

Chemoprevention Agents for Melanoma: A Path Forward Into Phase 3 Clinical Trials

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Recent progress in the treatment of advanced melanoma has led to unprecedented improvements in overall survival and, as these new melanoma treatments have been developed and deployed in the clinic, much has been learned about the natural history of the disease. Now is the time to apply that knowledge toward the design and clinical evaluation of new chemoprevention agents. Melanoma chemoprevention has the potential to reduce dramatically both the morbidity and the high costs associated with treating patients who have metastatic disease. In this work, scientific and clinical melanoma experts from the national Melanoma Prevention Working Group, composed of National Cancer Trials Network investigators, discuss research aimed at discovering and developing (or repurposing) drugs and natural products for the prevention of melanoma and propose an updated pipeline for translating the most promising agents into the clinic. The mechanism of action, preclinical data, epidemiological evidence, and results from available clinical trials are discussed for each class of compounds. Selected keratinocyte carcinoma chemoprevention studies also are considered, and a rationale for their inclusion is presented. These data are summarized in a table that lists the type and level of evidence available for each class of agents. Also included in the discussion is an assessment of additional research necessary and the likelihood that a given compound may be a suitable candidate for a phase 3 clinical trial within the next 5 years. **Cancer** 2019;125:18-44. © 2018 American Cancer Society.

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INTRODUCTION

Chemoprevention of cutaneous melanoma (CM) involves the use of a naturally occurring or synthetic agent to reduce risk for disease. Interventions can be made at different stages of carcinogenesis. Primary chemoprevention refers to inhibiting the formation or facilitating the repair of mutagenic molecular species in normal tissue. The objective of secondary prevention is to intervene in the progression of premalignant cells by slowing, blocking, or reversing their conversion to melanoma, whereas tertiary prevention refers to preventing melanoma recurrence in patients with treated disease.^{1,2} This work is focused on primary and secondary chemoprevention agents, although studies in animal or other models of advanced melanoma are included when they are relevant to safety. (see *Precis on the Melanoma Prevention Working Group*)

Malignant tumors develop through a multistep process that includes initiation, promotion, and progression.² Initiation occurs when mutations arise in otherwise normal cells. Many mutations occur because of faulty repair of DNA damage caused by exposure to carcinogens. In the case of melanoma, ultraviolet (UV) radiation (UVR), from both the sun and indoor tanning beds, is the most common carcinogen. Tumor promotion involves the accumulation of additional mutations and often occurs over many years.^{3,4} UVR also is involved in the promotion of melanoma. Progression refers to the final development of a tumor with invasive potential, which also may involve the acquisition of new mutations, epigenetic modifications, and loss of immune control of early oncogenic cellular changes. Potential chemoprevention agents *must* be evaluated at each step of tumor development, because an agent may exhibit inhibitory effects in the early stages of tumorigenesis but cancer-promoting effects in later stages.⁵⁻⁹

The potential mechanisms of action for melanoma chemoprevention agents are complex (Fig. 1) and include photoprotection, antioxidant activity, anti-inflammatory

effects, promotion of apoptosis, suppression of proliferation and angiogenesis, immunomodulatory effects, and promotion of DNA damage repair.¹⁰ In this work, we highlight the mechanisms of action for chemoprevention agents that have significant *in vivo* preclinical, epidemiologic, or clinical evidence for the prevention of UVR-induced DNA skin damage, tumor formation, or tumor growth in melanoma or keratinocyte carcinoma (KC) (including basal cell carcinoma [BCC] and squamous cell carcinoma [SCC]). Additional cohort studies are summarized in Supporting Table 1.

The inclusion of data from UVR-induced KC models is based on the shared etiologies and environmental risk factors of melanocytic and keratinocytic malignancies. UVR acts as a complete carcinogen in mouse models of KC, and individuals who have genetic defects in global genome repair. For example, patients with xeroderma pigmentosum (XP), have dramatically elevated rates of both melanoma (2000-fold) and KC (10,000-fold) originating from unrepaired, UV-induced DNA damage.¹¹ Although there are differences in biology that are reflected in the greater increase in risk for KC than for CM among patients who have XP, because these skin cancers have risk factors in common and are initiated and promoted by the same carcinogen, we propose that agents that decrease KC development should be considered as candidate melanoma prevention agents. The formation of keratinocyte tumors is commonly associated with UV-induced mutagenesis and immune suppression, and agents that decrease KC development could be considered as candidate melanoma prevention agents. We limit our discussion herein to studies that used malignant tumor formation as an endpoint rather than focusing on treatments aimed at reducing existing actinic damage or actinic keratoses (AKs).

An ideal melanoma chemoprevention agent not only would reduce melanoma risk but also would be safe,

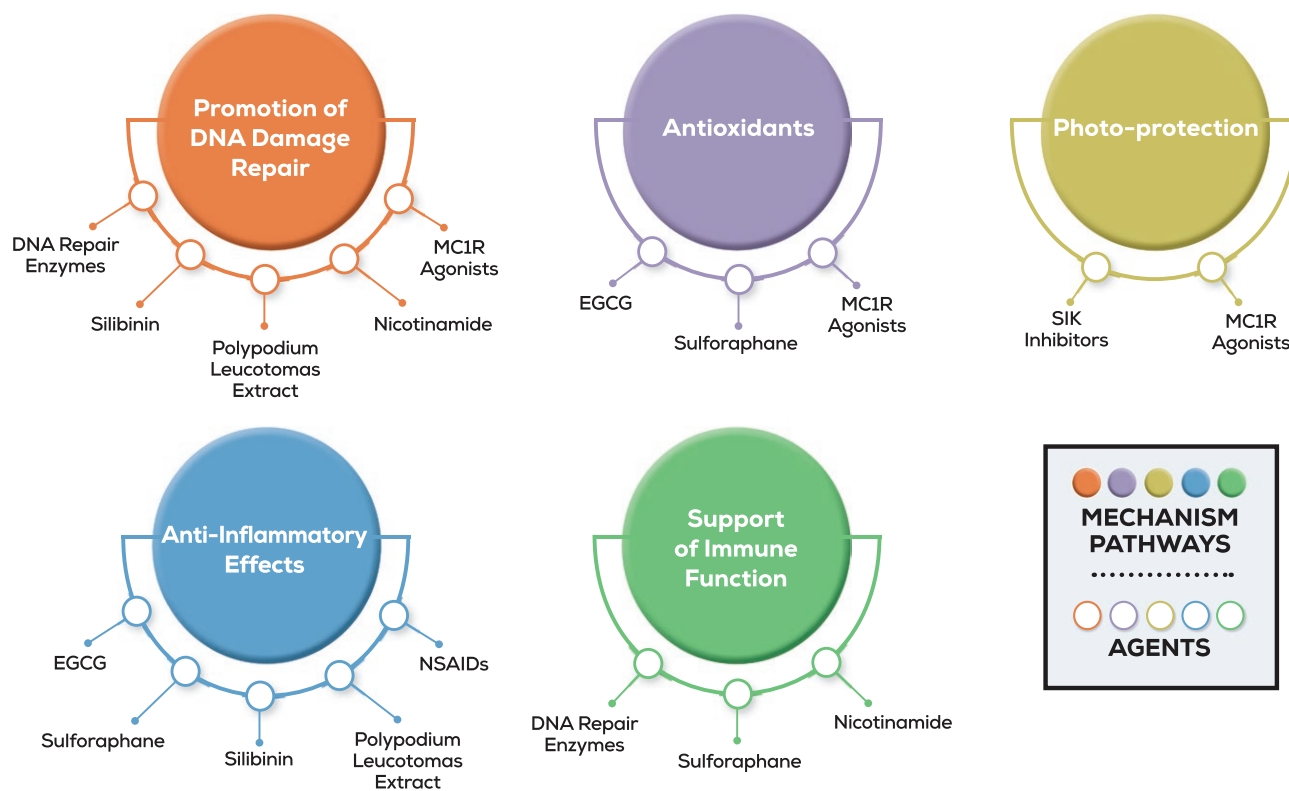


Figure 1. Modes of action of candidate chemoprevention of melanoma include photoprotection, promotion of DNA damage repair, reduction of metabolic and redox stress by antioxidants, anti-inflammatory effects, and support of immune function. Promising agents and target areas of impact for cancer prevention are shown. EGCG indicates epigallocatechin-3-gallate; MC1R, melanocortin 1 receptor; NSAIDs, nonsteroidal anti-inflammatory drugs; SIK, salt-inducible kinase.

cost effective, well tolerated, easy to use, and available in a standardized form.^{5-9,12} Defining a target population at risk for melanoma is important to maximize the population benefit of the intervention while reducing the risk of overtreatment. Considerations of melanoma biology, along with the mechanism of action of the chemoprevention agent, will inform the optimum age for an at-risk patient to begin melanoma chemoprevention. Finally, the success of a chemoprevention strategy ultimately would be gauged by the reduction in the incidence of invasive melanoma over the long term.

It is important to highlight 2 differences in the levels of evidence reported in the human epidemiologic and interventional studies included here. The highest level comes from studies that were specifically designed to assess the impact of a chemopreventive agent or intervention on CM (or KC). Lower levels of evidence are reported by post hoc analyses in which melanoma was a secondary endpoint of the study. The reason why it is important to make this distinction is that ad hoc study design and data analyses often lack considerations of many

of the variables that are pertinent to the establishment of an association with melanoma (eg, detailed history of sun exposure, pigmented phenotype, occupational and recreational UV exposure, temporal association with diagnosis, and the dose and schedule of administration of the agent). Taking these limitations into consideration, and in an effort to present a thorough review of the literature while being as concise as possible, we limit our discussion here to interventional studies in which CM or KC was the primary endpoint; observational studies that interrogated endpoints pertinent to the agents discussed are listed in Supporting Table 1.

Because melanoma has low incidence rates in the general population and often has long latency, early phase clinical trials cannot rely on tumor incidence as an endpoint. Consequently, biomarkers associated with melanoma initiation and/or progression, as well as the biologic activities of the agents, are necessary for clinical evaluations of the effectiveness of candidate agents and strategies.¹² Biomarker discovery often begins with in vitro cell culture studies; however, the sheer number of putative

melanoma chemoprevention agents described in the literature precludes consideration of each of those studies here. Therefore, discussion in this work is limited to those studies performed with human cell lines and to agents for which there is some indication of efficacy *in vivo* (for a summary of the development pipeline, see Fig. 2).

The objective of this work is to inform clinical and translational researchers about agents that have been evaluated in models relevant to melanoma prevention. The database at clinicaltrials.gov (National Institutes of Health, Bethesda, MD; accessed March 2, 2018 and September 9, 2018) also was interrogated, and ongoing studies of each agent are presented in Table 1. This synthesis of information (Table 2) provides the skin cancer prevention community with the tools to understand the potential applications of agents under development and to move forward in the translational research pipeline those agents with the highest potential to have an impact on the risk for melanoma. We begin the discussion with the standard of care: sunscreens.

SUNSCREENS

Exposure to solar UVR is the major environmental risk factor for melanoma; consequently, the gold standard for melanoma prevention is avoidance and/or minimization of exposure by wearing protective clothing and using sunscreen. Organic sunscreen ingredients act by absorbing UVR and converting energy to heat, whereas mineral sunscreens provide a physical barrier to UVR. Both act by preventing UV-induced DNA damage and immune suppression. The composition and efficacies of specific sunscreens have been discussed elsewhere.¹³ Studies done in mouse models have produced conclusive evidence of the benefit of sunscreen use for the prevention of melanoma. Three transgenic mouse studies demonstrated that the application of sunscreen to animals before UV irradiation significantly delayed the appearance of melanocytic tumors. These models included 1 in which mouse tissues overexpressed the melanocyte growth factor hepatocyte growth factor/scatter factor¹⁴; another in which a mutant B-raf proto-oncogene, serine/threonine kinase (*BRAF*) gene with a valine-to-glutamic acid substitution at position 600 (*BRAF*^{V600E}) is expressed specifically in melanocytes¹⁵; and a third in which melanocytes express an activated neuroblastoma RAS proto-oncogene guanosine triphosphatase (*NRAS*) glutamine-to-lysine substitution at position 91 (*NRAS*^{Q91K}).¹⁶

One randomized clinical trial presents evidence that routine, daily sunscreen use prevents melanoma.

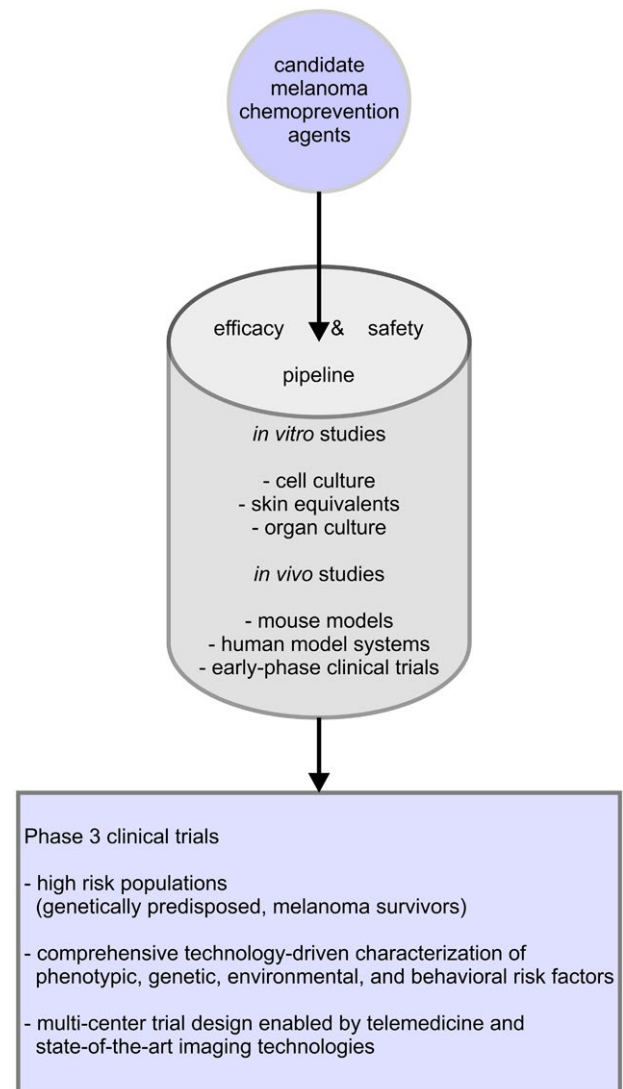


Figure 2. The pipeline for developing candidate agents is illustrated.

That Australian study of 1621 participants who were randomized to daily versus discretionary sunscreen (*broad spectrum* sun protection factor [SPF] 16) to the head and arms for a 4-year period (1992-1996) demonstrated a 50% reduction in melanoma at all body sites 10 years after the intervention (hazard ratio [HR], 0.50; 95% confidence interval [CI], 0.24-1.02; $P = .051$),¹⁷ with a 73% reduction in the risk of invasive CM (3 participants in the daily use group vs 11 in the discretionary use group; HR, 0.27; 95% CI, 0.08-0.97). The risk of melanoma *in situ* also was reduced, but the difference was not significant (HR, 0.73; 95% CI, 0.29-1.81). A more recent

TABLE 1. Summary of Ongoing Trials of Potential Melanoma Chemoprevention Agents From ClinicalTrials.Gov^a

Agent	Clinical Trial	Purpose	Primary Outcome Metrics
Sunscreen	NCT026668536	Evaluate durability, safety, and SPF characterization of bioadhesive nanoparticle encapsulated sunscreen	Determine MED, skin examinations to assess skin irritation, inflammation, and follicular occlusion
MC1R agonist	None pending	—	—
Salt-inducible kinase inhibitors	None pending	—	—
T4 endonuclease	NCT03224715	Investigate effects of T4 endonuclease treatment before treatment for actinic cheilitis	Blinded evaluation of photographs by dermatologists for partial or complete clearance
Vitamin A	None pending	—	—
Vitamin E	NCT00392561	Evaluate effects of vitamin E and selenium on preventing non-melanoma skin cancers	Incidence of skin cancer during a 6-y study period; secondary outcome metrics: occurrence of mortality and incidence of diabetes during study period
Vitamin D	NCT01748448	Investigate effects of vitamin D 100,000 IU/mo after surgery of first cutaneous malignant melanoma in patients with stage 1B-3 disease	Relapse-free survival; secondary endpoint 25-hydroxyvitamin D3 serum levels at diagnosis and at 6-mo intervals
Nicotinamide	NCT00301067	Evaluate the effects of using calcitriol to sensitize metastatic melanoma tumor cells to treatment with temozolomide	MTD of calcitriol, toxicity of treatment regimen with temozolomide and high-dose calcitriol; secondary outcome metrics: tumor response and time to progression, relation between vitamin-D receptor variants and tumor response
Selenium	NCT00392561	Evaluate effects of vitamin E and selenium on preventing non-melanoma skin cancers in Bangladesh	Incidence of skin cancer during a 6-y study period
Acetylsalicylic acid, NSAIDs	None pending	—	—
Statins	None pending	—	—
NAC	None pending	—	—
DFMO	NCT02636569	Assess effects of topical DFMO and diclofenac on reversing specific biomarkers in nonmelanoma skin cancer	Reduction in biomarkers associated with DFMO treatment; secondary outcome: determine whether individuals treated with diclofenac with or without DFMO have fewer AKs than placebo-treated individuals

(Continued)

TABLE 1. (Continued)

Agent	Clinical Trial	Purpose	Primary Outcome Metrics
EGCG	NCT02029352	Evaluate the effects of topical EGCG in patients with superficial BCCs	Percentage of patients with complete histologic clearance; secondary outcome metrics: no. of patient applications compared with prescribed applications, no. of local skin reactions or adverse events
Resveratrol	NCT02760160	Investigate effects of reconstituted grape powder on production of biomarkers for nonmelanoma skin cancer in response to UV	Changes in MED from baseline; secondary outcome: histologic changes in selected biomarkers and assessment of apoptosis
SFN	NCT03126539	Investigate effects of topical SFN on skin fragility associated with aging and UV exposure	Gene expression and histologic changes in keratins 16 and 17 in the basal epidermis
Lycopene/bixin	NCT03289832	Assess the effects of oral SFN and curcumin on skin exposed to UV	Changes in UV-induced erythema
PLE	None pending NCT02813902	— Evaluate efficacy, tolerability, and toxicity of PLE for the prevention of AK and keratinocytes in high-risk skin cancer populations	— Incidence of new, clinically visible AKs; secondary outcome metrics: histologic presence of UV-induced CPDs, solar elastosis, and sunburn cells
Silibinin	None pending	—	—

Abbreviations: AK, actinic keratoses; BCCs, basal cell carcinomas; CPDs, cyclobutane pyrimidine dimers; DFMO, difluoromethylornithine; EGCG, epigallocatechin-3-gallate; MCT1R, melanocortin 1 receptor; MED, minimal erythmal dose; MTD, maximum tolerated dose; NAC, N-acetylcysteine; NCT, National Clinical Trials identifier (clinicaltrials.gov); NSAIDs, nonsteroidal anti-inflammatory drugs; PLE, *Polypodium leucotomas* extract; SFN, sulforaphane; SPF, sun protection factor; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; UV, ultraviolet. The agent name and the terms "melanoma" and "skin cancer" were used to query the database on March 2, 2018, updated September 9, 2018.

TABLE 2. Summary of Evidence and Potential for Advancement Into Clinical Trials for Melanoma Prevention for Melanoma Prevention Agents With Scoring Matrix from 1-3, With 1 Being the Highest Level of Evidence

Agent	Preclinical Evidence ^a	Clinical Evidence ^b	Adverse Effects, Limitations	Potential for Clinical Impact in the Near Term, Next Steps, and/or Pending Trials
Sunscreens	1- Decreases DNA damage and delays tumor formation in 3 UV-induced mouse melanoma models	1- Reduced melanoma risk in interventional study 2- Reduced melanoma risk for sunscreen users in prospective cohort study	Skin irritation; efficacy can be limited by improper application	CURRENT STANDARD OF CARE: New formulations aimed at stabilizing active ingredients are being tested
MC1R agonists	2- α -MSH analogs stimulate pigment synthesis, protect against UV-induced DNA damage	1- Subcutaneous injection induces tanning and provides photoprotection in individuals with fair skin; NDP-MSH approved for use in humans in Europe	Significant side effects of analogs currently available for systemic administration humans include nausea, flushing, and loss of appetite; case reports of eruptive nevi in patients using unlicensed agents	MODERATE: Next-generation analogs have potential for greater selectivity and activity as topical agents; should be tested in a mouse model of melanoma
Salt-inducible kinase inhibitors	1- Increases pigmentation in transgenic mice 3- Increases pigmentation in melanocytes and human skin explants	None	None reported	MODERATE: Pigmentation effects do not require functional MC1R; should be tested in a mouse model of melanoma
DNA repair enzymes	2- Prevents UV-induced KC 3- Increases removal of UV-damaged DNA bases in keratinocytes	1- Reduces the no. of AKs and BCCs in high-risk patients	None reported	MODERATE-HIGH-Requires testing in a model of UV-induced melanoma
Vitamin A	3- Some growth inhibition, some growth promotion reported in human melanoma cell lines	1- Some benefit in patients with melanocytic lesions; one study reports benefit and two do not 2- One study reports benefit, 2 studies do not	Oral β -carotene increases lung cancer risk in smokers; topical vitamin A causes skin irritation; oral retinoids are teratogenic and cause liver and lipid abnormalities	LOW: No pending trials
Vitamin E	2- Topical vitamin E plus vitamin C reduces erythema and CPDs in UV-irradiated pig skin	1- Decreased MMP expression after topical treatment; topical vitamin E plus vitamin C plus ferulic acid reduces erythema, CPDs TP53, and cytokines in UV-irradiated skin	Oral vitamin E increases risk for prostate cancer; some indication of increased melanoma cell motility in vitro	LOW-MODERATE: Topical treatment in combination with vitamin C could be useful but requires testing in UV-induced carcinogenesis models for safety and efficacy

(Continued)

TABLE 2. (Continued)

Agent	Preclinical Evidence ^a	Clinical Evidence ^b	Adverse Effects, Limitations	Potential for Clinical Impact in the Near Term, Next Steps, and/or Pending Trials
Vitamin E, continued	3- Antioxidant effects in cell culture; an analog increases motility and invasion of melanoma cells in vitro	1- 50,000 IU biweekly × 9 wk modulated biomarker levels, but variability was high; 200K IU after UVR reduced skin inflammation 2- No effect of 400 IU plus calcium on melanoma risk 3- No effect of serum vitamin D on risk	None identified	LOW-MODERATE: Requires testing in a model of UV-induced melanoma; more clinical research into genetic determinants of response is needed
Vitamin D	3- Growth inhibition in human melanoma cell lines			
Nicotinamide	3- Enhances DNA damage repair in primary human melanocytes, ex vivo skin; inhibits proliferation but enhances invasiveness in melanoma cells	1- Nicotinamide 500 mg twice daily reduces risk for KC, decreases UV-induced immune suppression, decreases KC in transplantation recipients	Possible increased aggressive KC in human studies; increased invasiveness of melanoma cells in culture	MODERATE-HIGH: Should be examined in mouse model of UV-induced melanoma, followed by human clinical trial if safe and efficacious
Selenium	1- Topical selenomethionine delays onset of UV-induced melanoma; treatment of existing tumors increases growth rate	1- Oral selenomethionine increases risk for KC in some studies	Increases risk for KC in humans	VERY LOW: Supplementation likely important only in individuals who are nutritionally deficient
Acetylsalicylic acid and NSAIDS	2- Dramatic decrease in UV-induced NMSC in mice treated with low-dose sulindac; PGE ₂ levels correlate with efficacy 3- Decreased proliferation in human melanoma cells	1- Oral sulindac delivered to cutaneous nevi increases cleaved caspase-3 in atypical nevi; oral celecoxib reduces risk of KC ^c 2- Mixed results; no benefit for low-dose acetylsalicylic acid 3- Modest risk reduction for acetylsalicylic acid users in some studies	Oral NSAIDs have potential adverse GI and cardiac side effects	MODERATE-HIGH: Requires testing in a mouse model of UV-induced melanoma; clinical trials for effects of sulindac and other NSAIDS (topicals as well, especially sulindac) on UV-induced inflammation, skin cell proliferation, and PGE ₂ production should be performed
Statins	3- Beneficial effects in cell culture require dose that might be higher than that achieved clinically for cardiovascular disease risk reduction	1- Lovastatin treatment did not affect clinical or histologic features in patients with atypical nevi 2- Results are mixed	Low	LOW: No pending trials

(Continued)

TABLE 2. (Continued)

Agent	Preclinical Evidence ^a	Clinical Evidence ^b	Adverse Effects, Limitations	Potential for Clinical Impact in the Near Term, Next Steps, and/or Pending Trials
NAC	<p>1- Oral NAC before UV delays tumor appearance in mouse melanoma model; chronic NAC increases lymph node metastasis in BRAF^{V600E} mouse</p> <p>2- Topical NAC prevents NMSC in UV-induced mouse model</p>	<p>1- In phase 1 trial of nevi irradiated ex vivo, NAC relieved UV-induced glutathione depletion; phase 2 trial with in vivo irradiation failed to demonstrate modulation of study endpoints in treatment arm</p>	<p>Chronic treatment causes lung cancer progression in a mouse model</p>	<p>LOW: No pending clinical trials</p>
DFMO	<p>2- Oral and topical DFMO decrease NMSC in UV-induced mouse model</p> <p>3- Combination with γ-interferon arrests growth of melanoma cells</p>	<p>1- Placebo-controlled phase 3 trial demonstrated decrease in BCCs; phase 1 trial produced decrease in AKs with topical application; phase 2b trial with or without diclofenac (topical) failed to decrease polyamine levels</p>	<p>Oral DFMO associated with hearing loss; topical formulations can be effective without systemic exposure</p>	<p>LOW-MODERATE: Requires testing in a mouse model of melanoma; failure of phase 2b KC trial, which may have to do with topical formulation, must be addressed</p>
EGCG	<p>2- EGCG nanoparticles decrease xenograft tumor growth; oral and topical treatments prevent NMSC</p> <p>3- Cell growth inhibited in cell culture models</p>	<p>1- Two studies demonstrate that topical EGCG decreases UV-induced erythema</p> <p>2- Cohort studies of green tea consumption inconclusive</p>	<p>None reported</p>	<p>HIGH: Requires testing in a mouse model of UV-induced melanoma; topical EGCG preparation (Veregen) is approved for use in humans; phase 2 clinical trial has been completed for BCC treatment, no results yet published</p>
Resveratrol	<p>1- Inhibits growth of melanoma cells</p> <p>2- Topical resveratrol decreases KC and acute effects of UV; analog (pterostilbene) applied topically also decreases KC</p>	<p>None</p>	<p>None reported</p>	<p>LOW-MODERATE: Clinical trials examining effects of oral grape powder on UV-irradiated skin now recruiting; requires testing in a model of UV-induced melanoma</p>
SFN	<p>2- Oral and topical SFN prevents NMSC</p> <p>3- Inhibits growth of melanoma cells</p>	<p>1- Topical SFN decreases UV-induced erythema</p>	<p>None reported</p>	<p>MODERATE: Many clinical trials planned or not yet published; requires testing in a mouse model of UV-induced melanoma</p>

(Continued)

TABLE 2. (Continued)

Agent	Preclinical Evidence ^a	Clinical Evidence ^b	Adverse Effects, Limitations	Potential for Clinical Impact in the Near Term, Next Steps, and/or Pending Trials
Lycopene and bixin	<p>2- Lycopene metabolite (bixin)-treated mice had significantly decreased UV-induced oxidative DNA damage and inflammation</p> <p>3- Bixin upregulates antioxidant systems in keratinocytes</p>	<p>1- Lycopene from tomato supplements ameliorates markers of UV damage, including erythema, increased levels of mitochondrial DNA damage, and MMP-1 expression and reduces dermal fibrillin-1 levels</p>	<p>No reported adverse effect, although bixin doses used in mouse studies are high relative to the ADI for humans</p>	<p>LOW-MODERATE: Have not yet been examined in any skin cancer model; requires testing in a mouse model of melanoma</p>
PLES	<p>2- Inhibits NMSC in UV-irradiated mice</p> <p>3- Prevents UV-induced apoptosis of keratinocytes</p>	<p>1- Significant increase in MED among patients who have high-risk melanoma treated with Fernblock; a second study reports significant reductions in histologic markers of UV damage</p>	<p>None reported</p>	<p>MODERATE: Active ingredients are 1% (w/w) of extract (for discussion, see Conclusions); one clinical trial for the prevention of AKs and SCC is pending</p>
Silibinin	<p>1- Prevents growth of melanoma in mouse xenograft.</p> <p>2- Suppresses UV-induced KC in hairless mouse</p>	<p>None</p>	<p>Potential phototoxicity of minor component observed in cell culture</p>	<p>MODERATE-HIGH: Possibly available as well characterized cosmeceutical; requires testing in a mouse model of melanoma</p>

Abbreviations: ADI, acceptable daily intake; AKs, actinic keratoses; α -MSH, α -melanocyte-stimulating hormone; BCCs, basal cell carcinomas; BRAF^{V600E}, B-raf proto-oncogene, serine/threonine kinase (BRAF) gene valine-to-glutamic acid substitution at position 600; CPDs, cyclobutane pyrimidine dimers; DFMO, difluoromethylornithine; ECGC, epigallocatechin-3-gallate; GI, gastrointestinal; KC, keratinocyte carcinoma; MCT1R, melanocortin 1 receptor; MED, minimal erythema dose; MMP, matrix metalloproteinase; NAC, N-acetylcysteine; NDP-MSH, tridecapeptide α -melanocyte-stimulating hormone; NMSC, non-melanoma skin cancer; NSAIDs, nonsteroidal anti-inflammatory drugs; TP53, tumor protein 53; PGE₂, prostaglandin E₂; PLEs, *Polypodium leucotomas* extracts; SCC, squamous cell carcinoma; SFN, sulforaphane; UV, ultraviolet; UVR, ultraviolet radiation; w/w, weight/weight.

^aFor preclinical evidence, ratings are as follows, with 1 being the highest level of evidence: 1- mouse models of melanoma, or UV-irradiated pig skin; 2- mouse models of UV-induced KC and other non-melanoma endpoints; 3- cell culture and ex vivo skin culture studies.

^bFor clinical evidence, ratings are as follows, with 1 being the highest level of evidence: 1- interventional studies with a melanoma-relaxant endpoint; 2- cohort studies; 3- case-control studies. This was a trial for women only, and the primary outcome was a new invasive cancer diagnosis at any site except KC. Secondary endpoints were lung, colorectal, and breast cancers.

prospective cohort study of 143,844 Norwegian women indicated that the use of SPF >15 sunscreen by women aged 40 to 75 years potentially could reduce their melanoma incidence by 18% (95% CI, 4%-30%), despite reports by sunscreen users of more sunburns, more sunbathing vacations, and more indoor tanning bed use than never users.¹⁸

The US Food and Drug Administration has determined that broad-spectrum sunscreens can help reduce the risk of sun-induced skin cancer and premature skin aging when used as directed with other sun-protective measures.¹⁹ For individuals who spend time outdoors, the American Academy of Dermatology recommends daily application of a sunscreen that: 1) offers broad-spectrum protection (ie, absorbs in both the UVA and UVB regions of the solar spectrum), 2) has an SPF of at least 30, and 3) is water-resistant.²⁰

Melanocortin 1 Receptor Agonists

α -Melanocyte-stimulating hormone (α -MSH) is a melanocortin derived from the precursor polypeptide proopiomelanocortin, which is produced in the pituitary gland and by UV-irradiated keratinocytes in the skin. α -MSH binds to and activates the melanocortin 1 receptors (MC1Rs) located on the plasma membrane of melanocytes.²¹ There are 3 forms of MSH, α -MSH, β -MSH, and γ -MSH, which bind with different affinities to MC1Rs. α -MSH is a full agonist of the human MC1R. MC1R is polymorphic in human populations and is a determinant of hair and skin color as well as the risk for melanoma. MC1R activation by α -MSH produced in keratinocytes results in the stimulation of photoprotective eumelanin (brown-black pigment) synthesis in melanocytes. Exogenous delivery of α -MSH also can elicit tanning of the skin through the activation of MC1R. Therefore, α -MSH and its analogs have the potential to prevent both KC and melanoma by increasing photoprotective pigmentation in the skin. The best characterized synthetic α -MSH analog is the tridecapeptide (Nle,⁴D-Phe⁷) α -MSH (NDP-MSH), which differs from natural α -MSH by 2 amino acid substitutions.²² NDP-MSH and other tripeptide and tetrapeptide analogs of α -MSH are potent agonists of the MC1R in cultured human melanocytes that have wild-type MC1R but are not active (ie, they do not increase melanin synthesis or DNA damage repair) in melanocytes that harbor MC1R variants associated with red hair.²³ Given these in vitro data, it would seem reasonable to predict that non-Hispanic white individuals with red hair, 80% of whom harbor loss-of-function mutations in *MC1R*, would not tan when an MC1R

agonist is administered. However, there are reports that fair-skinned patients and those who are carriers of red hair color alleles of *MC1R* have a greater response to subcutaneous injections of NDP-MSH, as measured by changes in melanin density, than patients who have skin phototype III or greater and/or wild-type *MC1R*.²⁴ The reasons for the lack of concordance of in vitro and in vivo analyses of NDP-MSH are not clear and could have to do with the complex genetic and environmental factors that affect human pigmentation.

A randomized controlled trial of 28 white men who received 10 subcutaneous injections of either NDP-MSH or saline over 12 days demonstrated that NDP-MSH reliably tanned the skin, with the peak effect occurring 1 to 3 weeks after treatment.²⁵ However, the side effects of NDP-MSH (which are attributed to nonselective binding to other melanocortin receptors in tissues other than the skin) include nausea, flushing, and loss of appetite. A subsequent larger randomized controlled trial of 79 male and female patients who received subcutaneous injections of NDP-MSH demonstrated that melanin levels were increased by 41%; and, after receipt of 3 minimal erythema doses (MEDs) of UVR, epidermal sunburn cell formation was decreased by 50% in patients who had Fitzpatrick skin phototypes I and II.²⁶ Nausea was again noted as a common side effect, occurring in 85% of patients, as was flushing, which occurred in 74%. NDP-MSH, also called afamelanotide, is now marketed under the brand name SCENESSE by Clinuvel Pharmaceuticals (Melbourne, Victoria, Australia). In Europe, it is approved for the treatment of erythropoietic protoporphyria.²⁷ NDP-MSH also has been tested in patients with vitiligo, and more repigmentation was observed in those who received NDP-MSH monthly for 4 months after UVB radiation treatment compared with those who received UVB radiation alone.²⁸

Recently, it was demonstrated that analogs of γ -MSH, which had 16-fold selectivity for MC1R compared with other melanocortin receptors, induced rapid (1 minute) and reversible (1 day) pigmentation after intraperitoneal injection using the *Anolis carolinensis* lizard model of cutaneous pigmentation.²⁹ The development of more selective α -MSH analogs with the potential for topical administration is ongoing.²³ An α -MSH analog with increased specificity for the MC1R that can be delivered topically would be more convenient for patients than a drug administered by injection and has the potential for a decreased side-effect profile. Additional reports of side effects include patients who have presented with eruptive formation of nevi after using unlicensed melanotropic

peptides sold on the Internet under the names Melanotan I and II.³⁰ Finally, some studies conclude that the pro-oxidant properties of melanin could contribute to risk for melanoma; therefore, agents that increase pigmentation should be studied carefully for safety before use in patients who are at risk for melanoma.³¹

Salt-Inducible Kinase Inhibitors

Salt-inducible kinase (SIK) inhibitors act by increasing photoprotective cutaneous pigmentation. They do so by upregulating expression of the microphthalmia-associated transcription factor (MITF), which is the master regulator of pigment gene expression. The activity of MITF is positively regulated by signaling downstream of MC1R, which, in turn, is activated by α -MSH produced by UV-irradiated keratinocytes.³² Consequently, individuals who have loss-of-function mutations in *MC1R* often are unable to tan after exposure to UV light. SIK is a negative regulator of the cyclic-adenosine monophosphate (cAMP)-responsive element-binding protein (CREB)-regulated transcription coactivator, which enables activation of the transcription factor CREB, which is required for MITF expression in melanocytes. Mice harboring loss-of-function mutations in *Mc1r* have yellow hair; knockout of *Sik2* in this background results in animals with brown hair.³³ Mujahid and colleagues recently demonstrated that small-molecule inhibitors of SIK upregulate the CREB-MITF axis and induce melanin production in normal human melanocytes, melanoma cells, and transgenic mice without the need to activate *Mc1r*.³² Significant increases in epidermal pigmentation also were observed in human skin explants treated topically with SIK inhibitors. These compounds have the potential to prevent both KC and melanoma by increasing photoprotective pigmentation in the skin, even in individuals who cannot tan after exposure to UVR. No studies have been conducted in humans with this agent to date, and none are listed as pending on clinicaltrials.gov, although clinical development is being pursued (David E. Fisher, unpublished data).

DNA Repair Enzymes

Although it has been reported that human melanocytes possess a mechanism (nucleotide excision repair [NER]) for the repair of UV-induced DNA damage, mutagenesis still occurs when damaged DNA is replicated before this repair pathway can be activated. In melanocytes, NER is regulated by signaling downstream of both MC1R and endothelin receptors.³⁴ The efficiency of NER can

be significantly impacted by *MC1R* polymorphisms that are common among non-Hispanic whites with red hair. Although human cells have all the enzymes necessary to complete an alternative repair pathway (base excision repair), they lack a DNA glycosylase that can initiate base excision repair of dipyrimidine photoproducts by detecting and enzymatically removing damaged bases. Two groups have reported the topical delivery of liposome-encapsulated DNA glycosylases, derived from a prokaryote,³⁵ a virus,³⁶ and a yeast,³⁷ that are capable of both delivering this enzymatic activity and preventing SCC in mouse models. One of these products contains the bacterial T4 endonuclease (T4N5). This T4N5 formulation reportedly reduced DNA damage and epidermal proliferation after neonatal UVR treatment in a mouse melanoma model in which both alleles of the cyclin-dependent kinase 4 (*Cdk4*) contain the activating UV-signature arginine-to-cysteine substitution at position 24 (R24C), and melanocytes constitutively express activated *Nras*^{Q61R}. However, treatment with the endonuclease had no effect on penetrance or age of the mice at onset of melanoma.³⁸ The authors suggest that the melanoma-promoting effects of UVR in neonatal mice may not involve dipyrimidine photoproducts and that the melanocytes in their mouse model already may contain all of the UV-signature mutations (ie, *Cdk4*^{R24C}) necessary to drive tumorigenesis. Given the efficacy in UVR-induced KC models, we believe that it would be worthwhile to test DNA repair enzymes of this class using other models in which UVR induces melanoma in adult animals that harbor mutations in a single oncogene (see Evaluating Efficacy in Mouse Models, below).

A liposomal formulation of T4N5 also significantly decreased AKs in patients with XP.³⁵ The annualized rate of new AKs was 8.2 among patients assigned to T4N5 liposome lotion and 25.9 among those assigned to placebo (difference, 17.7; 95% CI, 11.8-26.5; $P = .004$). There was also a 30% reduction in new BCC among the patients who used T4N5 ($P = .006$). A recent study randomly assigned 15 patients who had AKs on their face or scalp to receive either topical DNA repair enzyme lotion or placebo.³⁹ There was a 46.6% reduction in AKs in the group that used the DNA repair enzyme lotion compared with a 32.7% decrease in the placebo group. Twelve weeks after the cessation of treatment, there was an additional 29.2% decrease in the number of AKs in the DNA repair enzyme-treated group, whereas those in the placebo group had a 31.4% increase in AKs ($P = .0026$).

Vitamins and Minerals

Vitamin A/retinoids

Exposure to vitamin A activates the nuclear retinoid acid receptor alpha (RARA) and retinoid X receptor alpha (RXRA) (for review, see Chhabra et al).⁴⁰ Preclinical studies of vitamin A and its precursors (retinol and the carotenoid provitamins for vitamin A, including β -carotene) for melanoma chemoprevention have demonstrated both growth-inhibiting and growth-promoting effects on human cell lines. These studies are discussed by Mounessa et al.⁴¹

Multiple case-control studies have been conducted to evaluate associations between vitamin A and the risk of melanoma. Analyses have assessed intake from food and supplements as well as total intake. The impacts of individual components within the vitamin A group also were determined. Overall, the results from those case studies have been mixed. Two of the larger studies demonstrated an inverse relation between vitamin A intake and melanoma risk, with up to a 54% reduction in risk, whereas the largest study reported no association (see Supporting Table 1, articles in the class *vitamin A* with a VIT_A prefix).

Two cohort studies also have produced conflicting results regarding vitamin A. In the Vitamins and Lifestyle (VITAL) cohort, individuals who received retinol supplements had a decreased risk of melanoma (HR, 0.60; 95% CI, 0.41-0.90); however, dietary or total intake of vitamin A or carotenoids was not associated with the risk of melanoma.⁴² Another study in this same cohort demonstrated no effect of β -carotene supplements on the risk of melanoma.^{43,44} Prospective data from the Nurses' Health Study also demonstrated no effect of vitamin A intake on melanoma incidence for total or dietary retinol or β -carotene.⁴⁵ The only group that had an inverse association between total retinol intake and melanoma risk was composed of women who were otherwise at low risk for melanoma at baseline, as determined by nondietary factors.

A meta-analysis of β -carotene supplementation and cancer risk included results from 9 randomized clinical trials.⁴⁵ Of these, 2 included data on melanoma incidence. The Women's Health Study reported no impact (relative risk [RR]) of 0.90 (95% CI, 0.49-1.68) for β -carotene use.⁴⁵ For the SU.VI.MAX (Supplementation en Vitamines et Mineraux Antioxydants) study, the results varied according to sex: men had an insignificantly decreased risk (RR, 0.49; 95% CI, 0.12-1.97), whereas the RR for women was elevated (RR, 4.31; 95% CI, 1.23-15.13).⁴⁶ However, it must be emphasized that other supplements in addition to β -carotene were included in the interventional arm of that study.

Several clinical trials of topical tretinoin (all-trans retinoic acid) in patients with melanocytic nevi have reported histologic and clinical *improvement* of dysplastic nevi and regression or the disappearance of benign nevi. Details of these studies are discussed in a report by Mounessa et al.⁴¹ Oral isotretinoin (13-cis-retinoic acid) also has been investigated in patients with dysplastic nevi, but no clinical or histologic benefit was evident.⁴⁷ Oral retinoids have significant side effects, including teratogenicity, dyslipidemias, and liver abnormalities.⁴⁸

With regard to dysplastic nevi and biomarkers of melanoma prevention, we note that individuals who have multiple dysplastic nevi are at elevated risk for melanoma,⁴⁹ and some melanomas are associated with melanocytic nevi (including acquired, congenital, and dysplastic nevi),^{50,51} but a significant portion of melanomas arise de novo.^{52,53} The role of dysplasia in nevi as a biomarker of efficacy for chemoprevention agents is not well defined. Pathologic assessments of dysplasia are subjective, and inter-rater reliability for dysplasia scoring is low.⁵⁴ Alternative molecular biomarkers of the effects of a therapeutic agent on dysplastic nevi, such as the ratio of phosphorylated signal transducer and activator of transcription 1 (STAT1) to phosphorylated STAT3 (which had a significant association with the degree of atypia),⁵⁵ could be used. However, each nevus on a patient is unique and has its own potential for tumorigenesis. In light of this finding, it appears that the ideal solution for monitoring treatment effects in dysplastic nevi likely will involve noninvasive methods, such as confocal microscopy,⁵⁶ which will be used to assess the evolution of molecular and structural features in individual lesions.

Vitamin E

Preclinical models suggest that vitamin E and its analogs might be useful for preventing melanoma. Many of the observed effects are thought to be mediated by the strong antioxidant properties of vitamin E and its ability to quench free radicals and inhibit lipid peroxidation (reviewed by Chhabra et al).⁴⁰ However, recent data have demonstrated that (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; Sigma-Aldrich, St Louis, MO), a vitamin E analog, increased migration and invasion in human melanoma cell lines through effects on the glutathione system.⁵⁷ Topical solutions containing vitamins E (1% α -tocopherol) and C (15% L-ascorbic acid) decrease erythema and cyclobutane pyrimidine dimer (CPD) formation in pig skin irradiated with simulated solar radiation.⁵⁸

Case-control studies examining the effects of vitamin E (VE) on melanoma incidence have produced mixed results and are summarized in Supporting Table 1 (articles in the class *vitamin E* with a VIT_E prefix). In addition, prospective data from the Nurses' Health Study addressed this question but demonstrated that total and dietary vitamin E were not associated with melanoma risk (multivariate RR, 1.11 [95% CI, 0.66-1.85] and 0.88 [95% CI, 0.59-1.32], respectively).⁵⁹ Oral supplementation daily for 3 months with vitamins E and C (1000 IU and 2 g, respectively) protected the skin of participants from the effects of UVR. Treatment effects included an increase in the MED and decreased UV-induced DNA damage.⁶⁰ The SU.VI.MAX trial discussed above demonstrated an increased risk for women who consumed antioxidant supplements, including supplementation with vitamin E. In addition, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) indicated an increased risk for prostate cancer (HR, 1.17; 99% CI, 1.004-1.36; $P = .008$) in men who consumed oral vitamin E supplements (400 IU daily as racemic α -tocopheryl acetate).⁶¹ Topical vitamin E treatment 24 hours before experimental UV irradiation provides a potentially protective effect in significantly reducing expression of the matrix metalloproteinase MMP-12 in UV-treated human skin.⁶² In a study of adults with Fitzpatrick skin phototypes II or III, a topical formulation containing 1% α -tocopherol, 15% L-ascorbic acid, and 0.5% ferulic acid (a plant-derived phenolic structurally related to cinnamic acid) potently increased the antioxidant capacity of skin treated daily for 4 days at a dose of 2 mg/cm². Treated skin exposed to simulated solar radiation had reduced erythema, sunburn cells, CPDs, and tumor protein 53 (*TP53*) induction. Quantitative polymerase chain reaction analysis of biopsied skin also identified reduced levels of UV-induced cytokine formation in treated skin versus controls.⁶³

Vitamin D

It is believed that the antiproliferative effects of vitamin D on melanoma cells are mediated by the vitamin D receptor (VDR). Activities associated with the ligand-bound vitamin D receptor include heterodimerization with the RXR and subsequent activation of the retinoid pathway (for review, see Chhabra et al).⁴⁰

Case-control studies of vitamin D intake and melanoma incidence are summarized in Supporting Table 1 (articles in the class *vitamin D* with a VIT_D prefix). A prospective cohort study of more than 12,000 individuals in Denmark did not detect statistically significant associations between serum vitamin D levels or vitamin

D intake and melanoma incidence. A meta-analysis of 6 studies with 721 individuals demonstrated a weak association between dietary vitamin D and the development of CM (standardized RR, 0.92; 95% CI, 0.25-3.44).⁶⁴ The sensitivity analysis of this group included assessment without the inclusion of data from the report by Weinstock et al⁶⁵ because of a lack of data specific to dietary intake alone without supplementation; this adjustment yielded a standardized RR of 0.63 (95% CI, 0.42-0.94). Another meta-analysis demonstrated no significant association between serum vitamin D levels and melanoma risk or prognosis, although an inverse relation between serum vitamin D levels and melanoma thickness was reported.⁶⁶

The Women's Health Initiative randomized 36,828 postmenopausal women to receive low-dose (400 IU) vitamin D and 1000 mg calcium (CaD supplementation) daily versus placebo. This study originally was designed to test the hypotheses that dietary CaD supplementation would reduce hip fractures and colorectal cancer in postmenopausal women.⁶⁷ A post hoc analysis of skin cancer incidence in study participants indicated no statistically significant difference in the incidence of melanoma (HR, 0.86; 95% CI, 0.64-1.16). However, a subgroup analysis demonstrated that, among women who had a history of KC, melanoma incidence was decreased in the supplementation arm (HR, 0.43; 95% CI, 0.21-0.90).

A prospective clinical study enrolled 25 individuals with serum 25-hydroxyvitamin-D levels <30 ng/mL and skin photodamage to receive 50,000 IU of cholecalciferol biweekly for 8 to 9 weeks.⁶⁸ Although serum levels of vitamin D metabolites were significantly elevated, VDR expression in skin biopsies of participants revealed minimal changes after supplementation. The expression of cytochrome P450-24 (CYP24) (a known target of vitamin D in skin) in photodamaged and photoprotected skin was increased by 186% ($P = .08$) and 134% ($P = .07$), respectively, after supplementation. In benign nevi from 11 participants, elevated VDR and CYP24 expression was observed (average, 20% [$P = .08$] and 544% [$P = .09$], respectively). Expression levels of caspase-14 (a marker of keratinocyte differentiation) were significantly increased (49%; $P < .0001$) in the basal layer of photodamaged skin. The authors noted that there was significant variation in the range of VDR and CYP24 expression at baseline, and they suggest that future studies of vitamin D for skin cancer prevention might include genotyping of genes encoding these proteins, which could provide further information on the role of these potential confounders and identify those individuals who would be more

likely to benefit from oral supplementation. A recent study in patients who received from 1 to 3 times their MED of simulated solar radiation indicated that those who received a very high dose (200,000 IU) of vitamin D₃ after irradiation had significantly higher serum levels of vitamin D₃, increased levels of anti-inflammatory mediator arginase-1, and a sustained reduction in skin erythema that correlated with significant expression of the genes related to skin-barrier repair.⁶⁹

Nicotinamide (niacinamide)

Nicotinamide and nicotinic acid are the major members of the vitamin B3 group. Details of in vitro studies of nicotinamide in melanocytes, melanoma cell lines, and human skin explants are discussed in a review by Minocha et al.⁷⁰ Those studies have reported not only inhibitory effects of nicotinamide on cell proliferation and vascular mimicry but also enhancement of invasiveness in melanoma cells. Nicotinamide enhances the repair of both oxidative and UV-induced DNA damage in primary human melanocytes, and the addition of 50 μM nicotinamide to culture medium increased the rates of CPD repair and oxidative DNA damage repair in human skin explants.

Oral nicotinamide (1500 mg or 500 mg daily for 3 days) decreases UV-induced immune suppression in human skin irradiated in vivo (also discussed by Minocha et al).⁷⁰ A double-blind, randomized, phase 3 clinical trial evaluated the effects of nicotinamide on the incidence of KCs.⁷¹ The receipt of 500 mg oral nicotinamide twice daily for 12 months resulted in a 13% reduction in AKs ($P = .001$) and a 23% reduction in KCs ($P = .02$). It has been noted that the development of aggressive BCCs and SCCs increased, rather than decreased, in the nicotinamide group, although those increases were not statistically significant.⁷² A secondary analysis demonstrated that the incidence rates of melanoma and melanoma in situ were similar between the groups that received nicotinamide daily versus those who received placebo. However, because melanoma incidence was a secondary endpoint, and only 10 melanomas were diagnosed in study participants (vs 801 nonmelanoma skin cancers), the study was likely underpowered and the analysis period too short to detect a difference in incidence of melanoma if it did exist.⁷⁰ Another possibility, which could be investigated in mouse models, is that a higher dose of nicotinamide might be required for melanoma prevention.

Selenium

Selenium is a trace element present in seafood, meats, grains, and nuts (primarily Brazil nuts). In humans,

selenium deficiency can lead to impaired muscular, cardiac, and immune functions as well as elevated cancer risk (for review, see Roman et al).⁷³ Selenium is incorporated into 25 human selenoproteins (many of which have antioxidant functions) by the addition of the amino acid selenocysteine to a polypeptide chain as it is synthesized on the ribosome. The presence of a unique 3' element (selenocysteine insertion sequence [SECIS]) in selenoprotein RNAs changes the translation of the UGA (opal) codon from stop to selenocysteine. At supranutritional levels (>400 μg daily), selenium metabolites such as methyl selenol are produced. It has been observed that human melanoma cells are more sensitive to the growth inhibitory and proapoptotic effects of the methyl selenol produg methylseleninic acid compared with primary human melanocytes.⁵ Treatment with topical l-selenomethionine results in a significant delay in the time required for UV-induced tumor development in KC⁷⁴ and melanoma mouse models, but continued treatment increases the rate of growth of melanomas once tumors appear.⁶

Numerous studies have evaluated the association between melanoma and selenium in humans, with mixed results. Most of the case-control studies have been negative (see Supporting Table 1), although selenium levels were assessed in different tissues by the various studies (eg, serum vs toenail specimens). Several cohort studies also examined the question of a potential chemoprevention or causative effect of selenium in melanoma, also with various results. An Italian cohort with exposure to high levels of selenium in their tap water had a statistically significant, 3.9-fold increase in melanoma incidence compared with an unexposed cohort.⁷⁵ The Nurses' Health Study also demonstrated that individuals with the highest tertile of toenail selenium levels had an increased risk of melanoma, but this was not statistically significant (multivariate RR, 1.66; 95% CI, 0.71-3.85). However, in the VITAL cohort, individuals with the highest levels of selenium intake were not significantly less likely to develop melanoma (multivariate RR, 0.98; 95% CI, 0.69-1.41).^{43,76} In contrast, in a cohort of patients with melanoma (stage I, n = 81; stage II, n = 63; stage III, n = 56), low serum selenium levels were associated with worse outcomes at 2 years.⁷⁷

Two randomized clinical trials evaluated selenium for its effect on melanoma risk. The SU.VI.MAX trial involved a combination of supplements that included selenium; its results are summarized above in the Vitamin A/retinoids section. The Nutritional Prevention of Cancer Trial evaluated the administration of selenized yeast as a chemopreventive agent for KC.⁷⁷ That study is widely

cited for the finding of a reduced risk for prostate cancer in men, but the multivariate-adjusted HR for melanoma was not significant (HR, 1.18; 95% CI, 0.49-2.85), and the risks for SCC and total KC were *elevated* (SCC: HR, 1.25; 95% CI, 1.03-1.51; total KC: HR, 1.17; 95% CI, 1.02-1.34). A 2018 Cochrane review concluded that selenium supplementation is associated with an increase in risk for melanoma.⁷⁸

Medications Used for Other Indications

Aspirin and nonsteroidal anti-inflammatory drugs

It is believed that acetylsalicylic acid (aspirin) and other nonsteroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory mechanisms primarily by the inhibition of cyclooxygenases/prostaglandin-endoperoxide synthase (PTGS1/COX1) and/or PTGS2/COX2. The cyclooxygenases convert arachidonic acid to prostaglandin (PG) H₂ (PGH₂), which is then transformed by prostaglandin synthases to the D-series (PGD), E-series (PGE), and F-series (PGF) prostaglandins.⁷⁹ Two NSAIDs, celecoxib and indomethacin, have been identified that can reduce proliferation in human A375 melanoma cells, whereas others (aspirin and piroxicam) did not have these effects.⁸⁰ Those authors presented evidence in support of their hypothesis that the activity of NSAIDs was mediated by COX2 inhibition, resulting in decreased levels of PGE₂ and reduced production of interleukin 6 (a pleiotropic inflammatory cytokine often associated with protumoral effects and/or aggressive malignancies).⁸⁰ Other work suggests that quinone metabolites of aspirin are responsible for the deleterious effects in SK-Mel-28 melanoma cells through intracellular glutathione depletion, reactive oxygen species formation, and mitochondrial toxicity.⁸¹ Goulet and colleagues reported that COX2 expression was consistently observed in keratinocytes, dermal fibroblasts, and inflammatory cells in regions adjacent to benign nevi and primary CM, but not in the cutaneous pigmented lesions themselves. The same authors observed that COX2 expression was high in melanoma metastases.⁸² A recent publication by Mikulec et al⁸³ indicated a dramatic (94%) reduction in UV-induced KC in mice treated daily with low-dose sulindac (160 mg daily human equivalent dose) in their feed. More modest effects were observed in animals treated with other NSAIDs, including aspirin. Their report noted that chemoprevention efficacy of the different tested NSAIDs correlated significantly with UV-induced PGE₂ production and keratinocyte proliferation.

Supporting Table 1 (articles in the class *acetylsalicylic acid* with an ASA prefix) summarizes the case-control

studies that have investigated the association between aspirin/other NSAIDs and the risk of melanoma. Several prospective cohort studies of NSAID use have been conducted. For many of these studies, melanoma incidence was not the primary endpoint of the study; therefore, results may be subject to confounding.

The first study, which was published in 2007, reported no association of aspirin with melanoma risk among 69,810 men and 76,303 women who participated in the Cancer Prevention Study II Nutrition cohort (current aspirin users with >5 years' duration: RR, 1.15; 95% CI, 0.83-1.59).⁸⁴ Past users and current users with <5 years' duration also did not have a reduced melanoma incidence. A subsequent report assessed the association between the risk of melanoma and the use of NSAIDs in the VITAL cohort study and indicated no association between these medications and melanoma risk.⁸⁵ An analysis of data from the Nurses' Health Study demonstrated that regular aspirin users had an increased incidence of melanoma (adjusted RR, 1.32; 95% CI, 1.03-1.70), although this effect was not observed among past users, and a dose-response effect was not observed.⁸⁶ However, a subsequent analysis from the Women's Health Initiative indicated a 21% reduced incidence of melanoma in aspirin users (HR, 0.79; 95% CI, 0.63-0.98),⁸⁷ with longer duration of use associated with lower risk (HR, 0.70; 95% CI, 0.55-0.94 for ≥5 years of use). No protective effect was observed among users of nonaspirin NSAIDs, although there was infrequent use of nonaspirin NSAIDs.^{87,88} Meta-analyses of this association have yielded negative results. One report of 13 studies (6 case-control studies with 93,432 cases and 401,251 controls, 6 cohort studies that included 563,380 participants, and 1 randomized controlled trial of 39,876 participants) revealed a lack of effect (RR, 0.97; 95% CI, 0.90-10.4) for ever-users of any NSAID.⁸⁹ The results did not differ between aspirin and nonaspirin NSAID users; however, case-control studies did demonstrate a slightly decreased risk of melanoma for aspirin users (RR, 0.88; 95% CI, 0.80-0.96). Another pooled analysis of 10 studies involving 490,322 participants demonstrated no impact on melanoma incidence (aspirin: RR, 0.96; 95% CI, 0.89-1.03; nonaspirin NSAIDs: RR, 1.05; 95% CI, 0.96-1.14).⁹⁰ Subgroup analyses of cohort studies, high-intensity NSAID use, and long-term NSAID use also failed to demonstrate a protective effect; although, again, a slight risk reduction was reported among aspirin users in case-control studies (RR, 0.86; 95% CI, 0.80-0.93). A meta-analysis of aspirin-only users produced similar results (odds ratio, 0.96; 95% CI, 0.82-1.12).⁹¹

A clinical trial in 2005 demonstrated that oral celecoxib at a dose of 200 mg twice daily for 10 days was associated with a significant reduction in erythema in 6 of 12 participants after irradiation of the skin with twice the MED.⁹² Several additional studies have been conducted to determine whether COX2 inhibitors might be effective preventive agents for KCs,^{93,94} including 2 that reported the incidence of KC as an endpoint.^{95,96} Elmets et al reported a double-blind trial in which 240 participants who had 10 to 40 AKs at baseline and a prior histologic diagnosis of at least 1 AK or KC were randomized to receive either celecoxib (200 mg twice daily) or placebo. Participants received treatment for 9 months and were followed for an additional 2 months off medication. There was no effect of celecoxib on the incidence of AKs. However, there was a dramatic decrease in the incidence of KCs. At 11 months, there was a 58% reduction in KCs relative to the placebo group.⁹⁵ In a trial conducted with 60 patients who had basal cell nevus syndrome, a trend toward a reduction of BCC burden by oral celecoxib was observed after an analysis of results from all participants ($P = .069$) was reported. Subgroup analysis that considered only the 60% of patients with less severe disease (<15 BCCs at study entry) indicated that celecoxib significantly reduced the number and burden of BCCs: participants who received placebo had a 50% increase in BCC burden per year, whereas those in the celecoxib group had a 20% increase ($P_{\text{difference}} = .024$).⁹⁶

To date, no clinical trials have directly assessed the impact of using aspirin or other NSAIDs on melanoma incidence. However, there is a report from a phase 2 randomized, placebo-controlled trial of oral sulindac 150 mg twice daily for 8 weeks. In that study, the primary endpoints were levels of sulindac and its metabolites in skin and serum. The analysis indicated that sulindac sulfone is delivered to keratinocytes and melanocytes, whereas the parent sulfide was the major sulindac-derived species detected in serum. Secondary endpoint analysis identified increased expression of the apoptotic marker cleaved caspase-3 in atypical nevi after treatment with sulindac, suggesting a possible therapeutic effect.⁹⁷

Statins

Statins inhibit 3-hydroxy 3-methylglutaryl coenzyme A reductase, an enzyme in the cholesterol biosynthetic pathway upstream of the prenyltransferase substrates farnesyl and geranylgeranyl diphosphate. Proteins known to be activated by prenylation include the Ras and Rho families, Rac, Rab, cell division control protein 42 homolog

(Cdc42), and nuclear lamins.⁹⁸ Cell culture studies have demonstrated that statins induce caspase-dependent apoptosis in multiple human melanoma cell lines through the inhibition of protein geranylgeranylation and the induction of cell-cycle arrest.^{99,100} However, the concentrations of simvastatin (1-10 μM) that were necessary to achieve these effects were orders of magnitude higher than the peak plasma concentrations observed at the highest dose (40 mg daily) commonly used for the treatment of hypercholesterolemia.¹⁰¹

Case-control studies of the effects of statins on melanoma incidence are listed in Supporting Table 1 (articles in the class *statins* with an STAT prefix). Prospective cohort analyses also have been conducted. The prospective Cancer Prevention Study II Nutrition Cohort of over 133,000 participants indicated that the use of cholesterol-lowering drugs for ≥ 5 years was associated with a lower risk of melanoma (RR, 0.79; 95% CI, 0.66-0.96).¹⁰² That study included data for multiple classes of cholesterol-lowering medications, although statins were the predominant medication represented. Effects were also observed for melanoma risk in former users (RR, 0.64; 95% CI, 0.46-0.89) and current users <5 years (RR, 0.89; 95% CI, 0.75-1.06). However, an analysis of prospective data from the Women's Health Initiative indicated no effect of statin use on melanoma risk in statin users and nonusers (multivariable adjusted HR, 1.14; 95% CI, 0.91-1.43).¹⁰³ Meta-analyses addressing this question have primarily indicated a null result for the association of statins with melanoma incidence (Supporting Table 1, Article index STAT_2),¹⁰⁴⁻¹⁰⁸ although 1 reported that an increased risk of melanoma was associated with statin use (median RR, 1.5; range, 1.3-1.7).¹⁰⁹ Subgroup analysis of 1 of the earlier meta-analyses indicated that lovastatin might have a drug-specific effect (odds ratio, 0.52; 95% CI, 0.27-0.99), but no data have confirmed this result on subsequent meta-analyses.^{105,106} However, unlike the Cancer Prevention Study II Nutrition Cohort study, the median follow-up for many of these studies was <5 years.

Although no clinical trials to date have directly assessed the impact of using statins on melanoma incidence, Linden et al conducted a randomized, double-blinded, placebo-controlled, phase 2 trial of lovastatin in 80 individuals who had a history of at least 2 clinically atypical nevi.¹¹⁰ Participants in that trial who received lovastatin did not have significant changes in histopathologic atypia, clinical atypia, or the number of nevi, nor did their nevi exhibit any effects of biomarkers of proliferation or progression to malignant disease.

N-acetylcysteine

N-acetylcysteine (NAC) is a well characterized antioxidant that has several current uses in medicine, including the treatment of acetaminophen toxicity and lung disorders.¹¹¹ NAC is cell permeable and can be given orally or topically. In vivo, NAC is deacetylated to produce L-cysteine, which is then converted into the potent antioxidant glutathione.¹¹² In mice, NAC delays primary tumor development of UV-induced melanoma¹¹³ and KC.¹¹⁴

A phase 1 study of NAC as a melanoma chemoprevention agent produced encouraging results.¹¹⁵ An ex vivo model was used in which patients at increased risk for melanoma (many or atypical nevi, personal or family history of melanoma) had nevi removed before and 3 hours after a single 1200-mg oral dose of NAC. The nevi were UV-irradiated ex vivo using a radiation source that emitted primarily in the UVB region of the spectrum. Signs of oxidative stress were evident in nevi from 24 to 48 hours after irradiation. NAC protected against UV-induced oxidative stress in nevi from 50% of patients. NAC was well tolerated, but a subsequent placebo-controlled phase 2 clinical trial involving 100 participants failed to demonstrate any protection of nevi that were irradiated in vivo with simulated solar radiation in patients who consumed a single 1200-mg oral dose of NAC.¹¹⁶ Included in the report of the second study is a discussion of potential reasons for the disparate results obtained in the phase 1 and 2 trials.

Although some in vitro and in vivo studies of NAC for the prevention of skin cancers, including melanoma, have produced evidence of beneficial effects, 2 reports indicate that NAC treatment increased metastasis of existing melanoma tumors. Transgenic mice with melanocyte-specific expression of oncogenic BRAF and deletion of the tumor-suppressor phosphatase and tensin homolog (PTEN) treated chronically with NAC and a soluble vitamin E analog developed more lymph node metastases⁵⁷ than control animals, whereas neither antioxidant had an effect on the number of tumors. In immune-compromised mice implanted with human-derived melanoma xenografts, subcutaneous injection of NAC (200 mg/kg daily) increased visceral metastases¹¹⁷ in grafts of 3 different tumors. These deleterious effects have dampened the enthusiasm for pursuing NAC in human trials and highlight the importance of studying chemoprevention agents at all stages of carcinogenesis, because the effects of a given agent may differ according to where on this continuum an intervention is made.

Difluoromethylornithine

Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC) (the rate-limiting enzyme in the synthesis of polyamines) and has been studied in combination with the NSAID sulindac for the prevention of sporadic colon adenomas in humans.¹¹⁸ A recent metabolomics study of tissue from intestinal tumors of *Apc*^{Min} mice and human colorectal cancer cells, both treated with DFMO, revealed that inhibition of ODC is associated with reduced levels of folate-dependent metabolites, including S-adenosylmethionine (SAM) and thymidine. Because decarboxylated SAM is required for polyamine biosynthesis, the authors proposed that depletion of polyamine levels elicits a futile SAM consumption/regeneration cycle that limits the tetrahydrofolate cofactor available for thymidylate synthase, thereby diminishing thymidine pools and restricting tumor growth. ODC and polyamine production are induced by UV exposure in the skin,¹¹⁹ and both oral and topical administration of DFMO reduce the number of KC tumors in UV-induced mouse models. DFMO in combination with interferon- γ treatment causes growth arrest in human melanoma cell lines.¹²⁰

No clinical or epidemiologic studies of the effects of DFMO on melanoma in humans have been reported. A randomized, double-blinded, placebo-controlled phase 3 trial for the prevention of KC in patients with a history of the disease randomized 291 participants to receive either oral DFMO (500 mg/m² daily) or placebo.¹²¹ Participants were followed for 4 to 5 years. The investigators reported a nonsignificant reduction in the primary endpoint of new KC in the DFMO-treated arm (260 in the DFMO-treated arm vs 363 in the placebo arm; $P = .069$). Separate evaluations of BCC and SCC revealed very little difference in SCC between treatment groups but a significant difference in new BCCs (163 in the DFMO-treated arm vs 243 in the placebo arm; $P = .03$). Adverse events included a significantly greater average hearing loss for DFMO-treated participants versus placebo (4-dB loss in the DFMO-treated arm vs 2-dB loss in the placebo arm; $P = .003$). A phase 1 study of topical 10% DFMO administered twice daily demonstrated delivery to the skin, ODC inhibition, absence of systemic exposure, and a decrease in AKs.¹²² However, similar effects were not observed in a phase 2b study in which 156 individuals who had sun-damaged skin were randomized to receive DFMO, or diclofenac, or a combination of both topically twice daily for 90 days.⁹³ The phase 2b study indicated no difference in polyamine levels in

the skin or in the primary endpoint (karyometric average nuclear abnormality) between levels at baseline and at the end of the study for any treatment group. The authors suggested that low baseline polyamine levels among participants in this study, relative to earlier studies, may have explained the lack of an observed effect on ODC activity.

Phytochemicals

The phytochemicals discussed here are plant-derived compounds with bioactivity that may benefit health and play a role in cancer prevention. Several of these compounds have significant in vivo preclinical or clinical evidence of their potential for melanoma chemoprevention. Liu-Smith and Meyskens provide an excellent review that discusses the effects of plant-derived flavonoid *nutraceuticals* on pigmentation and potential use as melanoma prevention agents.¹²³ Studies of compounds that have been examined in in vivo and clinical studies are reviewed below.

Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG) is a flavonoid that is abundant in green tea; lower levels are present in black tea. The mechanisms by which EGCG protects against skin cancers are diverse and include promotion of cell-cycle arrest and apoptosis and inhibition of angiogenesis as well as anti-inflammatory, immunomodulatory, and anti-oxidant effects (reviewed in Chhabra et al).⁴⁰ Studies in mice have indicated that both topical and oral delivery of EGCG can confer protection against KC. The efficacy of orally administered EGCG is limited by low bioavailability, but it was recently demonstrated that nanoparticle encapsulation significantly increased potency in a human melanoma xenograft model.¹²⁴

Cohort studies evaluating the effectiveness of green tea in melanoma prevention have not been conclusive. A prospective cohort study of approximately 35,000 postmenopausal women in the Iowa Women's Health Study indicated a small decrease in the overall incidence of cancer with nonherbal tea consumption but no specific association for melanoma.¹²⁵ Green tea was not differentiated from black tea in that study. Wu et al¹²⁶ evaluated melanoma incidence in the Women's Health Initiative, a prospective observational study of a cohort of 66,484 postmenopausal women. Three hundred ninety-eight cases of melanoma were reported in the group, with an average follow-up of 7.7 years. Questionnaires regarding coffee and tea consumption and melanoma incidence were used, and all information was self-reported. Tea

consumption was not associated significantly with a risk of melanoma (HR, 1.03; 95% CI, 0.81-1.31).

Human studies have demonstrated that the topical application of EGCG decreased erythema after UVR exposure,^{127,128} but clinical trials with other melanoma-related endpoints have not been conducted to date. Sin catechins 10% ointment (Veregen; PharmaDerm, a division of Fougera Pharmaceuticals Inc, Princeton, NJ) contains a standardized extract of green tea leaves of the species *Camellia sinensis* with 85% to 95% (weight/weight) green tea polyphenols (primarily catechins). The most abundant catechin in Veregen is EGCG. Veregen is approved by the US Food and Drug Administration for the treatment of genital warts in adults. A phase 2 trial of Veregen for the treatment of BCC has been completed according to clinicaltrials.gov, but the results are not yet available (clinicaltrials.gov identifier NCT02029352).

Resveratrol

Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol commonly identified in berry juices and in red wine; its antioxidant and anticancer properties are widely reported in both the popular and scientific literature. Topical resveratrol blocks many of the deleterious effect of UVR, including COX2 expression, keratinocyte proliferation, and KC tumor formation in mouse models (reviewed by Chhabra et al).⁴⁰ However, the benefits of resveratrol in preclinical models have not yet been realized in clinical studies, likely because of its low oral bioavailability. Numerous nanoparticle strategies that address this problem have been reported. The naturally occurring compound pterostilbene, an analog of resveratrol in which metabolism is blocked by methylation of the 3-hydroxy and 5-hydroxy groups, also is present in berry juices and has been studied as a more bioavailable alternative to resveratrol. The treatment of hairless mice with topical pterostilbene resulted in a dramatic decrease in both UV-induced erythema and KC tumor formation.¹²⁹

Sulforaphane

Sulforaphane (SFN) is an isothiocyanate compound that is present in its glucoraphanin prodrug form in cruciferous vegetables such as broccoli, brussels sprouts, and cabbage. In vitro experiments have demonstrated that SFN can reduce the growth of melanoma cells through apoptosis and by altering the activity of chromatin-modifying enzymes.¹³⁰ It has been reported that both topical application of SFN¹³¹ and a diet of broccoli sprouts¹³² protect against the development of nonmelanoma skin cancer in

mice exposed to UVR. This effect is likely caused by activation of the transcription factor NF-E2-related factor 2 (Nrf2) and downstream antioxidant enzymes, as well as inhibition of the transcription factor activator protein 1 (AP-1).¹³³

Topical application of sulforaphane increases the expression of antioxidant genes and decreases the MED in UV-irradiated human skin.¹³⁴ In a recent phase 1 study, 17 patients who had at least 2 atypical nevi and a history of melanoma were randomly allocated to 50, 100, or 200 μmol oral sulforaphane daily for 28 days. Atypical nevi were photographed on days 1 and 28, and plasma and nevus samples were taken on days 1, 2, and 28. The study indicated that oral sulforaphane is well tolerated at daily doses up to 200 μmol and achieves dose-dependent levels in plasma and skin.¹³⁵

Lycopene and related carotenoids

Lycopene is a lipophilic C-40 carotenoid antioxidant present at high concentrations in tomatoes and other red fruits. It is an efficient singlet-oxygen quencher. Dietary supplementation in the form of tomato paste increases the concentration of lycopene in human skin.¹³⁶ In a recent study, 20 healthy women aged 21 to 47 years were randomized to consume either 55 g of tomato paste (16 mg lycopene) in olive oil or olive oil alone spread daily on bread for 12 weeks.¹³⁷ The analysis of UV-irradiated skin of the participants demonstrated that the tomato paste treatment was associated with decreases in UVR-induced erythema ($P = .03$) and matrix metalloproteinase-1 expression ($P = .01$). UV-induced decreases in dermal fibrillin-1 and increased mitochondrial DNA 3895-base pair deletions also were ameliorated in the tomato paste arm ($P = .03$ and $P = .01$, respectively).

Bixin is an apocarotenoid that is present in a US Food and Drug Administration-approved natural food colorant derived from the seeds of the achiote tree (*Bixa orellana*; a native to tropical America). Bixin is formed by the oxidative cleavage of lycopene. It is used worldwide as a dietary additive and cosmetic ingredient known as annatto. There is evidence from studies in transgenic mouse models of prostate cancer that metabolites similar to bixin are the molecular species responsible for the cancer preventive effects of lycopene.¹³⁸ Bixin has an excellent safety record and good systemic bioavailability when orally administered. A team from the Arizona Cancer Center recently reported that intraperitoneal injection of bixin activates the transcription factor Nrf2 and thereby induces an antioxidant response in a mouse model of UV-induced photodamage and inflammation. Bixin-treated

animals had significantly decreased UV-induced oxidative DNA damage and inflammation compared with control animals.¹³⁹ However, 1 potential issue is the relatively high daily dose used (equivalent to 16 mg/kg in humans or 1200 mg for a 160-pound human),¹⁴⁰ which is 33% higher than the acceptable daily intake recommended by the World Health Organization.¹³⁹

Polypodium leucotomas extracts

An extract of the fern *Polypodium leucotomas* (PLE) is reported to have antioxidant and anti-inflammatory properties. It has been investigated in a variety of dermatologic applications, including prevention of UVR exacerbations of polymorphous light eruption, porphyria, and other photodermatoses, and as an adjunctive treatment for patients with melasma and atopic dermatitis (reviewed by Parrado et al).¹⁴¹ A study in a mouse model of UV-induced KC found that a dose equivalent to 7.5 mg/kg daily delayed tumor appearance.¹⁴¹

In a clinical study, patients at high risk for melanoma or melanoma recurrence ($n = 61$; familial or multiple melanomas, sporadic melanoma, atypical mole syndrome) were exposed to UVB radiation with or without 1080 mg oral PLE (240 mg every 8 hours 1 day before treatment, then 360 mg 3 hours before UV treatment).¹⁴² The MED was determined before and after PLE treatment. Participants had significantly higher MEDs post-treatment (ie, their skin required a higher UV dose to induce redness) compared with pretreatment MEDs. In a recent study, Kohli et al reported on the clinical and histologic effects of oral PLE on irradiation with a combination of UVA/UVB and visible light.¹⁴³ On day 1, 22 patients (Fitzpatrick skin type I-III) underwent irradiation, and the MED determinations were made on day 2. Participants were then treated on day 3 with 240 mg PLE 2 hours before and 1 hour before irradiation, and the MED was determined on day 4. Biopsies were performed on untreated skin and irradiated skin. For 7 of 22 patients, PLE treatment increased the MED ($P > .05$), but histologic differences in irradiated skin before and after treatment were highly significant in all participants. Markers of UV-induced damage, including proliferating cell nuclear antigen, sunburn cells (eg, dyskeratotic keratinocytes), CPDs, cyclin D1, COX2, and Ki67, all were reduced by at least 75% (with the exception of CPDs, which were reduced by 32%).

The branded *P. leucotomas* extract Fernblock (Cantabria Labs, Cantabria, Spain) contains several phenolic compounds, including 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid,

p-coumaric acid, ferulic acid, 4-hydroxycinnamoyl-quinic acid, and 5 isomers of chlorogenic acid. These compounds make up only 1% (weight/weight) of the extract's dry weight.¹⁴¹ There is no description in the literature of the other components of this extract, nor are there any published studies that examine the effects of the phenolic components alone at the doses contained in the extract. This leaves unanswered the possibility that some other component that has not been assessed in the standardization process might be important for its biologic activities. Therefore, there is some question regarding whether the characterization and standardization of the extract has been sufficiently rigorous to warrant testing in clinical trials for melanoma prevention.

Silibinin

Milk thistle extract has been shown in many models to have anticancer activity; silibinin is the main bioactive flavonolignan present in this mixture (reviewed in Kumar et al).¹⁴⁴ Silibinin is reported to suppress growth of xenografted human melanoma cells by directly targeting the mitogen-activated protein kinase kinase (MEK) and ribosomal s6 kinase (RSK)-mediated signaling pathways. In a hairless mouse model, treatment with topical silibinin significantly reduced UV-induced skin cancer by a mechanism that depends in part on TP53.¹⁴⁵ The skin of animals treated with silibinin before UV irradiation had lower levels of CPDs and inflammation. That study was done with chemically pure silibinin. There is 1 report in the literature of potential phototoxicity of 1 of the minor components in milk thistle extract (2,3-dehydrosilybin).¹⁴⁶

THE MELANOMA CHEMOPREVENTION PIPELINE

A recent commentary by Meyskens et al highlights the obstacles and challenges that confront the field of cancer prevention¹⁴⁷ and voices frustration over the repeated failures to translate promising preclinical results into successful human clinical trials. In the domain of melanoma prevention, where the latency of the disease is long and the biology of precursor lesions is still incompletely understood, successful translation of preclinical results into early stage (phase 0 and 1) clinical trials will require the identification of robust biomarkers of efficacy. These candidate biomarkers must come from preclinical studies done in cell cultures, human skin equivalents, *ex vivo* human tissues, animal models, and human pilot studies. These models not only should generate a test of whether

or not the agent prevents melanoma but also should identify relevant biomarkers that are both indicative of agent delivery to the target tissue and unambiguously tied to the mechanism of action of melanoma prevention.

In Vitro Systems

For cell culture evaluations of efficacy of prevention agents in the initiation or promotion stages, normal human melanocytes or immortalized yet nontumorigenic, melanocyte-derived cell lines, such as PIG1 cells,¹⁴⁸ are appropriate, whereas cell lines derived from frankly malignant lesions (melanoma cell lines) are much less informative. Experiments that examine the effects of candidate agents on melanocytes can be done in monoculture, human skin equivalents, or perhaps (in the future) in induced pluripotent stem cell derivatives.^{32,36,149,150} Melanoma cell lines can be informative regarding the safety of prevention agents. Examples include the safety concerns raised for both NAC and vitamin E, both of which increased tumor cell motility and invasive capacity *in vitro* and *in vivo*.⁵⁷

Evaluating Efficacy in Mouse Models

Efficacy considerations include studies that used UVR-induced models of KC and melanoma. Although mouse skin exhibits key differences in melanocyte localization, in which interfollicular melanocytes are not maintained in adult mice, they nonetheless have been used very effectively to demonstrate the principles and mechanisms of skin carcinogenesis and the roles of UVA, UVB, and simulated solar radiation in contributing to cutaneous carcinogenesis. Moreover, mice have been used successfully to demonstrate the effectiveness of sunscreen at preventing sunburn and UV-induced mutations. Mouse models are a well established preclinical tool in which proof of principle for a therapeutic strategy may be established; consequently, wherever possible, we have referenced extant mouse model data supporting each possible therapeutic approach. If an agent prevents tumor formation in these UV-induced models, then it is indicative of the potential to prevent melanoma, because both melanocytes and keratinocytes (and their microenvironments) are affected by UVR at both the initiation and promotion stages. However, before an agent is deployed in clinical trials for melanoma prevention, that agent should be tested in a mouse melanoma model that recapitulates as faithfully as possible the development of human melanoma so that a biomarkers specific to melanoma and the mechanism of action of the drug can be discovered and/or interrogated. Several good models of

UV-induced melanomas exist in mice that harbor activating mutations in oncogenes (*Braf*^{V600E} and *Nras*^{Q61R}) identified in human tumors^{15,151,152}; 2 of those models demonstrated protection from UVR-induced melanoma after sunscreen treatment. Therefore, it is reasonable to suggest that future studies should include a sunscreen arm so that the effects of the new treatment can be compared with the standard of care. It may also be appropriate for some agents to be tested in combination with sunscreen.

Evaluating Safety in Mouse Models

It is also vitally important that treatments continue past the initiation stage to determine effects on initiated tissues and early stage tumors. For studies of safety and efficacy at the post-initiation stage, the Cre-activated *Braf* mutant allele (CA) *Braf*^{CA}/*Pten*^{+/-} model, which does not require UVR for tumorigenesis, has demonstrated utility.⁵⁷ Animals with the *Braf*^{CA}/*Pten*^{+/-} genotype develop tumors with a latency intermediate between those with 2 wild-type and 2 mutant *Pten* alleles.¹⁵³ Thus, this system could model the genetic instability that drives promotion of a premalignant lesion to malignancy. Additional potentially useful mouse models are reviewed elsewhere.¹⁵⁴

Early Phase Clinical Trials and Human Model Systems

A valuable addition to early phase clinical trials of agents designed to ameliorate the effects of UV on tumor initiation and progression could include an examination of the effects of new drugs on the acute response of human skin to treatment with UVR. These studies can determine whether the drug modulates biomarkers associated with both the activity of the drug and DNA damage and/or tumorigenesis. Drugs that counteract the deleterious effects of UVR also might benefit immunosuppressed patients or those with XP. Because of their extremely high risk for developing UVR-induced precursor lesions in AKs and KCs, clinical trials in these populations can be statistically powerful, and they can rely on cancer development as an endpoint while requiring relatively few patients and short study duration. For example, the study of T4 endonuclease reported by Yarosh et al required only 30 patients in an 18-month study to demonstrate a significant reduction in both AKs and KCs.³⁴ Nicotinamide also has reduced these lesions in transplantation recipients.^{155,156} These studies provide invaluable evidence of efficacy in a human system that is supportive of the potential to prevent melanoma.

Phase 3 Trials

The initiation of phase 3 clinical trials in which melanoma is the endpoint will require the identification and recruitment of a cohort of patients who are at elevated risk for melanoma because of a personal or family history of melanoma or documented genetic and environmental risk factors. Study participants also must be well characterized with respect to family and personal history of other cancers, nevus and pigmentary phenotype, history of occupational sun exposure, and lifestyle-associated risk factors. Phase 3 trials also should assess changes in behavior (specifically, UVR exposure and use of sun protection) over the course of treatment. For example, by removing the threat of sunburn, pigmentation-enhancing agents may disinhibit unprotected UVR exposure in some patients, offsetting chemopreventive benefits. Recruitment, characterization, and monitoring of participants could be augmented and accelerated by the use of smart phone apps, such as Mole Mapper (using the Apple ResearchKit; Apple Inc, Cupertino, CA),¹⁵⁷ which currently helps individuals track the size and appearance of their nevi over time. Mole Mapper offers participants the opportunity to share their data with researchers under an institutional review board-approved protocol. This capability could be modified to accommodate the needs of a chemoprevention trial. Even with the use of Mole Mapper and teledermatology protocols, a phase 3 trial almost certainly will involve multiple academic institutions and the cooperation of individuals recruited through melanoma patient-advocacy groups and community registries.

CONCLUSIONS: THE MOST PROMISING AGENTS AND THE PATH FORWARD

Nicotinamide and NSAIDs

Candidates for the next phase 3 clinical trials for melanoma prevention likely will come from the agents discussed above and summarized in Table 1. In this summary the estimated potential of each agent to advance to a phase 3 trial for melanoma prevention in the near term (5 years) is based on the strength of preclinical and clinical evidence as well as the availability of a well characterized formulation (a major factor for natural products and so-called nutraceuticals or cosmeceuticals) or drug that is approved for use in humans. Nicotinamide is a strong candidate with convincing phase 3 evidence of efficacy for the prevention of KC, and preclinical and clinical studies support a mechanism of action that should be beneficial for melanoma prevention.⁷⁰ Convincing

evidence of both efficacy and safety in 1 or more mouse models of melanoma would further enhance enthusiasm for this agent. NSAIDs, especially sulindac, were extremely effective at reducing UV-induced KC in a mouse model.⁸³ In the same study, there was a strong correlation between decreased levels of PGE₂ and skin cancer prevention. A positive result in similar studies of sulindac in humans and mouse models of melanoma would support advancement of the compound into clinical trials for melanoma prevention.

Phytochemicals

Numerous natural products are in advanced stages of development for skin cancer prevention. The topical EGCG preparation Veregen is approved for use in humans and is well characterized chemically and pharmacologically; therefore, it should be available in the quantity and quality needed for large-scale human trials. Results from a clinical trial for the treatment of BCC are pending. Veregen should be tested in mouse models of melanoma. Other natural products for which there is evidence of potential utility for melanoma prevention are SFN, silibinin, and PLE. Topical SFN is now being tested in 2 human studies, and 1 will examine its effects on UV-irradiated skin. Silibinin is an active ingredient in the cosmeceutical product Difensa53 (ProTechSure Scientific, Inc, Aurora, CO), which could be tested in mouse models of melanoma. Although there are some reservations about the characterization of active ingredients in Fernblock, a clinical trial for the prevention of AKs and sun damage is planned. Other natural products listed in Table 1 have less clinical evidence of efficacy, and we have rated their potential for near-term advancement to melanoma chemoprevention clinical trials as low-to-moderate as a result.

New Agents That Promote DNA-Damage Repair and Photoprotective Pigmentation

MC1R agonists and SIK inhibitors are 2 new classes of drugs that have the potential to prevent melanoma by increasing DNA damage repair and/or epidermal pigmentation. These drugs could be formulated for topical application, thereby decreasing the potential for side effects. However, it will be necessary to test them in mouse models of melanoma, because these compounds will have potent effects on cells of the melanocyte lineage that could result in deleterious effects on tumor biology. DNA repair enzymes are another class of drugs that have produced promising effects in KC, both in patients with XP and in immunosuppressed transplantation recipients.

These agents should be tested in mouse models of melanoma and advanced to human trials for the disease if warranted.

There are several very promising agents in the melanoma prevention pipeline. Preclinical and early phase clinical trials have and will continue to produce a better understanding of mechanisms of action, optimal treatment schedules, and possible side effects for each agent. These data can be used to design statistically powerful phase 3 trials that not only will identify the drugs and natural products that can help prevent melanoma in individuals at risk for the disease but also will contribute to efforts toward understanding the genetic and environmental factors that contribute to that risk.

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In this work, experts from the national Melanoma Prevention Working Group^{3,158}, comprised of National Cancer Trials Network participants, discuss mechanisms of action, preclinical data, epidemiologic evidence, and results from available clinical trials for the most promising melanoma chemoprevention agents. Furthermore, the work provides an assessment of additional research necessary and the likelihood that a given compound may be a suitable candidate for a phase 3 clinical trial within the next 5 years.

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CONFLICT OF INTEREST DISCLOSURES

Joanne M. Jeter reports institutional support for clinical trials from Bristol-Myers Squibb and support for clinical trials from Merck outside the submitted work. Clara Curiel-Lewandrowski reports grants from Amgen and personal fees from Amgen and Novartis outside the submitted work. Fabian V. Filipp reports support from the Goethe Institute, Washington, DC, USA, and the Federal Foreign Office, Berlin, Germany grants from the National Cancer Institutes of Health/National Cancer Institute (CA154887), the University of California, Office of the President, Cancer Research Coordinating Committee (CRN-17-427258), and the National Science Foundation Graduate Research Fellowships Program during the conduct of the study. Zalfa Abdel-Malek reports 2 issued patents (10,738-320/113-004B [skin care compositions and methods comprising selective agonists of melanocortin 1 receptor] and 10,738-321/113-004 [pharmaceutical compositions and therapeutic methods of use comprising selective agonists of melanocortin 1 receptor]). Jeffrey E. Gershenwald reports personal fees from Castle Biosciences, Merck, and Syndax outside the submitted work. John Kirkwood reports grants from Merck and Prometheus and personal fees from Array Biopharma, Bristol-Myers Squibb, EMD Serrono, Genentec, Novartis, and Roche outside the submitted work. Pauline Funchain reports personal fees from Eisai Company Ltd outside the submitted work. David E. Fisher is a founding scientist of and holder of equity in Soltego Inc, a biotech company that is developing inhibitors of SIK as topical agents to induce skin pigmentation; he has a patent application on SIK inhibitors for skin darkening owned by Massachusetts General Hospital. Kim A. Margolin reports personal fees from Amgen, ImaginAb, and Iovance outside the submitted

work. Sherry L. Pagoto reports personal fees from Johnson & Johnson outside the submitted work. Mohammed Kashani-Sabet reports grants from Merck and personal fees from Cepheid outside the submitted work; holds stock in Melanoma Diagnostics; and has 2 patents issued (8492102 and 9920377) and 1 patent pending (20150211071). Aaron R. Mangold reports personal fees from Castle Biosciences outside the submitted work. Vernon K. Sondak reports personal fees from Array, Bristol-Myers Squibb, Genentech/Roche, Merck, Novartis, Oncolys, Pfizer, Polynoma, and Provectus outside the submitted work. Martin A. Weinstock reports personal fees from AbbVie and Cellegene outside the submitted work. Sancy A. Leachman is medical director for Skin Cancer Free, the non-profit arm of DermDetect, and also has participated in early access and registry programs with both Castle Biosciences and Myriad Genetics. The remaining authors made no disclosures.

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