b]OR and 95% confidence interval were calculated using logistic regression, adjusted for age and gender.
[c]The median score/ratio of each group was used to test the trend between score and spectrophotometer readings, using logistic regression adjusted for age and gender.

and hats as compared with 47.6% and 42.4% of the NHIS population, respectively.

Spectrophotometer readings of recent UVR exposure

A strong, intraindividual correlation was observed between the spectrophotometer readings obtained at the forearm and forehead (**Fig. 2**; $\rho = 0.61$; $P < 0.001$). Further analysis of baseline skin pigmentation readings revealed greater variation in the forearm spectrophotometer readings (median = 12.63, IQR = 7.46) as compared with the forehead readings (median = 10.76, IQR = 6.03). On the basis of past-week exposure, individuals in the highest tertile of self-reported sun exposure were 2.5 times more likely to have a higher than median forearm spectrophotometer readings compared with those in the lowest tertile of sun exposure (**Table 3**; ORs 2.48, 95% CI: 1.01–6.33, $P_{\text{trend}} = 0.06$), after adjustment for age and sex. Similarly, when a ratio of the summary measures of sun exposure and sun-protective behaviors was considered, a positive trend was observed between tertiles of the ratio and high versus low forearm spectrophotometer readings (**Table 3**; OR $_{\text{tertile 3 vs. 1}} = 2.89$, 95% CI: 1.24–6.95, $P_{\text{trend}} = 0.01$). No associations were observed with the forehead spectrophotometer readings (Supplementary Table S2).

### Baseline prevalence of cutaneous viral infections

On the basis of the completed analysis of baseline samples obtained from the 370 VIRUSCAN Study participants enrolled in the first year of the study who were eligible for follow-up, the prevalence of cuHPV and HPyV was consistently higher in skin swabs than eyebrow hairs, with strong virus-type specific correlations across eyebrow hairs and skin swabs in terms of prevalence, degree of infection and number of types (52). When expanding the analysis to include baseline samples obtained from all VIRUSCAN Study participants eligible for follow-up, similar findings were observed, with higher cuHPV and HPyV prevalence in skin swabs compared with eyebrow hairs and strong type-specific viral prevalence across both sample types (**Fig. 3**). For example, DNA corresponding to at least one β-HPV type was detected in the skin swabs of 95.6% of participants and in the eyebrow hairs of 64.4% of participants, with the same trends observed for β-species 1 and 2 (80.5% and 41.2% vs. 90.1% and 53.0%, respectively). Similarly, the γ-HPV prevalence was also higher in the skin swabs (75.6%) than in the eyebrow hairs (29.7%). HPyV prevalence rates followed the same pattern with 82.1% of swabs showing infection and only 35.8% of eyebrow hairs. The type-specific cuHPV and HPyV prevalence rates for VIRUSCAN Study participants are presented in Supplementary Table S3. β-HPV types with the highest prevalence rates in both the skin swab and eyebrow hair were types 5 (36.6% and 14.3%), 38 (47.4% and 18.4%), and 110 (35.7% and 13.3%), with types 4 (18% and 3.1%), 50 (15.3% and 3.5%), and SD2 (19.3% and 4.1%) being the most prevalent γ-HPV types (**Fig. 3**). In addition, among the subset of cuHPV/HPyV types tested in serology, the seroprevalence was 73.6% for any β-HPV, 57.3% for any γ-HPV and 98.6% for any HPyV.

Discussion

The VIRUSCAN Study is the first prospective cohort study to assess the association between cutaneous viral infections and keratinocyte carcinoma using multiple, repeated markers of viral infection and quantitative measures of UVR exposure. In this article, we present detailed information on the design, methods and baseline characteristics of the VIRUSCAN Study. VIRUSCAN Study participants are at higher risk for skin cancer than the general population, but tend to be

Figure 3.
Cutaneous human papillomavirus (HPV) prevalence in skin swabs and eyebrow hairs among VIRUSCAN Study participants, Tampa, FL, 2014–2017. The scatterplots display the correlation between type-specific HPV viral prevalence in skin swabs (x-axis) and eyebrow hairs (y-axis). The numbers within the rectangles indicate the HPV type tested for both β-HPV types (**A**) and γ-HPV types (**B**).
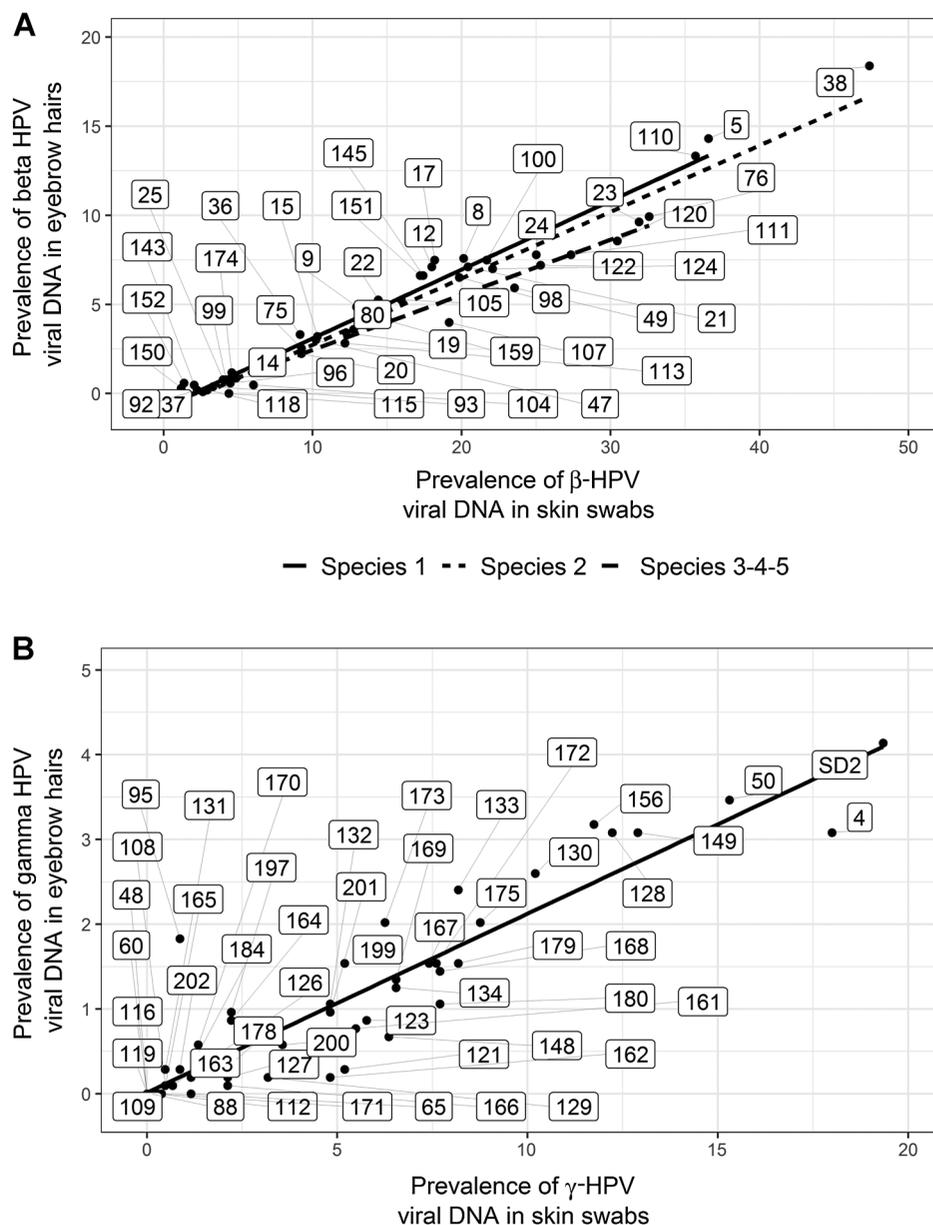
more conscious of skin health, engaging in sun protection and undergoing routine skin cancer screening exams. Therefore, this population is ideal for assessing the VIRUSCAN Study aims as we expect to observe a large number of incident keratinocyte carcinoma cases throughout the follow-up period.

The spectrophotometer-based skin pigmentation readings obtained at two anatomic sites (forehead and forearm) were highly correlated, although forearm readings varied more than forehead readings, perhaps reflecting differences in sun-protective behaviors, as 50.7% of participants used hats always/often to protect their forehead but only 31.8% of participants used long sleeves always/often to protect their forearms (**Table 2**). Measuring exposure at the forearm maybe a better surrogate for overall sun exposure than forehead measurements, further evidenced by the positive associations observed between forearm spectrophotometer measurements and self-reported recent sun exposure that were not observed for forehead spectrophotometer

measurements. Taken together, these results confirm that spectrophotometer-based measurements of skin pigmentation are objective measures that may be useful for tracking an individual's recent sun exposure over time in relation to other time-dependent biomarkers of disease risk and may be useful in future epidemiologic studies seeking to incorporate recent measures of UVR exposure.

A high prevalence of cutaneous viral infections was observed in both skin swabs and eyebrow hairs obtained from VIRUSCAN Study participants, consistent with previous epidemiologic studies of cutaneous viral infections (22, 27). Interestingly, regardless of the cuHPV species, viral prevalence was consistently higher in skin swabs than in eyebrow hairs, with HPyV types following this same pattern. We also observed a strong correlation between type-specific infections across skin swabs and eyebrow hairs. These full baseline viral prevalence results confirm the findings previously reported among a subset of VIRUSCAN Study participants enrolled in year one in which

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prevalence, as well as number of types and degree of infection, were correlated across skin swabs and eyebrow hairs (52).

The VIRUSCAN Study has some methodologic limitations. The study population is a select group, comprised of older adults residing in a geographic region with high ambient UVR exposure, engaging in sun protective behaviors more often than the general population. Therefore, the generalizability of study findings may be limited. However, if UVR is a necessary cofactor in virus-associated skin carcinogenesis, then examining virus-associated keratinocyte carcinoma risk in populations with higher UVR exposure maximizes the likelihood of observing such associations. Furthermore, should cutaneous viral infections be associated with keratinocyte carcinoma in the VIRUSCAN Study population, then future studies could examine similar associations in populations with lower UVR exposures. Importantly, the VIRUSCAN Study participants would be an ideal target population in which to test novel skin cancer prevention strategies, due to their high skin cancer risk profile and motivation to decrease their risk.

To minimize participant burden, blood samples were only collected at baseline. However, baseline seroreactivity will provide information about past viral infections, while the DNA-based markers of infection in eyebrow hairs and skin swabs will provide insight on current infection. The tumor samples collected were typically small in size since they are screen-detected cancers. However, β-HPV types have been shown to be more prevalent in earlier stages of skin carcinogenesis (61) and therefore, the viral DNA detection may be more feasible in smaller tumors compared with more advanced, larger tumors. We were unable to collect tissue samples from premalignant lesions such as actinic keratosis because the standard treatment protocol at the University of South Florida Dermatology Clinic is cryodestruction. However, incident actinic keratosis lesions are documented throughout follow-up, thus enabling the assessment of actinic keratosis risk associated with baseline viral infections. Finally, while VIRUSCAN Study participants were only followed for up to four years; longer term follow-up may be possible with additional grant funding.

A major strength of the VIRUSCAN Study is the incorporation of repeated measures of UV exposure and cutaneous viral infections that will be used to assess temporal patterns of exposure and their associations with incident keratinocyte carcinomas, as well as acquisition of new viral infection. In addition, the longitudinal biospecimens were tested for over 100 cutaneous virus types, facilitating the investigation of keratinocyte carcinoma risk associated with persistence of viral infections, an aspect of HPV infection known to be associated with increased risk of other cancers (62). Furthermore, the banked samples will facilitate multi-disciplinary research involving measures of immune function and subsequent cancer development. Finally, our subsample validation study indicates that the spectrophotometer readings yield an objective and quantifiable measure of recent UVR exposure which will be used in the VIRUSCAN study to assess the possible interaction between UVR exposure and cuHPV/HPyV infection in relation to keratinocyte carcinoma risk.

While the VIRUSCAN Study is ongoing, the longitudinal results will provide critical temporal evidence as to the role of cutaneous viral infections in the development of keratinocyte carcinomas, as well as the potential interaction between viral infections and UVR. These findings will inform future keratinocyte carcinoma prevention efforts, such as the development of a vaccine which could serve as a primary prevention strategy among individuals at high risk for keratinocyte carcinomas.

Disclosure of Potential Conflicts of Interest

V.K. Sondak is a consultant for Regeneron, Merck, Bristol-Myers Squibb, Novartis, Array, Genentech/Roche, and Replimune and is a member of the data monitoring committee for Pfizer. A.R. Giuliano is an advisory board member for and reports receiving a commercial research grant from Merck & Co., Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Pawlita, D.E. Rollison
Development of methodology: J.L. Messina, T. Waterboer, M. Pawlita, T. Gheit, D.E. Rollison
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.A. Fenske, B.S. Cherpelis, L. Vijayan, J. Balliu, J.L. Messina, V.K. Sondak, T. Waterboer, M. Pawlita, T. Gheit, D.E. Rollison
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Zhao, J. Balliu, V.K. Sondak, A.R. Giuliano, M. Pawlita, M. Tommasino, D.E. Rollison
Writing, review, and/or revision of the manuscript: R.P. Amorrortu, B.S. Cherpelis, L. Vijayan, Y. Zhao, J.L. Messina, V.K. Sondak, A.R. Giuliano, T. Waterboer, T. Gheit, M. Tommasino, D.E. Rollison
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.P. Amorrortu, L. Vijayan, Y. Zhao, J. Balliu, V.K. Sondak, D.E. Rollison
Study supervision: R.P. Amorrortu, M. Pawlita, D.E. Rollison

Acknowledgments

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Rossybelle P. Amorrortu, Neil A. Fenske, Basil S. Cherpelis, et al.

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