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Chemoprevention Agents for Melanoma: A Path Forward into Phase III Clinical Trials

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Abstract

Recent progress in the treatment of advanced melanoma has led to unprecedented improvements in overall survival. 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 towards the design and clinical evaluation of new chemoprevention agents. Melanoma chemoprevention has the potential to dramatically reduce both the morbidity and high costs associated with treating patients with metastatic disease. In this work, scientific and clinical melanoma experts from the national Melanoma Prevention Working Group comprised of National Cancer Trials Network (NCTN) participants, discuss research aimed at discovering and developing (or re-purposing) 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, pre-clinical data, epidemiological evidence and results of available clinical trials are discussed for each class of compounds. Selected keratinocyte carcinoma chemoprevention studies are also 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 likelihood that a given compound might be a suitable candidate for a Phase III clinical trial within the next 5 years.

Precis:

In this work, experts from the national Melanoma Prevention Working Group, [156,157] 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.

Keywords

chemoprevention; melanoma; biomarkers; natural products; human model systems

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. Secondary prevention aims to intervene in the progression of premalignant cells by slowing, blocking, or reversing their conversion to melanoma, while tertiary prevention refers to preventing melanoma recurrence in patients with treated disease [1]. The following work will focus on primary and secondary chemoprevention agents, although studies in animal or other models of advanced melanoma will be included when relevant to safety.

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 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]. UVR is also involved in promotion of melanoma. Progression refers to the final development of a tumor with invasive potential, which may also 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, as an agent may show inhibitory effects in the early stages of tumorigenesis but cancer-promoting effects at later stages. [4–9]

The potential mechanisms of action for melanoma chemoprevention agents are complex (Figure 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]. This work will highlight the mechanisms of action for chemoprevention agents that have significant *in vivo* pre-clinical, epidemiologic, or clinical evidence for prevention of UVR-induced DNA damage in skin, 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 Supplementary Table 1. Inclusion of data from UVR-induced KC models is based on the shared etiologies and environmental risk factors between melanocytic and keratinocytic malignancies. UVR acts as a complete carcinogen in mouse models of KC, and individuals with genetic defects in global genome repair (e.g., xeroderma pigmentosum or XP) have dramatically elevated rates of both melanoma (2,000 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 in XP patients than for CM, 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 with malignant tumor formation as an endpoint rather than focus on treatments aimed at reducing existing actinic damage or actinic keratoses (AKs).

An ideal melanoma chemoprevention agent would not only reduce melanoma risk but also be safe, cost effective, well-tolerated, easy to use, and available in a standardized form [4–8, 12]. Defining a target population at risk for melanoma is important in order to maximize the population benefit of the intervention while reducing the risk of over-treatment. 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 would ultimately be gauged by the reduction of incidence of invasive melanoma over the long-term.

It is important to highlight two differences in the levels of evidence that are found in the human epidemiological and interventional studies reported 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 found in post hoc analyses where melanoma is 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 establishment of an association with melanoma, e.g., detailed history of sun exposure, pigmentary 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 consice as possible, we have limit our discussion here to interventional studies with CM or KC as the primary endpoint; observational studies that interrogate endpoints pertinent to the agents discussed are listed in supplementary data.

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. As a consequence, biomarkers associated with melanoma initiation and/or progression as well as the biological activity of the agent 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. Discussion in this work is thus limited to those studies performed with human cell lines and to agents for which there is some indication of efficacy in vivo (see Figure 2 for summary of development pipeline).

The goal of this work is to inform clinical and translational researchers as to the array of agents that have been evaluated in models relevant to melanoma prevention. The database at Clinicaltrials.gov was also 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 move forward in the translational research pipeline those agents with the

highest potential for impacting 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, while 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 found conclusive evidence of the benefit of sunscreen use for prevention of melanoma. Three transgenic mouse studies show that application of sunscreen to animals before UV irradiation significantly delays appearance of melanocytic tumors. These models include one in which mouse tissues overexpress the melanocyte growth factor HGF/SF [14], another in which mutant BRAF ($BRAF^{V600E}$) is expressed specifically in melanocytes [15], and a third in which melanocytes express activated NRAS ($NRAS^{Q91K}$) [16].

One randomized clinical trial presents strong evidence that routine daily sunscreen use prevents melanoma. This Australian study of 1621 participants randomized to daily versus discretionary sunscreen (“broad spectrum” SPF 16) to the head and arms for a 4-year period (1992–1996), showed a 50% reduction in melanoma at all body sites 10 years following the intervention ([HR 0.50; 95% CI, 0.24 to 1.02; P=0.051) [17], with 73% reduction in the risk of invasive CM (3 in the daily use group versus 11 in the discretionary use group; HR, 0.27; 95% CI, 0.08 to 0.97), The risk of melanoma in situ (MIS) was also reduced, but the difference was not significant (HR, 0.73; 95% CI, 0.29 to 1.81). A more recent prospective cohort study of 143 844 Norwegian women found that use of SPF > 15 sunscreen by women aged 40 to 75 years could potentially reduce their melanoma incidence by 18% (95% CI 4–30%), despite the fact that sunscreen users reported more sunburns, more sunbathing vacations, and more indoor tanning bed use than did never users [18].

The FDA has determined that “broad spectrum sunscreens … can help reduce the risk of sun-induced skin cancer and premature skin aging when used with other sun protective measures, as directed” [19]. For persons spending time outdoors, the American Academy of Dermatology recommends daily application of a sunscreen that 1) offers broad spectrum protection, i.e., absorbs in both the UVA and UVB region of the solar spectrum; 2) has an SPF of at least 30; and 3) is water resistant [20].

MC1R Agonists

α-Melanocyte-stimulating hormone (α-MSH) is a melanocortin, derived from the precursor polypeptide proopiomelanocortin (POMC), 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 (MC1R) located on the plasma membrane of melanocytes [21]. There are three forms of MSH, α,β γ, which bind with different affinities to MCRs. α-MSH is a full agonist of the human MC1R. The MC1R is polymorphic in human populations and is a

determinant of hair and skin color as well as risk for melanoma. MC1R activation by the α-MSH produced in keratinocytes results in stimulation of synthesis of photoprotective eumelanin (brown-black pigment) in melanocytes. Exogenous delivery of α-MSH can also elicit tanning of the skin through 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^4,$D\text{-}Phe^7$] α-MSH (NDP-MSH), which differs from natural α-MSH by two amino acid substitutions [22]. NDP-MSH and other tri- 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 (i.e., 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, one would predict that non-Hispanic white people 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 do patients who have skin phototypes III and above and/or are wild-type for *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 given 10 subcutaneous injections of NDP-MSH or saline over 12 days showed that NDP-MSH reliably tanned the skin, with the peak effect occurring 1 to 3 weeks after treatment [25]. However, side effects of NDP-MSH, which are attributed to non-selective binding to other MCRs 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 given subcutaneous injections of NDP-MSH showed that melanin was increased by 41% and epidermal sunburn cell formation after administration of 3 MED of UV radiation was decreased by 50% in patients with 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. In Europe, it is approved for the treatment of erythropoietic protoporphyria [27]. NDP-MSH has also been tested in vitiligo patients, where more repigmentation was observed in patients receiving NDP-MSH monthly for 4 months after UVB radiation treatment compared to patients receiving UVB radiation alone [28].

Analogs of γ-MSH, with 16-fold selectivity for MC1R versus other melanocortin receptors, were recently shown to induce rapid (1 minute) and reversible (1 day) pigmentation after intraperitoneal injection using the *Anolis carolinensis* lizard model of cutaneous pigmentation [29]. 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 conventient 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 carefully studied for safety before use in patients 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 the expression of the microphthalmia-associated transcription factor (MITF), the master regulator of pigment gene expression. The activity of MITF is positively regulated by signaling downstream of the MC1R, which are in turn activated by α-MSH produced by UV-irradiated keratinocytes [32]. As a consequence, individuals with loss-of-function mutations in *MC1R* often are unable to tan after exposure to UV light. SIK is a negative regulator of the CREB-regulated transcription co-activator (CRTC), which enables activation of the transcription factor cyclic-AMP-responsive-element-binding protein (CREB) that is in turn 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 et al. have recently shown 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 for activation of MC1R [32]. Significant increases in epidermal pigmentation were also seen 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 UV radiation. No studies have been conducted in humans with this agent to date, and none are listed as pending on Clinicaltrials.gov (accessed October 23, 2017) though clinical development is being pursued (David E. Fisher, unpublished data).

DNA Repair Enzymes

Despite the fact that human melanocytes possess a mechanism (nucleotide excision repair [NER]) for 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 in non-Hispanic whites with red hair. Although human cells have all the enzymes necessary to complete an alternate repair pathway (base excision repair [BER]), they lack a DNA glycosylase that can initiate BER 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 was shown to reduce DNA damage and epidermal proliferation after neonatal UVR treatment in a mouse melanoma model wherein both alleles of *CDK4* contain the activating UV-signature mutation 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 this mouse model may already contain all of the UV-signature mutations (i.e.

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 in other models where UVR induces melanoma in adult animals that harbor mutations in a single oncogene (see below “Evaluating efficacy in mouse models”).

A liposomal formulation of T4N5 was shown to significantly decrease AKs in xeroderma pigmentosum (XP) patients [35]. The annualized rate of new AKs was 8.2 among the patients assigned to T4N5 liposome lotion and 25.9 among those assigned to placebo (difference 17.7 [95% CI 11.8–26.5]; p=0.004). There was also a 30% reduction in new BCC in patients using the T4N5 (p=0.006). A recent study randomly assigned 15 patients with AKs on their face or scalp to receive topical DNA repair enzyme lotion or placebo [39]. There was a 46.6% percent reduction in AKs in the group treated with DNA repair enzyme lotion compared to a 32.7% decrease in the placebo group. Twelve weeks after cessation of treatment, there was an additional 29.2% decrease in the number of AKs in the DNA repair enzyme-treated group, while those treated with placebo had a 31.4% increase in AKs (p=0.0026).

Vitamins and Minerals

Vitamin A/Retinoids—Exposure to vitamin A activates the nuclear retinoid receptors RAR and RXR (reviewed in Chhabra et al. [40]). Pre-clinical studies of vitamin A and its precursors (retinol and the carotenoid pro-vitamins for vitamin A including beta-carotene) for melanoma chemoprevention have found both growth inhibiting and growth promoting effects on human cell lines. These studies are discussed in Mounessa et al. [41].

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

Two cohort studies have also shown conflicting results regarding vitamin A. In the Vitamins and Lifestyle (VITAL) cohort, users of 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 melanoma risk [42]. Another study in this same cohort showed no effect of beta-carotene supplements on melanoma risk [43]. Prospective data from the Nurses’ Health Study also demonstrated no effect of vitamin A intake on melanoma incidence for total or dietary retinol or beta-carotene [44]. The only group that had an inverse association between total retinol intake and melanoma risk included women who were otherwise at low risk for melanoma at baseline, as determined by non-dietary factors.

A meta-analysis of beta-carotene supplementation and cancer risk included results from 9 randomized clinical trials [44]. Of these, two included data on melanoma incidence. The Women’s Health Study reported no impact (RR) of 0.90 (95% CI 0.49–1.68) for beta carotene use [45]. For the SU.VI.MAX 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 beta-carotene were included in the interventional arm of this 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 disappearance of benign nevi. Details of these studies are discussed in Mounessa et al [41]. Oral isotretinoin (13-cis-retinoic acid) has also 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].

A note about dysplastic nevi and biomarkers of melanoma prevention: individuals with 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 biomarker of the effects of a therapeutic agent on dysplastic nevi such as the ratio of phosphorylated Stat1/phosphorylated Stat3, which was shown to be significantly associated with degree of atypia [55], could be used. However, each nevus on a patient is unique and has its own potential for tumorgenesis. In light of this, it seems that the ideal solution for monitoring treatment effects in dysplastic nevi will likely involve non-invasive methods, such as confocal microscopy [56], which will be used to assess the evolution of molecular and structural features in individual lesions.

Vitamin E—Pre-clinical 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 in Chhabra et al. [40]). Recent data, however, have shown that Trolox, 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% alpha-tocopherol) and C (15% L-ascorbic acid) decrease erythema and CPD formation in pig skin irradiated with simulated solar radiation (SSR) [58].

Case-control studies examining the effects of vitamin E on melanoma incidence have shown mixed results and are summarized in Supplementary Table 1 (VE). In addition, prospective data from the Nurses’ Health Study addressed this question but showed that total and dietary vitamin E were not associated with melanoma risk (multivariate RRs 1.11 (0.66–1.85) and 0.88 (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 UV radiation. Treatment effects included an increase in MED and decreased UV-induced DNA damage [60]. The SU.VI.MAX trial discussed above found an increased risk for women consuming antioxidant supplements, including supplementation with vitamin E. Additionally, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) found an increased risk for prostate cancer ([HR], 1.17; 99% CI, 1.004–1.36, P=.008) in men consuming oral vitamin E supplements (400 IU daily as racemic alpha-tocopheryl acetate)

[61]. Topical vitamin E treatment 24 hours prior to experimental UV irradiation provides a potentially protective effect in significantly reduced expression of the matrix metalloprotease MMP-12 in UV-treated human skin [62]. In a study of adults with Fitzpatrick skin phototypes II-III, a topical formulation containing 1% alpha-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 four days at the dose of 2gm/cm^2. Treated skin exposed to SSR had reduced erythema, sunburn cells, CPDs, and p53 induction. qPCR analysis of biopsied skin also found reduced levels of UV-induced cytokine formation in treated skin versus controls [63].

Vitamin D—The anti-proliferative effects of vitamin D on melanoma cells are thought to be mediated by the vitamin D receptor. Activities associated with the ligand-bound vitamin D receptor include heterodimerization with the RXR and subsequent activation of the retinoid pathway (reviewed in Chhabra et al. [40]).

Case-control studies of vitamin D intake and melanoma incidence are summarized in Supplementary Table 1 (VD). A prospective cohort study of over 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 cases found a weak association between dietary vitamin D and the development of CM(SRR 0.92; 95% CI 0.25–3.44) [64]. Sensitivity analysis of this group included assessment without inclusion of the Weinstock paper [65] due to lack of data specific to dietary intake alone without supplementation; this adjustment yielded an SRR of 0.63 (95% CI 0.42–0.94). Another meta-analysis found no significant association between serum vitamin D levels and melanoma risk or prognosis, although an inverse relationship between serum vitamin D levels and melanoma thickness was reported [66].

The Women's Health Initiative randomized 36,828 postmenopausal women to use of low dose (400 IU) vitamin D and 1000 mg calcium (CaD) supplementation daily versus placebo. This study was originally 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 found no statistically significant difference in the incidence of melanoma (HR = 0.86, 95% CI 0.64–1.16). However, subgroup analysis showed that in women with a history of KC, melanoma incidence was decreased in the supplementation arm (HR, 0.43; 95% CI,0.21to 0.90).

A prospective clinical study enrolled 25 individuals with serum 25-hydroxyvitamin-D levels <30 ng/mL and with skin photodamage to take 50000 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 showed minimum changes after supplementation. Cytochrome P450–24 (CYP24, a known target of vitamin D in skin) expression in photodamaged (PD)- and photoprotected-skin was increased after supplementation by 186%, p = 0.08, and 134%, p = 0.07, respectively. In benign nevi from 11 participants elevated VDR and CYP24 expression was observed (average of 20%, p = 0.08, and 544%, p = 0.09, respectively). Caspase-14 expression, a marker of keratinocyte differentiation, was significantly increased (49%, P < 0.0001) in the basal layer of PD skin.

The authors noted that there was significant variability 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 treated with 1 to 3 times their MED of simulated solar radiation found that those given a very high dose (200 000 IU) of Vitamin D_3 after irradiation had significantly higher serum levels of Vitamin D_3, increased levels of anti-inflammatory mediator arginase-1, and a sustained reduction in skin erythema that correlated with significant expression of 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]. These studies have not only reported 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 increases the rate of repair of CPDs and oxidative DNA damage 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 in Minocha et al.)[70]. A double-blind, randomized Phase III clinical trial evaluated the effects of nicotinamide on the incidence of KCs [71]. Use of 500 mg oral nicotinamide twice daily for 12 months resulted in a 13% reduction in AKs (p=0.001) and 23% reduction in KCs (p=0.02). 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]. Secondary analysis showed that the incidence rates of melanoma and melanoma in situ were similar between the groups receiving nicotinamide daily versus those receiving placebo. However, given that melanoma incidence was a secondary endpoint and only 10 melanomas were diagnosed in study participants (versus 801 non-melanoma skin cancers), the study was likely underpowered and the analysis period too short to detect a difference in incidence of melanoma if one were to 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 found 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 (reviewed in Rowan et al. [73]). Selenium is incorporated into 25 human selenoproteins, many of which have antioxidant functions, via addition of the amino acid selenocysteine to a polypeptide chain as it is synthesized on the ribosome. Presence of a unique 3'- element (selenocysteine insertion sequence, or SECIS) in selenoprotein RNAs changes the translation of the UGA codon from "stop" to selenocysteine. At supranutritional levels (above 400 μg/day), selenium metabolites such as methyl selenol are produced. Human melanoma cells have been found to be more sensitive to the growth inhibitory and pro-apoptotic effects of the methyl selenol

pro-drug methyl seleninic acid than are primary human melanocytes [5]. Treatment with topical l-selenomethionine results in a significant delay in the time required for UV-induced tumor development in KC [74] and melanoma mouse models, but continued treatment increases the rate of growth of melanomas once tumors appear [5].

Numerous studies have evaluated the association between melanoma and selenium in humans, with mixed results. The majority of case-control studies are negative (see Supplementary Table 1), although selenium levels were assessed in different tissues in the various studies (e.g., serum versus toenail specimens). Several cohort studies have also examined the question of a potential chemoprevention or causative effect of selenium in melanoma, with varying results. An Italian cohort with exposure to high levels of selenium in their tap water was found to have a statistically significant 3.9-fold increase in melanoma incidence as compared to an unexposed cohort [75]. The Nurses' Health Study also found that subjects 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 melanoma patients (81 stage I, 63 stage II, 56 stage III), 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 in the Vitamin A section. The Nutritional Prevention of Cancer Trial evaluated the administration of selenized yeast as a chemopreventive agent for KC [75]. This study is widely cited for the finding of reduced risk for prostate cancer in men, but the multivariate-adjusted HR for melanoma was not significant (1.18 (95% CI 0.49–2.85)), and risk for squamous cell carcinoma and total KC were elevated; (HR = 1.25, 95% CI = 1.03 to 1.51 and HR = 1.17, 95% CI = 1.02 to 1.34), respectively. A 2018 Cochrane review concluded that "Randomized controlled trials with low risk of bias suggested increased melanoma risk" in study participants treated with selenium supplements [78].

Medications used for other indications

Aspirin and Nonsteroidal Anti-inflammatory Drugs (NSAIDs)—Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are believed to exert their anti-inflammatory activities primarily by the inhibition of cyclooxygenases 1 and/or 2 (COX-1 and COX-2). The cyclooxygenases convert arachidonic acid to prostaglandin H2 (PGH2), which is then transformed by prostaglandin synthases to the D- E- and F-series prostaglandins (PGDs, PGFs and PGEs) [79]. Two NSAIDs, celecoxib and indomethacin, have been found to reduce proliferation in human A375 melanoma cells, while others (aspirin and piroxicam) did not show these effects [80]. Study authors presented evidence in support of their hypothesis that the activity of NSAIDs was mediated by COX-2 inhibition and resulting decrease in levels of prostaglandin E2 (PGE2), and reduced production of IL-6, a pleiotropic inflammatory cytokine with 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 via intracellular

glutathione depletion, ROS formation, and mitochondrial toxicity [81]. Goulet et al. report that COX-2 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 study found that COX-2 expression was high in melanoma metastases [82]. A recent publication by Mikulec et al. [83] reports a dramatic (94%) reduction in UV-induced KC in mice treated daily with a low dose (160 mg/day human equivalent dose) of sulindac in their feed, with more modest effects of other NSAIDs, including aspirin. This report found that chemoprevention efficacy of the different NSAIDs tested correlated significantly with UV-induced PGE2 production and keratinocyte proliferation.

Supplementary Table 1 (A) summarizes the case-control studies investigating the association between aspirin other NSAIDs and melanoma risk. 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, published in 2007, found no association of aspirin with melanoma risk in 69810 men and 76303 women participating in the Cancer Prevention Study II Nutrition Cohort (RR 1.15 in current users of over 5 years duration, 95% CI 0.83 to 1.59) [84]. Past users and current users with less than 5 years duration also did not have a reduced melanoma incidence. A subsequent paper assessed the risk association for NSAIDsin the VITAL cohort study and found no association between use of these medications and melanoma risk [85]. Analysis of data from the Nurses' Health Study showed regular aspirin users had an increased incidence of melanoma (adjusted RR = 1.32, 95 % CI 1.03–1.70), although this effect was not seen in past users and a dose-response effect was not observed [86]. However, a subsequent analysis from the Women's Health Initiative found 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 in users of non-aspirin NSAIDs, although there was infrequent use of non-aspirin NSAIDs[87, 88]. Meta-analyses of this association have yielded negative results. One report of 13 studies (six case-control studies with 93,432 cases and 401,251 controls, six cohort studies consisting of 563,380 subjects, and one randomized controlled trial of 39,876 participants) showed a lack of effect (RR.0.97 (95 % CI = 0.90–10.4)) for ever-users of any NSAID [89]. Results did not differ between aspirin and non-aspirin NSAID users; however, case-control studies did show a slightly decreased risk of melanoma in aspirin users (RR = 0.88, 95 % CI = 0.80–0.96). Another pooled analysis of ten studies involving 490,322 participants demonstrated no impact on melanoma incidence (RR of 0.96 for aspirin (95% CI, 0.89–1.03) and 1.05 for non-aspirin NSAIDs (95% CI, 0.96–1.14)) [90]. Subgroup analyses of cohort studies, high-intensity NSAID use, and long-term NSAID use also failed to show a protective effect, although again a slight risk reduction was seen in aspirin users in case-control studies (RR=0.86; 95% CI, 0.80–0.93). A meta-analysis of aspirin-only users showed similar results (OR = 0.96, 95% CI 0.82–1.12) [91].

A clinical trial in 2005 showed that celecoxib administered orally at a dose of 200 mg twice daily for 10 days was associated with a significant reduction in erythema in six of the twelve participants after irradiation of the skin with 2 times the MED [92]. A number of additional studies have been conducted to determine whether COX-2 inhibitors might be effective

preventive agents for KCs [93, 94]; two of these report incidence of KC as endpoints [95, 96]. Elmets et al. reported a double-blind trial in which 240 participants with 10–40 AKs at baseline and a prior histological diagnosis of at least one AK or KC were randomized to receive celecoxib (200 mg twice daily) or placebo. Participants were treated for 9 months and were followed up 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 with basal cell nevus syndrome, a trend for reduction of BCC burden by oral celecoxib was seen after analysis of results from all subjects ($p = 0.069$) was reported. Subgroup analysis that considered only the 60% of patients with less severe disease (<15 BCCs at study entry) showed that celecoxib significantly reduced BCC number and burden: subjects receiving placebo had a 50% increase in BCC burden per year, whereas subjects in the celecoxib group had a 20% increase ($p(\text{difference}) = 0.024$) [96].

To date, no clinical trials have directly assessed impact of the use of aspirin or other NSAIDs on melanoma incidence. However, there is a report from a Phase II randomized placebo-controlled trial of oral sulindac 150 mg twice a day for 8 weeks. In this study, the primary endpoints were levels of sulindac and its metabolites in skin and serum. The analysis found sulindac sulfone is delivered to keratinocytes and melanocytes, while the parent sulfide was the major sulindac-derived species detected in the serum. Secondary endpoint analysis found 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 HMG-CoA 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 familes, Rac, Rab, Cdc42, and nuclear lamins [98]. Cell culture studies showed that statins induced caspase-dependent apoptosis in multiple human melanoma cell lines via 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 affects are orders of magnitude higher than peak plasma concentrations observed at the highest dose (40 mg/day) commonly used for treatment of hypercholesterolemia [101].

Case-control studies of the effects of statins on melanoma incidence are shown in Supplementary Table 1 (ST1). Prospective cohort analyses have also been conducted. The prospective Cancer Prevention Study II Nutrition Cohort of over 133,000 subjects showed use of cholesterol-lowering drugs for five or more years was associated with a lower risk of melanoma (RR 0.79, 95% CI 0.66–0.96) [102]. This 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 for current users for less than 5 years (RR 0.89, 95% CI 0.75–1.06). However, analysis of prospective data from the Women's Health Initiative showed no effect of statin use on melanoma risk in statin users and nonusers, and the multivariable adjusted HR was 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 (Supplementary Table 1 (ST2) [104–108], although one found increased melanoma risk

associated with statin use (median RR 1.5, range 1.3 −1.7) [109]. Subgroup analysis of one of the earlier meta-analyses indicated that lovastatin might have a drug-specific effect with OR 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, median follow-up for many of these studies was less than 5 years.

While no clinical trials to date have directly assessed impact of the use of statins on melanoma incidence, Linden et al conducted a randomized, double-blinded, placebo-controlled Phase II trial of lovastatin in 80 subjects with a history of at least two clinically atypical nevi [110]. Subjects receiving lovastatin did not have significant changes in histopathologic atypia, clinical atypia, or number of nevi, nor did their nevi show 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 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 to the potent antioxidant glutathione [112]. In mice, NAC delays primary tumor development of UV-induced melanoma [113] and KC [114].

A Phase I study of NAC as a melanoma chemoprevention agent showed 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 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 II clinical trial involving 100 participants failed to show any protection of nevi 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 I and II trials.

While some in vitro and in vivo studies of NAC for prevention of skin cancers, including melanoma, have shown evidence of beneficial effects, two 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 PTEN treated chronically with NAC and a soluble vitamin E analog developed more lymph node metastases [57] than did control animals, while neither antioxidant had an effect on the number of tumors. In immune-compromised mice implanted with melanoma patient-derived xenografts, subcutaneous injection of NAC (200 mg/kg/day) increased visceral metastases [117] in grafts of three different tumors. These deleterious effects have dampened the enthusiasm for pursuit of NAC in human trials and highlight the importance of studying chemoprevention agents at all stages of carcinogenesis, as effects of a given agent may differ according to where in this continuum intervention is made.

Difluoromethylornithine—Difluoromethylornithine (DFMO) is an irreversible inhibitor of the rate-limiting enzyme in the synthesis of polyamines, ornithine decarboxylase (ODC), that 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, showed 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 gamma treatment causes growth arrest in human melanoma cell lines [120].

No clinical or epidemiologic studies of the effects of DMFO on melanoma in humans have been reported. A randomized, double-blinded, placebo-controlled phase 3 trial for prevention of KC in patients with a history of the disease randomized 291 participants to receive oral DFMO (500 mg/m^2/day) or placebo [121]. Participants were followed for 4 to 5 years. The authors reported a non-significant reduction in the primary endpoint of new KC in the DFMO treated arm (260 in DFMO treated versus 363 in placebo, p=0.069). Evaluation of BCC and SCC separately showed very little difference in SCC between treatment groups but a significant difference in new BCCs (163 for DFMO versus 243 for placebo, p=0.03). Adverse events included a significantly greater average hearing loss for DMFO-treated participants versus placebo (4 dB loss for DMFO versus 2 dB for placebo, p=0.003). A phase I study of topical 10% DFMO administered twice daily demonstrated delivery to the skin, ODC inhibition, absence of systemic exposure, and decrease in AKs [122]. However, similar effects were not observed in a phase IIb study where a total of 156 subjects with sun-damaged skin were randomized to receive DFMO or diclofenac or a combination of the two topically twice daily for 90 days [93]. The phase IIb study found no difference in polyamine levels in the skin or in the primary endpoint (karyometric average nuclear abnormality) between baseline and end of study for any treatment group. The authors suggested that low baseline polyamine levels in participants in this study, relative to earlier studies, may have explained the lack of observed effect on ODC activity.

Phytochemicals

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 pre-clinical 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 “nutriceuticals” on pigmentation and potential use as melanoma prevention agents [123]. Studies of compounds that have been examined in in vivo and clinical studies are discussed below.

Epigallocatechin-3-gallate—Epigallocatechin-3-gallate (EGCG) is a flavonoid that is abundant in green tea; lower levels are found 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 shown that both topical and oral delivery of EGCG can confer protection against KC. Efficacy of orally-administered EGCG is limited by low bioavailability, but nanoparticle encapsulation has recently been shown to significantly increase 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 showed a small decrease in the overall incidence of cancer with non-herbal tea consumption but no specific association for melanoma [125]. Green tea was not differentiated from black tea in this study. Wu et al. [126] 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 this group, with an average follow-up of 7.7 years. Questionnaires regarding coffee and tea consumption and melanoma incidence were given; all information was self-reported. Tea consumption was not found to have a significant association with melanoma risk (HR 1.03; 95% CI 0.81–1.31).

The topical application of EGCG was shown in human studies to decrease erythema after UV radiation exposure [127, 128], but clinical trials with other melanoma-related endpoints have not been conducted to date. Sinecatechins 10% ointment (Veregen®) contains a standardized extract of green tea leaves of the species *Camellia sinensis* with 85–95% (w/w) green tea polyphenols (primarily catechins). The most abundant catechin in Veregen® is EGCG. Veregen® is approved by the US Food and Drug Administration (FDA) for treatment of genital warts in adults. A Phase II trial of Veregen® for treatment of BCC has been completed per Clinicaltrials.gov, but results are not yet available (NCT02029352).

Resveratrol—Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a polyphenol commonly found in berry juices and in red wine; its antioxidant and anti-cancer properties are widely reported in both the popular and scientific literature. Topical resveratrol blocks many of the deleterious effect of UV radiation, including Cox-2 expression, keratinocyte proliferation, and KC tumor formation in mouse models (reviewed in Chhabra et al. [40]). However, benefits of resveratrol in pre-clinical models have not yet been realized in clinical studies, likely due to 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- and 5-hydroxy groups, is also found in berry juices and has been studied as a more bioavailable alternative to resveratrol. 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 found in its glucoraphanin pro-drug form in cruciferous vegetables such as broccoli, brussel sprouts, and

cabbage. In vitro experiments have shown that SFN can reduce the growth of melanoma cells via apoptosis and by altering activity of chromatin-modifying enzymes [130]. Both topical application of SFN [131] and a diet of broccoli sprouts [132] have been shown to protect against the development of non-melanoma skin cancer in mice exposed to UV radiation. This effect is likely due to activation of the transcription factor Nrf2 and downstream antioxidant enzymes, as well as inhibition of the transcription factor AP-1 [133].

Topical application of sulforaphane increases expression of antioxidant genes and decreases MED in UV-irradiated human skin [134]. In a recent Phase I study seventeen patients with 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 found that oral sulforaphane is well-tolerated at daily doses up to 200 μmol and achieves dose-dependent levels in plasma and skin [Tahata et al., Cancer Prevention *In Press*].

Lycopene and related carotenoids—Lycopene is a lipophilic C-40 carotenoid antioxidant found in 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 [135]. In a recent study, 20 healthy women ages 21 to47 were randomized to consume 55 g of tomato paste (16 mg lycopene) in olive oil or olive oil alone, spread daily on bread for 12 weeks [136]. Analysis of UV-irradiated skin of participants showed that the tomato paste treatment was associated with decreases in UVR-induced erythema ($p=0.03$) and matrix metalloprotease-1 expression ($p=0.01$). UV-induced decreases in dermal fibrillin-1 and increased mitochondrial DNA 3895-bp deletion were also ameliorated in the tomato paste arm ($p=0.03$ and 0.01, respectively).

Bixin is an apocarotenoid that is present in an FDA-approved natural food colorant derived from the seeds of the achiote tree (*Bixa orellana*, 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 [137]. Bixin has an excellent safety record and good systemic bioavailability when administered orally. A team from the Arizona Cancer Center has recently reported that intra-peritoneal 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 to control animals [138]. However, one potential issue is the relatively high daily dose used (equivalent to 16 mg/kg in humans or 1,200 mg for a 160 pound human [139]), which is 33% higher than acceptable daily intake (ADI) recommended by the World Health Organization [138].

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. [140]). A study in a mouse model of UV-induced KC found that a dose equivalent to 7.5 mg/kg/day delayed tumor appearance [140].

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 1,080 mg oral PLE (240 mg every 8 hours 1 day before and then 360 mg 3 hours before UV treatment) [141]. MED was determined before and after PLE treatment. Participants had significantly higher MED post-treatment (i.e., their skin required a higher UV dose to induce redness) compared to pre-treatment. In a recent study, Kohli et al. reported on the clinical and histological effects of oral PLE on irradiation with a combination of UVA/UVB and visible light [142]. On day 1, 22 patients (Fitzpatrick skin type I–III) were irradiated and MED determinations were made on day 2. Participants were then treated on day 3 with 240 mg of PLE 2 hours before and 1 hour before irradiation; MED was determined on day 4. Biopsies were performed on untreated skin and irradiated skin at the MED. For 7 out of 22 patients, PLE treatment increased MED ($p>0.05$), but histological differences in pre-and post-treatment irradiated skin were highly significant in all participants. Markers of UV-induced damage including PCNA, sunburn cells (e.g., dyskeratotic keratinocytes), CPDs, Cyclin D1, Cox-2, and Ki67 were all reduced by at least 75% (with the exception of CPDs, which were reduced by 32%).

The branded *Polypodium leucotomas* extract Fernblock® contains a number of phenolic compounds, including 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 4-hydroxycinnamoyl-quinic acid, and five isomers of chlorogenic acid. These compounds make up only 1% (w/w) of the extract's dry weight [140]. 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 in the doses contained in the extract. This leaves unanswered the possibility that some other component not assessed in the standardization process might be important for its biological activities. Therefore, there is some question as to 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 anti-cancer activity; silibinin is the main bioactive flavonolignan present in this mixture (reviewed in Kumar et al. [143]). Silibinin is reported to suppress growth of xenografted human melanoma cells by directly targeting MEK- and RSK-mediated signaling pathways. In the hairless mouse model, treatment with topical silibinin significantly reduces UV-induced skin cancer by a mechanism dependent in part on p53 [144]. The skin of animals treated with silibinin before UV irradiation had lower levels of CPDs and inflammation. This study was done with chemically-pure silibinin. There is one report in the literature of potential phototoxicity of one of the minor components in milk thistle extract (2,3-dehydrosilybin [145]).

The Melanoma Chemoprevention Pipeline

A recent commentary by Meyskens et al. highlights the obstacles and challenges that confront the field of cancer prevention [146] and voices frustration over the repeated failures to

translate promising pre-clinical 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 pre-clinical results into early stage (Phase 0 and I) clinical trials will require the identification of robust biomarkers of efficacy. These candidate biomarkers must come from pre-clinical studies done in cell culture, human skin equivalents, ex vivo human tissues, animal models and human pilot studies. These models should generate not only a test of whether or not the agent prevents melanoma but also 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 non-tumorigenic melanocyte-derived cell lines such as PIG1 [147] are appropriate, while 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, 148, 149]. Melanoma cell lines can be informative regarding the safety of prevention agents. An example is the safety concerns raised for both NAC and vitamin E, both of which were found to increase tumor cell motility and invasive capacity both in vitro and in vivo [57].

Evaluating efficacy in mouse models—This work includes studies that use UVR-induced models of KC and melanoma. While mouse skin exhibits key differences in melanocyte localization, where interfollicular melanocytes are not maintained in adult mice, they have nonetheless 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 both KC and melanoma. Moreover, mice have been successfully used to show the effectiveness of sunscreen at preventing sunburn and UV induced mutations. Mouse models are a well established preclinical tool where proof of principle for a therapeutic strategy may be established, and as a result, we have referenced wherever possible extant mouse model data supporting each possible therapeutic approach.If an agent prevents tumor formation in these UV-induced models, 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, in order that 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 (B-Raf^{V600E} and N-Ras^{Q61R}) found in human tumors [15, 150, 151]; two of these 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 to 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 in order to determine effects on initiated tissues and early stage tumors. For studies of safety and efficacy at the post-initiation stage, the $BRAF^{CA/PTEN+/-}$ 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 two wild-type and two mutant PTEN alleles [152]. Thus, this system could model the genetic instability that drives promotion of a pre-malignant lesion to malignancy. Additional potentially useful mouse models are reviewed elsewhere [153].

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 might also benefit immunosuppressed patients or those with XP. Because of their extremely high risk for developing UVR-induced precursor lesions (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 a 18 month study to demonstrate a significant reduction in both AKs and KCs [35]. Nicotinamide has also been shown to reduce these lesions in transplant patients [48, 154]. These studies provide invaluable evidence of efficacy in a human system that is supportive of the potential to prevent melanoma.

Phase III trials—Initiation of Phase III clinical trials where melanoma is the endpoint will require the identification and recruitment of a cohort of patients who are at elevated risk for melanoma due to personal or family history of melanoma, or documented genetic and environmental risk factors. Study participants must also 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 III trials should also assess change in behavior, specifically, UVR exposure and use of sun protection, over the course of treatment. For example, pigmentation enhancing agents, by removing the threat of sunburn, 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 MoleMapper™ [155], which currently helps individuals track the size and appearance of their nevi over time. MoleMapper™ offers participants the opportunity to share their data with researchers under an IRB-approved protocol. This capability could be modified to accommodate the needs of a chemoprevention trial. Even with the use of MoleMapper™ and teledermatology protocols, a Phase III trial will almost certainly involve multiple academic institutions and the participation of subjects 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 III clinical trials for melanoma prevention will likely come from the agents discussed above and summarized in Table 1. In this work, the estimation of the potential of each agent for advancement to Phase III trial for melanoma prevention in the near term (5 years) is based on the strength of pre-clinical and clinical evidence as well as the availability of a well-characterized formulation (a major factor for natural products and so-called nutriceuticals or cosmeceuticals) or drug that is approved for use in humans. Nicotinamide is an strong candidate with convincing Phase III evidence of efficacy in prevention of KC, as well as pre-clinical and clinical studies that support a mechanism of action that should be beneficial for melanoma prevention [70]. Convincing evidence of both efficacy and safety in one or more mouse models of melanoma would further enhance enthusiasm for this agent. NSAIDs, especially sulindac, are extremely effective at reducing UV-induced KC in a mouse model [83]. In this 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 for a clinical trial for 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 sulforaphane (SFN), silibinin, and *Polypodium leucotomas* extract. Topical SFN is in now being tested in two human studies; one will examine its effects on UV-irradiated skin. Silibinin is an active ingredient in the cosmeceutical product Difensa53™, 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 prevention of AKs and sun damage is planned. Other natural products 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-moderate as a result.

New agents that promote DNA-damage repair and photoprotective pigmentation—MC1R agonists and SIK inhibitors are two 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, testing in mouse models of melanoma is necessary 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 shown promising effects in KC, both in XP patients and immunosuppressed transplant patients. These agents should be tested in mouse models of melanoma and advanced to human trials for the disease if warranted.

There are number of very promising agents in the melanoma prevention pipeline. Pre-clinical 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 III trials that will not only identify the drugs and natural products that can help prevent melanoma in individuals at risk for the disease but also contribute to efforts to understand the genetic and environmental factors that contribute to that risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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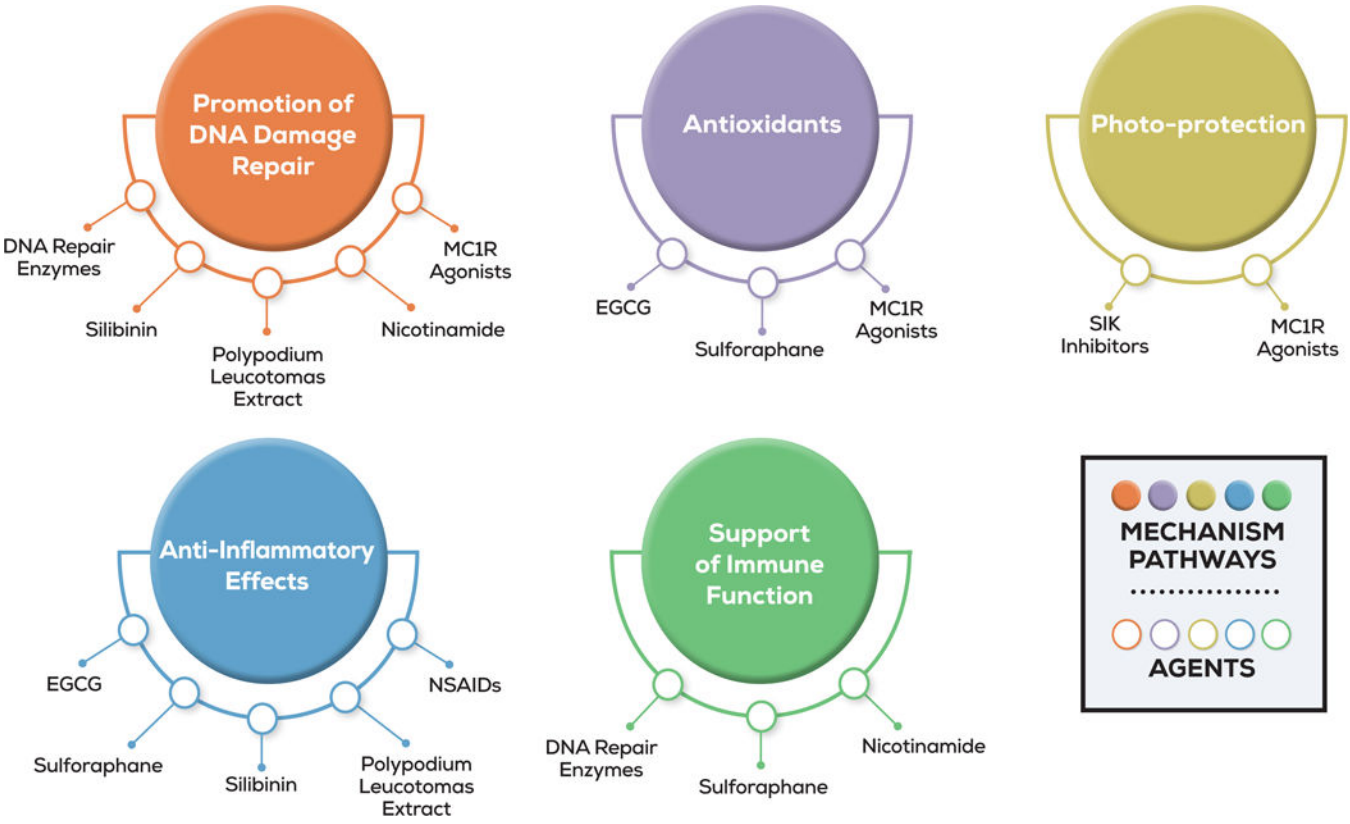

Figure 1:

Modes of action of candidate chemoprevention of melanoma include photo-protection, promotion of DNA damage repair, reduction of metabolic and redox stress by anti-oxidants, anti-inflammatory effects, and support of immune function. Some of the most promising agents are shown.

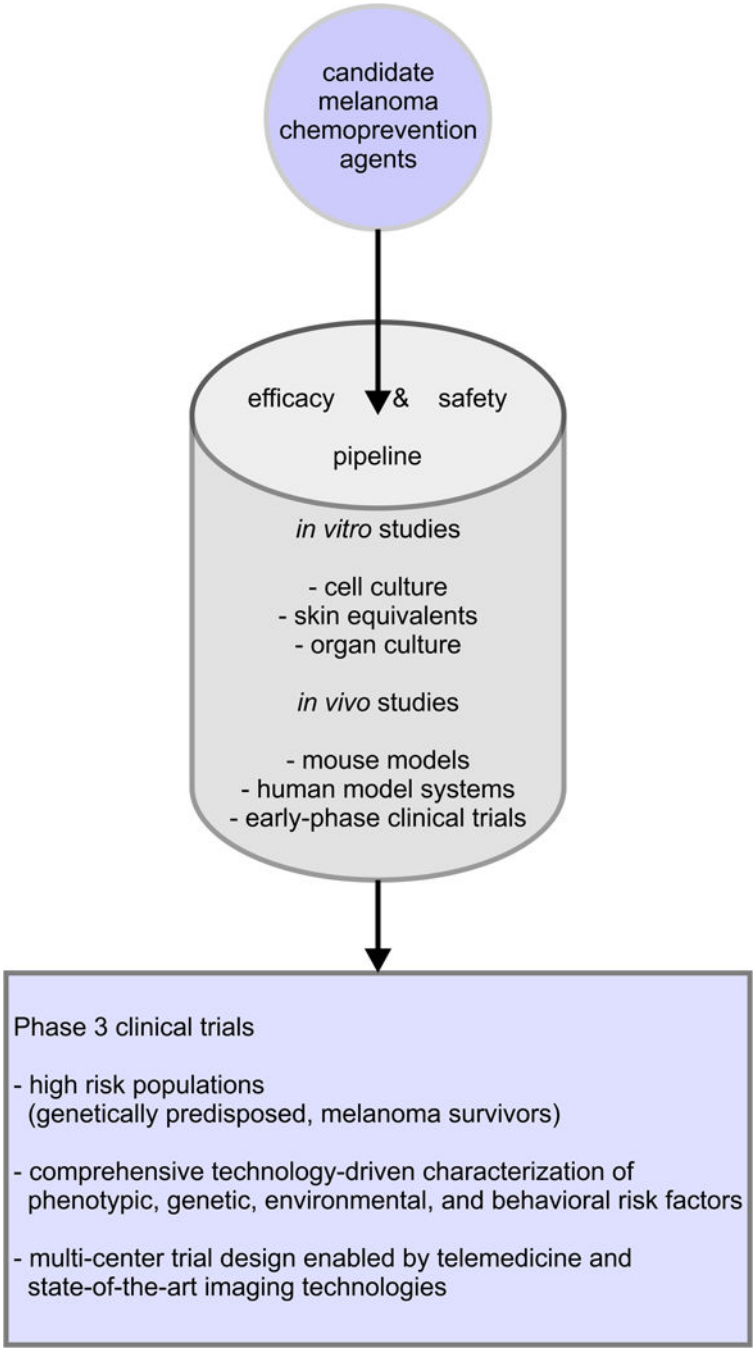

Figure 2:
Pipeline for developing candidate agents

Table 1.

Summary of ongoing trials of potential melanoma chemoprevention agents from Clinicaltrials.gov

The agent name and the terms “melanoma” and “skin cancer” were used to query the database on March 2, 2018.

Agent	Clinical Trial	Description	Primary Outcome Metrics
Sunscreen	NCT02668536	Evaluate durability, safety, and SPF characterization of bioadhesive nanoparticle encapsulated sunscreen	Determine minimal erythemal dose (MED), skin exams 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 prior to treatment for actinic chelitis	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-year 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/month following surgery of first cutaneous malignant melanoma in Stage 1B-III patients	Relapse-free survival. 2^{o} endpoint 25-hydroxyvitamin D3 serum levels at diagnosis and at 6 month intervals
	NCT00301067	Evaluate the effects of using calcitriol to sensitize metastatic melanoma tumor cells to treatment with temozolomide	Maximum tolerated dose of calcitriol, toxicity of treatment regimen with temozolomide and high-dose calcitriol. Secondary Outcome Metrics: Tumor response and time to progression, relationship between vitamin-D receptor variants and tumor response.
Nicotinamide	None Pending	Not Available	Not Available
Selenium	NCT00392561	Evaluate effects of vitamin E and selenium on preventing non-melanoma skin cancers in Bangladesh	Incidence of skin cancer during a 6-year study period. Secondary Outcome Metrics: Occurrence of mortality and incidence of diabetes during study period.
Aspirin, NSAIDs	None Pending	-	-
Statins	None Pending	-	-
N-acetylcysteine (NAC)	None Pending	-	-
Difluoromethylornithine (DFMO)	NCT02636569	Assess effects of DFMO and diclofenac on reversing specific biomarkers in non-melanoma skin cancer	Reduction in biomarkers associated with DFMO treatment. Secondary Outcome: Determine if subjects treated with diclofenac +/− DFMO have fewer AKs than placebo treated subjects
Epigallocatechin-3-gallate (EGCG)	NCT02029352	Evaluate the effects of topical EGCG in humans with sBCC	Percentage of patients with complete histological clearance. Secondary Outcome Metrics: Number of patient applications compared to prescribed applications, number of local skin reactions or adverse events.
Resveratrol	NCT02760160	Investigate effects of reconstituted grape powder on production of biomarkers for non-melanoma skin cancer in response to UV	Changes in MED from baseline. Secondary Outcome: Histological changes in selected biomarkers and assessment of apoptosis.
Sulforaphane (SFN)	NCT01568996	Assess if SFN has an effect on the progression of atypical nevi to melanoma	Assess adverse events associated with SFN treatment, visual and cellular changes in atypical nevi. Secondary Outcome: SFN levels

			in blood following 3 doses, effects of SFN on STAT1 and STAT3 expression.
	NCT03126539	Investigate effects of topical SFN on skin fragility associated with aging and UV exposure	Gene expression and histological changes in Keratin 16 and 17 in the basal epidermis.
	NCT03289832	Assess the effects of SFN and curcumin on skin exposed to UV	Changes in UV-induced erythema
Lycopene/Bixin	None pending	-	-
Polypodium leucotomas extract (PLE)	NCT02813902	Evaluate efficacy, tolerability, and toxicity of PLE for prevention of actinic keratosis and keratinocytes in high risk skin cancer populations	Incidence of new clinically visible AKs. Secondary Outcome Metrics: Histological presence of UV induced CPDs, solar elastosis, and sunburn cells
Silibinin	None pending	-	-

Table 2.

Summary of evidence and potential for advancement into Phase III clinical trials for melanoma prevention agents.

For **Pre-clinical evidence: 1**-mouse models of melanoma or UV-irradiated pig skin. **2-** in vivo mouse models of UV-induced KC; **3-** cell culture and ex vivo skin culture studies; For **Clinical Evidence- 1**-interventional studies with melanoma-relevant endpoint including KC;. **2-** cohort studies and interventional studies with melanoma incidence as secondary endpoint; **3-** case-control studies;

Table 2	Pre-clinical evidence	Clinical evidence	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 activing ingredients are being tested
MC1R agonists	**2-** α-MSH analogs stimulate pigment synthesis, protect against UV-induced DNA damage.	**1**-Sub-cutaneous injection induces tanning and provides photoprotection in individuals with fair skin. NDP-MSH approved for use in humans in Europe.	Significant side effects for analogs currently available for 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	**1-** prevents UV-induced NMSC **3-** increase removal of UV-damaged DNA bases in keratinocytes	**1-** Reduces number 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	**2**-one reports benefit, 2 do not **1-** some benefit in patients with melanocytic lesions	Oral beta-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	**1-** topical vitamin E plus vitamin C reduces erythema and CPDS in UV-irradiated pig skin **3-** antioxidant effects in cell culture. An analog increases motility and invasion of melanoma cells in vitro	**1-** decreased MMP expression after topical treatment. Topical vitamin E plus vitamin C plus ferrulic acid reduces erythema ,CPDS p53, 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
Vitamin D	**3-** growth inhibition in human melanoma cell lines	**1**-50,000 IU biweekly X 9 weeks modulated biomarkers but variability was high; 200K IU after UVR reduced skin inflammation. **2-**No effect of 400 IU + calcium on melanoma risk. **3-** no effect of serum Vit 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

Table 2	Pre-clinical evidence	Clinical evidence	Adverse effects, limitations	Potential for clinical impact in the near term, next steps and/or pending trials
Nicotinamide	**3-** Enhances DNA damage repair in primary human melanocytes, ex vivo skin; inhibits proliferation but enhan-ces invasiveness in melanoma cells.	**1**-500 mg 2x daily reduces risk for KC, decreases UV-induced immune suppression; decreases KC in transplant patients	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	**1**-Increases risk for KC in humans	**VERY LOW**-supplementation likely only important in individuals that are nutritionally deficient
Aspirin and NSAIDS	**2**- dramatic decrease in UV-induced NMSC in mice treated with low dose sulindac. PGE2 levels correlate with efficacy. **3**- decreased proliferation in human melanoma cells.	**1**- **. Oral sulindac is delivered to cutaneous nevi, increased cleaved caspase-3 in atypical nevi. Oral celecoxib reduces risk of KC. **2**- mixed results; No benefit for low-dose aspirin **3**- modest risk reduction for aspirin 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 PGE2 production should be performed.
Statins	**3**-benificial effects in cell culture require dose that might be higher than achieved clinically for cardiovascular disease risk reduction	**1**-lovistatin treatment did not affect clinical or histologic features in patients with atypical nevi **2**- results are mixed	Low	**LOW**- no pending trials
NAC	**1**-oral NAC before UV delays tumor appearance in mouse melanoma model. Chronic NAC increases lymph node metastasis in $BRAF^{V600E}$ mouse **2**-topical NAC prevents NMSC in UV-induced mouse model	**1**-Phase I trial of nevi irradiated ex vivo showed NAC relived UV-induced glutathione depletion. Phase II trial with in vivo irradiation failed to show modulation of study endpoints in treatment arm.	Chronic treatment causes lung cancer progression in a mouse model	**LOW**- no pending clinical trials
DFMO	**2**-Oral and topical DFMO decrease NMSC in UV-induced mouse model **3**-Combo with γ-interferon arrests growth of melanoma cells.	**1**-Placebo-controlled Phase 3 trial showed decrease in BCCs. Phase 1 showed decrease in AKs with topical application. Phase IIb +/− dichlofenac (topical) failed to decrease polyamine levels.	Oral DFMO associated with hearing loss. Topical formulations can be effective without systemic exposure	**LOW-MODERATE**- Requires testing in a mouse model of melanoma. Failure of Phase IIb KC trial, which may have to do with topical formulation, must be addressed.
EGCG	**2**- EGCG nanoparticles decrease xenograft tumor growth. Oral and topical treatment prevents NMSC. **3**- cell growth inhibited in cell culture models.	**1**- Two studies show topical EGCG decreases UV-induced erythema **2**- cohort studies of green tea consumption inconclusive	None reported	**HIGH**-Requires testing in a mouse model of UV-induced melanoma. Topical EGCG preparation (Veregen®) is approved for use in humans; Phase II clinical trial has been completed for basal cell carcinoma treatment no results yet published.
Resveratrol	**1**- inhibits growth of melanoma cells. **2**-topical resveratrol decreases KC and acute effects of UV; analog (ptero-stilbene) applied topically also decreases KC	None	None reported	**LOW-MODERATE**-Clinical trials examining effects of oral grape powder on UV-irradiated skin is now recruiting. Requires testing in a model of UV-induced melanoma
Sulforaphane	**2**- oral and topical SFN prevents NMSC **3**- inhibits growth of melanoma cells	**1**- Topical SFN decreases UV-induced erythema	None reported	**MODERATE**-Many clinical trials planned or not yet published. Requires testing in a mouse model of UV-induced melanoma.

Table 2	Pre-clinical evidence	Clinical evidence	Adverse effects, limitations	Potential for clinical impact in the near term, next steps and/or pending trials
Lycopene and bixin	**2-** Bixin (lycopene metabolite) treated mice had significantly decreased UV-induced oxidative DNA damage and inflammation **3-** Bixin upregulates antioxidant systems in keratinocytes	**1-** Lycopene from tomato supplements ameliorates markers of UV damage including erythema, increased levels of mitochondrial DNA damage and MMP-1 expression, and reduction in dermal fibrillin-1.	No reported adverse effect although bixin doses used in mouse studies are high relative to the acceptable daily intake (ADI) for humans.	**LOW-MODERATE-**Have not yet been examined in any skin cancer model. Requires testing in a mouse model of melanoma.
Polypodium leucotomas extracts	**2-** inhibits NMSC in UV-irradiated mice **3-** prevents UV-induced apoptosis of keratinocytes	**1-** significant increase in MED in high-risk melanoma patients treated with Fernblock®. A second study finds significant reduction of histologic markers of UV damage.	None reported	**MODERATE-**Active ingredients are 1% (w/w) of extract. See Conclusions for discussion. One clinical trial for prevention of AKs and SCC is pending.
Silibinin	**2-** prevents growth of melanoma in mouse xenograft; suppresses UV-induced KC in hairless mouse	None	Potential phototoxicity of minor component observed in cell culture	**MODERATE-HIGH** Possibly available as well-characterized cosmeceutical; requires testing in a mouse model of melanoma

** Women only, primary outcome was new invasive cancer diagnosis at any site except KC. Secondary endpoints were lung, colorectal and breast cancers.