A critical Review of the Preliminary Opinion Report of the European Commission`s Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) on *„Biological effects of ultraviolet radiation relevant to health with particular reference to sunbeds for cosmetic purposes“*

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Summary

On 22-01-2016, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) of the European Commission published a preliminary opinion on the biological effects of ultraviolet radiation relevant to health with particular reference to sunbeds for cosmetic purposes, for public consultation, that was supposed to look at the rationale for and risks associated with the use of sunbeds. In their report, that was announced to be based on the available scientific evidence, the SCENIHR concludes that: (i) ultraviolet radiation (UVR) is a complete carcinogen, both an initiator, and a promoter; (ii) there is strong evidence that sunbed exposure causes skin melanoma, squamous cell carcinoma and, to a lesser extent, basal cell carcinoma, especially when exposure starts young; (iii) there is also some evidence that sunbed exposure may cause ocular melanoma; (iv) sunbed use is responsible for a noticeable proportion of both melanoma and non-melanoma skin cancers and for a large percentage of melanomas arising before the age of 30. SCENIHR also concludes that: (i) sunbed exposure has little health benefits, and there is no need to use sunbeds for optimal Vitamin D levels; and that (ii) because of evidence of the carcinogenic effects of sunbed exposure and of the nature of skin cancer induction (there are no indications for threshold levels of UV-irradiance and UV–dose), there is no safe limit for UV irradiance from sunbeds. Unfortunately however, this report on the preliminary opinion of SCENIHR shows an alarming tendency for an unbalanced view and must be criticized because of many shortcomings, weaknesses, and incorrectnesses, that include the following : (i) main conclusions are not supported by our present scientific knowledge; (ii) it underappreciates the body of evidence from epidemiological and animal studies demonstrating no increase in melanoma risk following chronic UV exposure; (iii) it ignores the large body of evidence demonstrating beneficial health effects of UV radiation; and (iv) it does not recognize the importance of the UV-induced cutaneous vitamin D synthesis for human health and ignores consequences of vitamin D deficiency.

Introduction

When preparing its policy and proposals relating to food safety, consumer safety, public health and the environment, the European Commission relies on independent Scientific Committees that should provide it with sound scientific advice and draw its attention to new and emerging problems (1; p.2., l. 1-13 of the SCENIHR opinion). These Scientific Committees can call on additional expertise from a pool of scientific advisors and a database of experts (1). Directorate Health and Food Safety manages work of three Scientific Committees responsible for non-food issues, including the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (1). On 22-01-2016, SCENIHR published a preliminary opinion on the biological effects of ultraviolet radiation relevant to health with particular reference to sunbeds for cosmetic purposes (2), for public consultation, that was supposed to look at the rationale for and risks associated with the use of sunbeds (1,2). In their report (2), that was announced to be based on the available scientific evidence, the SCENIHR concludes (p.6, l. 24-37) that: (i) ultraviolet radiation (UVR) is a complete carcinogen, both an initiator, and a promoter; (ii) there is strong evidence that sunbed exposure causes skin melanoma, squamous cell carcinoma and, to a lesser extent, basal cell carcinoma, especially when exposure starts young; (iii) there is also some evidence that sunbed exposure may cause ocular melanoma; (iv) sunbed use is responsible for a noticeable proportion of both melanoma and non-melanoma skin cancers and for a large percentage of melanomas arising before the age of 30. SCENIHR also concludes that: (i) sunbed exposure has little health benefits, and there is no need to use sunbeds for optimal Vitamin D levels; and that (ii) because of evidence of the carcinogenic effects of sunbed exposure and of the nature of skin cancer induction (there are no indications for threshold levels of UV-irradiance and UV–dose), there is no safe limit for UV irradiance from sunbeds.

Unfortunately however, this report on the preliminary opinion of SCENIHR (2) shows an alarming tendency for an unbalanced view and must be criticized because of many shortcomings,

weaknesses, and incorrectnesses, that include the following (selection): (i) main conclusions are not supported by the data presented and are not supported by our present scientific knowledge; (ii) it underappreciates the body of evidence from epidemiological and animal studies demonstrating no increase in melanoma risk following chronic UV exposure; (iii) it ignores body of evidence demonstrating beneficial health effects of UV radiation; and (iv) it ignores consequences of vitamin D deficiency.

Comments (C.) 1-5: Main conclusions of the SCENIHR report are not supported by our present scientific knowledge

C. 1: As a major conclusion, the SCENIHR report states repeatedly that "there is strong evidence that sunbed exposure causes skin melanoma" (2; e.g. p. 6., l. 26; p. 11, l. 45-46). In contrast to the SCENIHR report (e.g. p. 34, l. 29- p. 42, l. 31), we conclude, based on all the available scientific evidence, that there is at present no convincing evidence that solarium use increases melanoma risk. Our present scientific knowledge (3-54) is based on observational studies (30 case-control (cc) and 2 cohort (co) studies) with poor quality, several meta-analyses, (e.g. Boniol et al. 2012 (44): summary relative risk of 1·20 (95% CI 1·08-1·34) for the association of ever exposure to UV radiation from sunbeds with melanoma risk (based on 27 studies)) and other reports, which report associations but do not prove causality (3-54). Overall quality of these studies is poor, due to lack of interventional studies and severe limitations of cc and co studies that include unobserved or unreported confounding. Moreover, study-limitations cause overestimation of the association of solarium use with melanoma risk because of: (i) Recall bias; (ii) dermatologic phototherapy is often included (e.g. PUVA – Landi et al. (20)); (iii) control selection bias (e.g. subgroups of individuals with presumably high UV exposure in the past (e.g. individuals with "non-melanoma skin cancer" or "dermatologic conditions" are in many studies excluded in controls but not in cases).

C. 2: Additionally, and in contrast to the SCENIHR opinion (e.g. p.5, l. 31-34; p. 10, l. 43- p. 11., l.

1; p. 60, l. 38-41), it has to be noted that studies available are characterized by high heterogeneity and by difficulties in adjusting for important confounding factors, including solar UV and life style: only a minority of studies report odds ratios (ORs) adjusted for the same confounding factors, 12 studies not for a single confounder. 20 studies adjusted for age (n=15), gender (n=11), skin colour (n=11), hair colour (n=10), sun exposure (n=8), sunburns (n=8), family history of melanoma (n=7), naevi (n=7), freckles (n=5) and/or education (n=5). Moreover, because of individual confounders were assessed using different techniques, these studies are only partly comparable. It has to be emphasized that one has to distinguish between associations, as reported in these cc/co studies and meta-analyses, and causation. In this context, we are convinced that same results and risk estimates as in Boniol et al. (44) and Colantonio et al. (47) could be obtained in the following scenario: Solarium use does not have any effect on melanoma risk, life style factors such as extensive sunbathing in the summer as sun worshipper and/or "unhealthy lifestyle" (e.g. alcohol, smoking), do increase melanoma risk with true OR=1.2 (it has been reported previously that sun worshippers and individuals with "unhealthy lifestyle go more frequently to tanning salons). In this context, it has to be emphasized that many confounding factors, including extensive sunbathing in the summer and "unhealthy lifestyle", were not systematically considered in studies so far.

C. 3: In strong contrast to the SCENIHR-report (who uses a highly questionable classification of Evidence levels; e.g. p. 17, l. 11-28; who states "strong evidence"; e.g. p. 6., l. 26), we therefore postulate (due to lack of interventional studies and severe limitations including unobserved or unrecorded confounding) for all main outcomes (association of ever exposure, first exposure at younger age and high/low exposure to UV radiation from a solarium with melanoma risk) reported in meta-analyses (44,46,47), according to generally accepted recommendations of the Oxford Centre for Evidence-based Medicine (55), a resulting level four of evidence (poor quality cohort and case-control studies) and a resulting grade C of recommendation. In conclusion, our present scientific knowledge (also considering poor study quality, poor levels of evidence and grades of recommendation) does not support the hypothesis that solarium use may increase melanoma risk.

C. 4: Therefore, many statements of the SCENIHR report (e.g. p. 6, l. 26, l. 29-31, l. 35-37) are in contrast with our present scientific knowledge, including the attempts of the SCENIHR report (p.6, l. 14-23; p. 11, l. 33-42; p. 60, l. 42-44) and others (44,48) to attribute melanoma cases to solarium use, that are speculative and are scientifically not sufficiently supported.

C. 5: SCENIHR should provide the European Commission with the scientific advice it needs when preparing policy for the European population (1,2; e.g. p. 2, l. 1-10). In this context, it has to be emphasized, that the conclusions of the SCENIHR report are based on data that do not reflect the present situation in Europe. It is well known that regional differences, including impact of confounding factors (e.g. solar UV exposure), technical differences of UV-emitting devices, and/or differences in their operating, strongly influence the association of ever exposure to UV radiation from sunbeds with melanoma risk (3-54). Therefore, it is alarming that this SCENIHR report conceals the very important finding, that meta-analyses of studies performed in Europe do not show an association of ever exposure to UV radiation from sunbeds with increased melanoma risk (47). Because of the high number of participants in European studies, this result is most likely not due to a loss of power, but reflects regional differences concerning impact of confounding factors (e.g. solar UV exposure), technical differences of UV-emitting devices, and/or differences in their operating (47).

It has also to be noted that the conclusions of the SCENIHR report are based on historical data that do not reflect the present situation in Europe. Many studies included individuals with skin type I, who are at present in Europe not allowed to use a solarium. Moreover, many studies included data obtained by technical devices that are at present not allowed to be used in Europe.

C. 6, 7: The SCENIHR report underappreciates the large body of evidence from epidemiological and animal studies demonstrating no increase in melanoma risk following chronic UV exposure

C. 6 (p. 11, l. 13-32): Experimental animal models, including genetically engineered mice, the Xiphophorus hybrid fish, the south american oppossum, and human skin xenografts, constitute important platforms upon which to build strategies designed to further elucidate the pathogenesis of UV-induced melanomagenesis. It underlines the unbalanced view of the SCENIHR report, that it underappreciates the large body of evidence from epidemiological and animal studies demonstrating no increase in melanoma risk following suberythemal UV exposure. As an example, important informations were obtained analyzing UV-inducible melanomagenesis in the HGF/SF transgenic mouse (56-58). Using this model, it was demonstrated that dermal melanomas arise in untreated mice with a mean onset age of approximately 21 months, a latency that was not overtly altered in response to chronic suberythemal, or skin non-reddening UV irradiation (p. 43, l. 11-20; Ref. 56-58). In contrast, a single erythemal dose to 3.5-day-old-neonatal HGF/SF mice induced cutaneous melanoma with significantly reduced latency (56-58). Moreover, the UV-induced murine melanomas frequently resembled their human counterparts with respect to histopathological appearance and graded progression. Many other studies also support the concept that exposure with suberythemal UV-doses not only does not increase melanoma risk, but may even be protective (e.g. 59-62).

C. 7 (p. 11, l. 21-28; p. 44, l. 14-16; p. 60, l. 20-22): The SCENIHR report conceals that the relevance of UV signature mutation patterns has not been shown for the BRAF gene and for other important drivers of melanomagenesis.

C. 8: The SCENIHR report ignores the large body of evidence demonstrating beneficial health effects of UV radiation

C. 8: It further underlines the unbalanced view of the SCENIHR report (e.g. concerning term of reference 1; p. 15, l. 9-15), that it conceals the large body of evidence demonstrating beneficial health effects of UV radiation (e.g. 63-105). As an example, a large cohort study reported recently a longer life expectancy amongst participants with active sun exposure habits, that was related to a decrease in cardiovascular disease (CVD) and non-cancer mortality (64). Many of the well documented beneficial health effects of UV radiation are mediated via vitamin D (see following chapter), or via other factors. Melatonin is involved in the circadian system with higher levels during the night than in the daytime. Light information from the retina influences the production of melatonin via the suprachiasmatic nuclei of the hypothalamus. A mutation of the melatonin receptor affecting the melatonin system (MTNR1B) is known to be related to increased risk of type 2 diabetes, through the inhibition of insulin release (rev. in 64). Thus, sun exposure may affect susceptibility to type 2 diabetes mellitus by interfering with the melatonin system. Hypertension is a major determinant of CVD. Observational data support the notion that lack of UVB radiation is involved in the pathogenesis of hypertension and CVD by (i) suppression of the renin–angiotensin–aldosterone system, (ii) a direct effect on endothelial cells and (iii) effects on calcium metabolism (rev. in 64). A lack of either UVB or UVA light produced a short-term reduction in blood pressure (rev. in 64). Solar UVA radiation may also produce systemic NO with a sustained reduction in blood pressure and has been suggested to act in a cardioprotective manner (rev. in 64). Both high acute and chronic stress levels have a role in the activation of coagulation and may increase the risk of CVD (rev. in 64). The finding that UV radiation induces β-endorphin synthesis, which may attenuate stress levels and have a cardioprotective effect, is interesting (rev. in 64).

C. 9: The SCENIHR report does not recognize the importance of the UV-induced cutaneous vitamin D synthesis for human health and ignores the consequences of vitamin D deficiency

C. 9: The SCENIHR report does not consider the large body of evidence demonstrating hazardous effects of vitamin D deficiency (73-105). The statement of the SCENIHR report: "Usual exposure to UVR from the sun (even on cloudy days) and a normal diet are sufficient to achieve a sufficient vitamin D level" (p. 10, l. 28-30; p. 34, l. 18-20) is not correct. How does SCENIHR explain that approximately 60% of the population in many European countries, including Germany (106), are vitamin D deficient/insufficient? It has been estimated that at present, although oral vitamin D supplements are easily available, approximately one billion people worldwide are vitamin D-deficient or –insufficient (74). This epidemic causes serious health problems that are still widely under-recognized (73-77). Apart from well documented problems for bone and muscle function, there are associations between vitamin D-deficiency and increased incidence of and/or unfavourable outcome for a broad variety of independent diseases including various types of malignancies (e.g. colon-, skin-, and breast cancer), autoimmune-, infectious- and cardiovascular-diseases (73-105).

A large meta-analysis has assessed the beneficial and harmful effects of vitamin D supplementation for prevention of mortality in healthy adults and adults in a stable phase of disease (102). In that study, 56 randomised trials with 95,286 participants provided usable data on mortality. The age of participants ranged from 18 to 107 years. Most trials included women older than 70 years. The mean proportion of women was 77%. Forty-eight of the trials randomly assigned 94,491 healthy participants. Of these, four trials included healthy volunteers, nine trials included postmenopausal women and 35 trials included older people living on their own or in institutional care. The remaining eight trials randomly assigned 795 participants with neurological, cardiovascular, respiratory or rheumatoid diseases. Vitamin D was administered for a weighted mean of 4.4 years. More than half of the trials had a low risk of bias. All trials were conducted in high-income countries. Forty-five trials (80%) reported the baseline vitamin D status of participants based on

serum 25-hydroxyvitamin D levels. Participants in 19 trials had vitamin D adequacy (at or above 20 ng/mL). Participants in the remaining 26 trials had vitamin D insufficiency (less than 20 ng/mL). Vitamin D decreased mortality in all 56 trials analysed together (5,920/47,472 (12.5%) vs 6,077/47,814 (12.7%); RR 0.97 (95% confidence interval (CI) 0.94 to 0.99); P = 0.02; I(2) = 0%). More than 8% of participants dropped out. 'Worst-best case' and 'best-worst case' scenario analyses demonstrated that vitamin D could be associated with a dramatic increase or decrease in mortality. Trial sequential analysis supported the findings regarding vitamin D3, with the cumulative Z-score breaking the trial sequential monitoring boundary for benefit, corresponding to 150 people treated over five years to prevent one additional death. Vitamin D3 statistically significantly decreased cancer mortality (RR 0.88 (95% CI 0.78 to 0.98); P =0.02; I(2) = 0%; 44,492 participants; 4 trials). (102)

Notably, a large body of evidence now clearly demonstrates the relevance of the vitamin D endocrine system (VDES) for skin cancer prevention and therapy. Some aspects of this topic are outlined in the following paragraphs, that represent a citation of reference 105.

“*Cross-talk between VDR and p53 signaling pathways*

Increasing evidence indicates an important role of the VDES for skin carcinogenesis. It has been stated that the VDR, mostly due to its ligand-induced growth-regulatory effects, acts as a tumor suppressor in skin (13,71). Both vitamin D- and p53-signaling pathways have a significant impact on spontaneous or carcinogen-induced malignant transformation of cells, with vitamin D receptor (VDR) and p53 representing important tumor suppressors (13,71). VDR and the p53/p63/p73 proteins (the p53 family) all function typically as receptors/sensors-that-turn-into-transcriptional-regulators-upon-stimulus, with the main difference being that the nuclear VDR is transcriptionally activated after binding its naturally occurring ligand $1,25(OH)_2D_3$ with high affinity while the p53 clan, mostly in the nucleoplasm, responds to a large number of alterations in cell homeostasis commonly referred to as stress (13). Interestingly, an increasing body of evidence now convincingly

demonstrates a cross talk between vitamin D- and p53 signalling that occurs at different levels, has genome-wide implications and that should be of high importance for many malignancies, including non-melanoma skin cancer (13,17). One interaction involves the ability of p53 to regulate skin pigmentation (13). It has been shown that p53 upregulates skin pigmentation via POMC derivatives including alpha-MSH and ACTH (13). Increased pigmentation protects the skin against UV-induced DNA damage and skin carcinogenesis, but on the other hand reduces cutaneous synthesis of vitamin D (13). A second level of interaction may be through the ability of $1,25(OH)_2D_3$ to increase the survival of skin cells after UV irradiation (13). UV irradiation-surviving cells show significant reductions in thymine dimers in the presence of $1,25(OH)_2D_3$ that are associated with increased nuclear p53 protein expression, and significantly reduced NO products (13). A third level of interaction is documented by the ability of vitamin D compounds to regulate the expression of the murine double minute (MDM2) gene in dependence of the presence of wild type p53 (13,17). MDM2 has a well established role as a key negative regulator of p53 activity (17). The E3 ubiquitin ligase and transcriptional repressor MDM2 is a potent inhibitor of the p53 family of transcription factors and tumor suppressors (17). It was reported that VDR is also bound and inhibited by MDM2 (17). This interaction was not affected by vitamin D ligand (17). VDR was ubiquitylated in the cell and its steady-state level was controlled by the proteasome (17). Strikingly, overproduced MDM2 reduced the level of VDR whereas knockdown of endogenous MDM2 increased the level of VDR (17). In addition to ubiquitin-marking proteins for degradation, MDM2, once recruited to promoters by DNA-binding interaction partners, can inhibit the transactivation of genes (17). Transient transfections with a VDR-responsive luciferase reporter revealed that low levels of MDM2 potently suppress VDR-mediated transactivation. Conversely, knockdown of MDM2 resulted in a significant increase of transcript from the CYP24A1 and p21 genes, noted cellular targets of transactivation by liganded VDR (17). These findings (17) suggest that MDM2 negatively regulates VDR in some analogy to p53 (17). Finally, p53 and its family members have been implicated in the direct regulation of the VDR (13).

VDES in non-melanoma skin cancer

Using immunohistochemical techniques and real-time PCR, strong expression of key components of the VDES (VDR, CYP24A1, CYP27A1, CYP27B1) has been demonstrated in cutaneous basal (BCC) and squamous (SCC) cell carcinomas previously (72-74). Interestingly, expression of VDR, CYP24A1, and CYP27B1 is stronger in BCCs and SCC as compared to unaffected, normal skin (72-74). These findings provide supportive evidence for the concept that endogeneous synthesis and metabolism of vitamin D metabolites as well as VDR expression may regulate growth characteristics of BCCs and SCCs. It has been shown that mouse and human BCC and SCC cell lines respond well against the antiproliferative effects of biologically active vitamin D compounds (72,75). Additionally, it has been demonstrated that calcitriol inhibits proliferation and growth of BCCs of patched (Ptch) mutant mice in vitro and in vivo (75). As assessed by reduced Gli1 transcription, it has recently shown that calcitriol inhibits canonical Hh signaling independently of VDR signaling and downstream of Ptch. An obvious molecular target of this VDR-independent effect of calcitriol is Smo, because Smo-deficient cells show no decreased Gli1 transcription in response to this substance. A similar observation has been made for the inactive form of calcitriol, vitamin D3 (76). According to this work, Ptch might function as an efflux pump for vitamin D-related compounds with hedgehog (Hh)-inhibitory potential.

Considering the importance of the VDES for carcinogenesis of BCCs and SCCs that is outlined in this review, it is no surprise that low 25(OH)D serum concentrations and genetic variants of the VDES have recently drawn attention as potential risk factors for occurrence and prognosis of non-melanoma skin cancer. Expression and function of the VDR protein can be affected by SNPs in the VDR gene (77,78). Associations indicate that the Apa1 and Taq1 genotypes of VDR may be of importance for carcinogenesis of BCCs, but not for SCCs (79). Associations of the BSM1 polymorphism with BCC (80) and SCC (81) have also been reported. In conclusion, an increasing body of evidence now indicates that the VDES is of relevance for carcinogenesis and progression of

non-melanoma skin cancer and that vitamin D compounds may hold promise as effective agents for the prevention and treatment of these malignancies.

VDES in melanoma

The relevance of the VDES for tumorigenesis and prognosis of malignant melanoma has been realized for several decades (82). The presence of the VDES (VDR, CYP27A1, CYP27B1, CYP24A1) in normal melanocytes and in malignant melanoma has been characterized in vitro and in situ (83), indicating that endogeneous synthesis and metabolism of vitamin D metabolites as well as VDR expression may modulate growth both of normal melanocytes and of melanoma cells in vitro and in vivo (83).

When the effects of $1,25(OH)_2D_3$, its analog seocalcitol (EB 1089), and 25(OH)D3, on the proliferation of seven melanoma cell lines were analysed in vitro (83), three cell lines (MeWo, SK-Mel-28, SM) responded to antiproliferative effects of active vitamin D analogs, while the remaining (SK-Mel-5, SK-Mel-25, IGR, MelJuso) were resistant. A strong increase (up to 7000-fold) of CYP24A1 mRNA was observed in responsive cell lines after stimulation with $1,25(OH)_2D_3$, indicating functional integrity of VDR-mediated transcription. In contrast, induction of CYP24A1 was much lower in resistant melanoma cells (70-fold). VDR mRNA was induced up to 3-fold both in responsive and resistant cell lines after stimulation with $1,25(OH)_2D_3$. In that study, RNA for vitamin D-activating enzymes CYP27A1 and CYP27B1 was detected in all melanoma cell lines analyzed, additionally splicing variants of CYP27B1 were shown in SK-Mel-28 cells. Expression of CYP27A1 and CYP27B1 was marginally modulated along with treatment. Growth of melanoma cells was not inhibited by treatment with $25(OH)D_3$, indicating no induction of endogeneous production of $1,25(OH)_2D_3$. In conclusion, the VDES has been characterized in melanoma cells and it was demonstrated that the majority of melanoma cell lines analyzed is resistant to antiproliferative effects of $1,25(OH)_2D_3$. The authors concluded, that only a minority of cases with metastasizing melanoma may represent a promising target for palliative treatment with new vitamin D analogs that exert little calcemic side effects or for pharmacological modulation of endogeneous

$1,25(OH)_2D_3$-synthesis/metabolism. Remarkably, it was previously that $1,25(OH)_2D_3$-sensitivity of melanoma cells can, at least partially, be restored by co-stimulation with the histone deacetylase inhibitor (HDACI) trichostatin A (TSA) or with the DNA methyltransferase inhibitor (DNMTI), 5-azacytidine (5-Aza) (84). It was shown that stimulation with $1,25(OH)_2D_3$ and/or epigenetic drugs (5-Aza, TSA) modulated the VDR mRNA expression in $1,25(OH)_2D_3$-responsive and -resistant melanoma cell lines and in cultured normal human melanocytes (NHM). Treatment with 5-Aza, but not with TSA, reduced the expression of a VDR regulating microRNA (miR-125b) in $1,25(OH)_2D_3$-responsive and -resistant melanoma cell lines and in NHM. Treatment with $1,25(OH)_2D_3$ and/or epigenetic drugs (5-Aza, TSA) reduced the expression of another VDR-regulating microRNA (miR 27b) in three out of four melanoma cell lines. It was concluded that responsiveness to $1,25(OH)_2D_3$ corresponds to the expression level of VDR mRNA which in turn might be regulated by VDR microRNAs (miR-27b, miR-125b) or by epigenetic modulation (84).

Considering the importance of the VDES for cancer, it is no surprise that low 25(OH)D serum concentrations and genetic variants of the VDES have drawn attention as potential risk factors for occurrence and prognosis of melanoma. In 2000, an association of Fok 1 restriction fragment length polymorphisms (RFLP) of the VDR gene with occurrence and outcome of malignant melanoma, as predicted by Breslow thickness, was reported (85). The same laboratory demonstrated thereafter that a SNP in the promotor region of VDR (A-1012*G*, adenine-guanine substitution -1012 bp relative to the exon 1a transcription start site) is associated in melanoma patients with greater Breslow thickness and with the development of metastatic disease (86). The authors concluded that polymorphisms of the VDR gene, which can be expected to result in impaired function of biologically active vitamin D metabolites, are associated with susceptibility and prognosis in malignant melanoma. In recent years, many studies have convincingly reported an association of VDR SNPs with occurrence and outcome of malignant melanoma, although it has to be noted that a few investigations showed negative results. The interaction between VDR polymorphisms and sun exposure was investigated in a population-based multinational study comparing 1138 patients with

a multiple (second or subsequent) primary melanoma (cases) to 2151 patients with a first primary melanoma (controls) (87). This was essentially a case-control study of melanoma in a population of melanoma survivors. Sun exposure was assessed using a questionnaire and interview, and was shown to be associated with multiple primary melanoma. VDR was genotyped at the FokI and BsmI loci and the main effects of variants at these loci and their interactions with sun exposure were investigated. The authors reported that only the BsmI variant was associated with multiple primary melanoma (odds ratio [OR]=1.27, 95% confidence interval [CI], 0.99-1.62 for the homozygous variant genotype) and concluded that these findings indicate a higher risk of multiple primary melanomas in people who have the BsmI variant of VDR.

The association of VDR polymorphisms and the risk of cutaneous melanoma was analyzed in a meta-analysis (88). Six studies (cases, 2152; controls, 2410) that investigated the association between 5 VDR polymorphisms (TaqI, FokI, BsmI, EcoRV, and Cdx2) and the risk of melanoma were retrieved and analyzed. The model-free approach was applied to meta-analyze these molecular association studies. Available data suggested a significant association between the BsmI VDR polymorphism and melanoma risk (pooled OR, 1.30; 95% CI, 1.11-1.53; P= .002; heterogeneity Cochran Q test, P> .1), and the population-attributable risk was 9.2%. In contrast, the FokI polymorphism did not appear to be associated with such risk (OR, 1.09; 95% CI, 0.99-1.21; P= .07; heterogeneity Cochran Q test, P> .1). For the TaqI and the EcoRV polymorphisms, significant between-study heterogeneity did not support genotype data pooling. Only 1 study investigated the Cdx2 variant, and the findings were negative. Current evidence is in favor of an association between 1 VDR gene polymorphism (BsmI) and the risk of developing melanoma. These findings prompt further investigation on this subject and indirectly support the hypothesis that sun exposure may have an anti-melanoma effect through activation of the VDES.

Several studies reported a strong inverse correlation between serum 25(OH)D concentrations and Breslow thickness (89-92). Among the patients with malignant melanoma, significantly reduced serum 25(OH)D levels were found in the stage IV patients as compared to stage I patients, and

those with low 25(OH)D serum levels (<10 ng/ml) may develop earlier distant metastatic disease compared to those with higher 25(OH)D serum levels (>20 ng/ml) (90). In a follow up study (91), serum 25(OH)D concentrations were retrospectively analyzed in a cohort of melanoma patients (n=324) and healthy controls (n=141) to test the hypothesis that serum 25(OH)D concentrations are predictive of melanoma risk, thickness of primary melanomas, and overall survival (OS). Median serum 25(OH)D concentrations were significantly lower (p=0.004) in melanoma patients (median=13.6 ng/ml) as compared to controls (median=15.6 ng/ml) (91). Primary tumors of patients with low serum 25(OH)D concentrations (<10 ng/ml) had significantly (p=0.006) greater Breslow thickness (median: 1.9 mm) as compared to patients with higher levels (>20 ng/ml; median: 1.00 mm). Patients with 25(OH)D serum concentrations in the lowest quartile had inferior overall survival (median: 80 months) comparing with the highest quartile (median: 195 months; p=0.049).

Our results are in agreement with a recent study that analyzed plasma samples from 1,042 prospectively observed patients with melanoma for 25(OH)D serum concentration and CRP (92). The associations of demographics and CRP with 25(OH)D serum concentration were determined, followed by a determination of the association between 25(OH)D serum concentration and stage and outcome measures from the date of blood draw (92). The median follow-up time was 7.1 years (92). In that study, a lower 25(OH)D serum concentration was associated with the blood draw during fall/winter months (P < .001), older age (P = .001), increased CRP (P < .001), increased tumor thickness (P < .001), ulcerated tumor (P = .0105), and advanced melanoma stage (P = .0024) (92). On univariate analysis, lower 25(OH)D serum concentration was associated with poorer overall (OS; P < .001), melanoma-specific survival (MSS; P = .0025), and disease-free survival (DFS; P = .0466) (92). The effect of 25(OH)D serum concentration on these outcome measures persisted after adjustment for CRP and other covariates (92). Multivariable hazards ratios per unit decrease of 25(OH)D serum concentration were 1.02 for OS (95% CI, 1.01 to 1.04; P = .0051), 1.02 for MSS (95% CI, 1.00 to 1.04; P = .048), and 1.02 for DFS (95% CI, 1.00 to 1.04; P = .0427) (92).

Although lower 25(OH)D serum concentration was strongly associated with higher CRP, the associations of lower 25(OH)D serum concentration with poorer OS, MSS, and DFS were independent of this association (92). The authors concluded that lower 25(OH)D serum concentrations in melanoma patients were associated with poorer outcomes and that analysis of mechanisms responsible for these associations may be of value to patients with melanoma (92).

In summary, these findings support the concept that serum 25(OH)D concentrations are associated with risk and prognosis of melanoma. However, it has to be noted that most of these investigations are association studies that do not allow a conclusion of a causal relationship and that randomized controlled trials are still lacking. Whether normalizing serum 25(OH)D concentrations in these patients improves outcomes will require testing in future clinical trials.

In light of inverse relationships reported in observational studies of vitamin D intake and serum 25(OH)D concentrations with risk of nonmelanoma skin cancer (NMSC) and melanoma, the effects of vitamin D (400 IU daily) combined with calcium supplementation (1000 mg daily) on skin cancer were recently evaluated in a randomized placebo-controlled trial analyzing postmenopausal women age 50 to 79 years (N = 36,282) enrolled onto the Women's Health Initiative (WHI) calcium/vitamin D clinical trial (mean follow-up period of 7.0 years) (93). Neither incident NMSC nor melanoma rates differed between treatment hazard ratio [HR], 1.02; 95% CI, 0.95 to 1.07) and placebo groups (HR, 0.86; 95% CI, 0.64 to 1.16) (93). In subgroup analyses, women with history of NMSC assigned to CaD had a reduced risk of melanoma versus those receiving placebo (HR, 0.43; 95% CI, 0.21 to 0.90; P(interaction) = .038), which was not observed in women without history of NMSC (93). The authors concluded that vitamin D supplementation at a relatively low dose plus calcium did not reduce the overall incidence of NMSC or melanoma (93). However, in women with history of NMSC, CaD supplementation reduced melanoma risk, suggesting a potential role for calcium and vitamin D supplements in this high-risk group (93). The authors concluded that results from this post hoc subgroup analysis should be interpreted with caution but warrant additional investigation (93). It can be speculated whether vitamin D supplementation at a more appropriate,

higher dose (e.g. 1000 -2000 IU) would have reduced the overall incidence of NMSC or melanoma in that study."

C. 10: (p. 11, l. 33-42) It should be noted that decreases of melanoma mortality rates during the last decades do not support the hypothesis that UV radiation from sunbeds may have increased melanoma risk. While melanoma death rates had more than doubled in light-skinned populations between 1955 and 1985, decreases of melanoma mortality rates have been observed from 1985-1990 in Australia, the United States and in many European countries. It also should be noted that the authors of a recent paper analyzing the forthcoming inexorable decline in light-skinned populations concluded that independently from screening or treatment, death from malignant melanoma is likely to become an increasingly rare event (106).

C. 11: We disagree with the final conclusion of the SCENIHR report: "New studies would therefore not be a priority for future work." (p. 62, l. 4-5) We are convinced that well-designed studies are urgently needed and would like to strengthen the fact that, at present, meta-analyses of studies performed in Europe do not show an association of ever exposure to UV radiation from sunbeds with increased melanoma risk (47).

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