ABSTRACTS OF THE JOINT INTERNATIONAL SYMPOSIA "VITAMIN D IN PREVENTION AND THERAPY" AND "BIOLOGIC EFFECTS OF LIGHT"

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Oral presentations (OP)

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OP No. 1
LIGHT, THE CIRCADAIN CLOCK, AND BEHAVIORAL DESPAIR

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Background/Aim: In humans, day-length has a remarkable effect on mood status. It is generally accepted that short winter days are often associated with a form of depression termed seasonal affective disorder. Light therapy is effective in alleviating the symptoms of this disorder, but the mechanisms how light exerts these therapeutical effects are unknown. *Materials and Methods/Results:* Applying a mouse model system, we were able to demonstrate that mice show similar responses to light as humans. We observed beneficial effects of light on the mood-related behaviors of mice that correlated with the positive effects of light on the depressive behavior of humans. We were able to identify the clock gene *Period 1* as an important component, necessary to exert the beneficial effects of light on the depressive behavior of mice. *Conclusion:* Light has beneficial effects on the mood-related behaviors of mice and humans. *Period 1* mediates the beneficial effects of light on the depressive behavior of mice.

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OP No. 2
COLORECTAL CANCER, VITAMIN D AND MICROBIOTA: A DOUBLE-BLIND PHASE II RANDOMIZED TRIAL IN COLORECTAL CANCER PATIENTS

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Background/Aim: In recent years, several studies have shown microbial composition changes in colorectal cancer (CRC) patients and supported the idea that gut microbiota may play an important role in cancer initiation and progression. Moreover, CRC risk was found to be inversely correlated with vitamin D levels, with data showing a significant reduction in risk when comparing the highest levels *versus* the lowest levels of 25(OH)D. However, little is still known on the interplay between these factors. *Patients and Methods:* In total, 74 CRC patients after standard treatment were randomly assigned to 2000 IU/day vitamin D supplementation or placebo and treated for 1 year. Randomization was stratified according to whether the patient had received chemotherapy during treatment or not. For each patient, fecal microbiota (shotgun metagenomics sequencing) was available at baseline and post-treatment. *Results:* Alpha diversity change overtime was not significantly different between the two arms (p=1), and beta diversity did not differ between the two groups either at baseline (p=0.99) and post treatment (p=0.70). At follow-up, supervised and unsupervised analysis showed a higher abundance of beneficial species in the supplemented group, such as *Faecalibacterium prausnitzii* and *Holdemanella biformis*, and a higher abundance of species usually associated to human infections in the placebo group, such as *Hafnia alvei*, *Shigella boydii,* and *Raoultella ornithinolytica*. For each patient, information on microbiota composition was summarized into a score using principal component analysis. In multivariate analysis, vitamin D supplementation was significantly associated with the microbiota score (p=0.02). We also found sex differences in 25(OH)D levels bewtween the two arms: specifically, in the placebo group we observed a higher increase in 25(OH)D levels in men compared to women, whereas in the supplemented group of patients, the opposite was observed. *Conclusion:* These preliminary results suggest that intestinal microbiota and vitamin D may interact and affect each other; however, further investigation is necessary to understand whether this interplay affects cancer development and progression.

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OP No. 3
FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF CHOLESTEROL-, VITAMIN D_2- AND D_3- HYDROXYLASES FROM *BACILLUS MEGATERIUM*

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Background/Aim: Not only mammalian, but also bacterial cytochromes P450s can catalyse the conversion of cholesterol and vitamin D to their hydroxyderivatives. Since it is easier to apply bacterial P450s as biocatalysts than the corresponding mammalian ones, the use of bacterial P450s for industrial production may offer an important biotechnological advantage. *Materials and Methods/Results:* Here, we report the identification of two P450s from *Bacillus megaterium* DSM319, namely CYP109E1 and CYP109A2, which can hydroxylate cholesterol, vitamin D_3, and vitamin D_2, as well as the functional and structural characterization of these enzymes. Very recently, it was demonstrated that CYP109E1 can hydroxylate cholesterol to its highly important derivatives 24(S)- and 25-hydroxycholesterol. As demonstrated by nuclear magnetic resonance analysis, it also converts vitamin D_3 into several hydroxyderivatives, including 24(S)-hydroxyvitamin D_3, 25-hydroxyvitamin D_3, and 24S,25-dihydroxyvitamin D_3 as major products. In addition, we demonstrated that vitamin D_3 is converted by CYP109A2 with high regio-selectivity, producing 25-hydroxyvitamin D_3 as the major metabolite (90% of total products). Moreover, vitamin D_2 was hydroxylated by CYP109E1, leading to a regio- and stereo-selective production of 24(R), 25-diOH vitamin D_2. *Conclusion:* The crystal structures of CYP109A2 and CYP109E1, as well as docking calculations, provide a promising tool to better understand the structural background for the reaction selectivity of these P450s and to generate mutants with improved activities and changed selectivity of hydroxylation.

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OP No. 4
HOW TO RAPIDLY SUBSTITUTE VITAMIN D IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background/Aim: Vitamin D deficiency is a common problem in patients with diffuse large B-cell lymphoma (DLBCL). Prognosis is significantly worse with vitamin D levels <8 ng/ml (1). This is likely due to decreased efficacy of the therapeutic anti-CD20 antibody rituximab. Translational experiments showed that the optimum effect of rituximab on CD20 positive Daudi lymphoma cells was at the vitamin D concentration of 65 ng/ml (2). *Materials and Methods:* We provided a recommendation for rapid vitamin D substitution in the current OPTIMAL study of our DSHNHL study group (OPTIMAL>60; NCT01478542). A total of 99 patients, enrolled from 2014 to 2015, received a weight-based dose of vitamin D after baseline determination: 100× (65-serum level) × kg body weight (3). 20,000 IU vitamin D capsules were administered, with a maximum dose of 200,000 IU. If the level measured in the patients remained <65 ng/ml, additional cycles were administered. *Results*: The baseline vitamin D level was 17±12 ng/ml. A total of 14% of patients could be saturated to 65 ng/ml in the first cycle, requiring 386±137 k IU. On average, the level was increased by 28 ng/ml, to 45±18 ng/ml. In the 2nd cycle, vitamin D serum levels of 53±14 ng/ml were achieved with a mean dose of 188±102 k IU. After the 3rd cycle with 91±56 kIU, vitamin D levels of 56±10 ng/ml were achieved. *Conclusion:* Vitamin D substitution in a weight-based high dose was able to raise vitamin D levels rapidly and safely.

1 Bittenbring JT, Neumann F, Altmann B, Achenbach M, Reichrath J, Ziepert M, Geisel J, Regitz E, Held G and Pfreundschuh M: Vitamin D deficiency impairs rituximab-mediated cellular cytotoxicity and outcome of patients with diffuse large B-cell lymphoma treated with but not without rituximab. J Clin Oncol *32(29)*: 3242-3248, 2014. PMID: 25135997. DOI: 10.1200/JCO.2013.53.4537

2 Neumann F, Acker F, Schormann C, Pfreundschuh M and Bittenbring JT: Determination of optimum vitamin D3 levels for NK cell-mediated rituximab- and obinutuzumab-dependent cellular cytotoxicity. Cancer Immunol Immunother *67(11)*: 1709-1718, 2018. PMID: 30132083. DOI: 10.1007/s00262-018-2224-y

3 van Groningen L, Opdenoordt S, van Sorge A, Telting D, Giesen A and de Boer H: Cholecalciferol loading dose guideline for vitamin D-deficient adults. Eur J Endocrinol *162(4)*: 805-811, 2010. PMID: 20139241. DOI: 10.1530/EJE-09-0932

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OP No. 5

POTENTIAL OF VITAMIN D SUPPLEMENTATION OR VITAMIN D FOOD FORTIFICATION FOR PREVENTING CANCER DEATHS IN GERMANY

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Background/Aim: Meta-analyses of randomized controlled trials (RCTs) have recently demonstrated that vitamin D supplementation significantly reduced cancer mortality (1). We estimated the potential of supplementation of vitamin D or of fortification of foods with vitamin D for avoiding cancer deaths in Germany and for saving cancer-related costs. *Materials and Methods:* We reviewed RCTs investigating the influence of vitamin D supplements on cancer mortality, literature on increases of vitamin D levels by either food fortification or supplementation, and literature on costs of fortification or supplementation. We estimated numbers of prevented cancer deaths, savings, and costs from preventing cancer deaths in Germany by either supplementation of the older adult population (aged 50 years or older) or by fortification of foods. We derived the number of preventable cancer deaths by multiplying sex- and age-specific numbers of cancer deaths with the estimated proportionate reduction in cancer mortality derived by vitamin D supplementation of food fortification. Estimations were based on national data on cancer mortality in 2016. Preventable cancer deaths were multiplied with estimated end-of-life cancer care costs to estimate saved costs. Costs of vitamin D supplementation were estimated at 25€ per person above the age of 50 per year. Yearly costs of vitamin D food fortification were derived from previous estimates based on production costs of cholecalciferol, food control and monitoring costs, marketing and education costs, and further recurrent production costs (2). We performed comprehensive sensitivity analyses. *Results:* In our main analysis, we estimated that vitamin D supplementation could prevent almost 30,000 cancer deaths per year at approximate costs of €900 million and savings of €1.154 billion, implying net savings of €254 million (3). Food fortification is expected to have a similar effect on reducing cancer mortality as supplementation but might achieve that decrease at even substantially larger net savings (4). *Conclusion*: Our findings support the promotion of supplementation of vitamin D among older adults and food fortification as cost-saving approaches to reduce cancer mortality substantially.

1 Keum N, Lee DH, Greenwood DC, Manson JE and Giovannucci E: Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials. Ann Oncol *30*: 733-743, 2019. PMID: 30796437. DOI: 10.1093/annonc/mdz059

2 Sandmann A, Amling M, Barvencik F, König HH and Bleibler F: Economic evaluation of vitamin D and calcium food fortification for fracture prevention in Germany. Public Health Nutr *20*: 874-1883, 2017. PMID: 26568196. DOI: 10.1017/S1368980015003171

3 Niedermaier T, Gredner T, Kuznia S, Schöttker B, Mons U and Brenner H: Vitamin D supplementation to the older adult population in Germany has the cost-saving potential of preventing almost 30 000 cancer deaths per year. Mol Oncol *15*: 1986-1994, 2021. PMID: 33540476. DOI: 10.1002/1878-0261.12924

4 Niedermaier T, Gredner T, Kuznia S, Schöttker B, Mons U and Brenner H: Potential of vitamin D food fortification in prevention of cancer deaths - A modeling study. Nutrients *13*: 3986, 2021. PMID: 34836241. DOI: 10.3390/nu13113986

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OP No. 6

CUTANEOUS MELANOMA: SHEEP IN WOLVES CLOTHING?

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Cutaneous melanoma incidence in European-origin populations has risen steeply; however, according to 73 years of Danish cancer data, mortality has not. Welch *et al*. (1) attributed these divergent trends to overdiagnosis from the increasing diagnostic scrutiny of suspicious lesions. There is also consensus that the increase in melanoma is attributable to increased sun exposure, and therefore infer that the large majority of these melanomas are non-lethal (in stark contrast to melanomas diagnosed in the 1950s), especially at ages <50 years. Icelandic data show an increase in melanoma incidence paralleling increasing sunbed use, which remitted after a campaign against sunbeds. Melanoma mortality remained constant, and was virtually zero in young women, who showed the most pronounced peak in incidence. UV-irradiation of naevi produces transient clinical and pathological features of melanoma that may explain some of these overdiagnoses. Ways to distinguish non-lethal from potentially lethal melanomas are needed.

1 Welch HG, Mazer BL and Adamson AS: The rapid rise in cutaneous melanoma diagnoses. N Engl J Med *7384(1)*: 72-79, 2021. PMID: 33406334. DOI: 10.1056/NEJ Msb2019760

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OP No. 7

IMPACTS OF UV AND VITAMIN D ON SKIN CANCER; MOUSE EXPERIMENTS

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Skin cancers are clearly related to excessive exposure to solar UV radiation, but the UV etiology differs between the types of skin cancer. Squamous cell carcinoma (SCC) can be induced in wild-type (hairless) mice by (chronic) exposure. Basal cel carcinoma (BCC), and cutaneous melanoma (CM), can be promoted by UV exposure in transgenic mice primed to develop these cancers. Vitamin D, its metabolites, and/or its receptor, VDR, were found to suppress these skin cancers in mouse models. Early on, the DNA damage response was shown to be enhanced by vitamin D, in particular repair of UV-induced DNA damage, and the UV-induced immunosuppression was counteracted; both of which should suppress formation of skin cancers. However, dietary vitamin D was shown to be ineffective against BCC raised in Ptch1+/- mice exposed to ionizing radiation, in contrast to topical vitamin D or UV exposure (of female mice; males are not proficient in vitamin D production from UV exposure). Interestingly, next to SCCs, BCCs were raised in skin-carcinogenic experiments with RXR-alpha null and VDR null mice, indicating that these receptors act as repressors; VDR repressing the hedgehog pathway (crucial in BCC). In combination with $1,25(OH)_2D$, the VDR also represses beta-catenin signaling in the Wnt pathway and removes it from the E-cadherin complex. The impact on the Wnt pathway most likely explains the suppressive effect of VDR over-expression in melanoma lung metastases in mice. Evidently, UV radiation can also exert an anti-carcinogenic effect; the impact may depend on the type of skin cancer and the exposure schedule (through vitamin D, moderate regular UV exposure is probably beneficial against melanoma).

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OP No. 8

VITAMIN D AND THE HEART: CONTROVERSIES STILL EXIST

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Background/Aim: Epidemiological and experimental studies have indicated a link between the serum level of vitamin D and the risk of cardiovascular diseases. The deficiency of vitamin D may be involved in the arterial hypertension' pathogenesis. *Materials and Methods/Results:* Researchers have raised the hypothesis of a relationship between vitamin D deficiency and high blood pressure considering the key modulating effects of vitamin D on the renin-angiotensin-aldosterone system axis. Hyperlipidemia is an important risk factor for cardiovascular diseases, especially for ischemic heart disease. The serum lipid levels have seasonal variations, even after changes in diet and physical activity. Although studies confirmed a relationship between normal vitamin D levels and normal serum lipids, the consequences of vitamin D treatment remain unclear. Experimental studies highlighted the mechanisms by which vitamin D can be involved in glycemia control, its deficiency promoting insulin resistance and increasing the diabetes mellitus risk. Although there is strong evidence about the etiological link between the deficiency of vitamin D and the risk of cardiovascular diseases, low 25-OH D levels may be a consequence of cardiovascular diseases, and not the cause, therefore, this link may be an epiphenomenon. *Conclusion:* An insufficient level of serum vitamin D is associated with the majority of cardiovascular risk factors and with cardiovascular morbidity and mortality, but the causal link between them has to be further investigated, especially in the light of vitamin D therapy for cardiovascular protection.

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OP No. 9

VITAMIN D SUPPLEMENTATION MAY IMPROVE COVID-19 PROGNOSIS? EVIDENCE FROM A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background/Aim: The association of SARS-CoV2 infection and COVID-19 prognosis with vitamin D supplementation and serum levels (25OHD) has been widely investigated but the evidence is not consistent. *Materials and Methods:* In order to clarify the source of heterogeneity and have more solid evidence, we conducted a systematic review up to April 2021. *Results:* We calculated summary estimates from 2 randomised controlled trials and 27 cohort-studies including a total of 205,565 patients, 1,197 admitted to the intensive care unit or who needed invasive mechanical ventilation or intubation and hospital stay, and more than 910 COVID-19 deaths. Random effects models showed that more than 60% of cases had a significantly reduced risk of both COVID-19 severe disease (SRR=0.38, 95%CI=0.20-0.72, 6 studies) and mortality (SRR=0.35, 95%CI=0.17-0.70, 8 studies) with vitamin D supplementation. We also did not find a statistically significant difference among doses. A greater reduction in mortality risk was found in association with vitamin D supplementation in older individuals and at higher latitudes. We investigated the quality of studies using the New Castle-Ottawa quality scale and in most cases, we did not find statistically significant differences between low-, medium- or high-quality studies. A greater association of vitamin D supplementation with COVID-19 was found especially in seasons characterized by low 25OHD levels and patients with no severe disease. *Conclusion:* Well-designed randomized clinical studies are needed to confirm these results.

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OP No. 10

PHOTOTOXIC SKIN REACTIONS: AN UPDATE

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Many artificial or naturally occurring substances (medicines, sun filters, fragrances, and substances derived from plants) are included under the term photosensitizer. In addition, new therapeutic agents have been identified in recent years for which phototoxic potentials could be detected, *e.g.*, B-Raf inhibitors. After ultraviolet light exposure, such agents can lead to increased photosensitivity and subsequently to phototoxic skin reactions. Therefore, patients with an erythema in sunlight-exposed skin areas should always be considered for photosensitive disease. The diagnosis is based on a careful anamnesis, clinical examination and, if necessary, phototesting for minimal phototoxicity dose as well as histopathological examinations. Sometimes, it might be difficult to distinguish phototoxic reactions from photoallergic skin reactions. Initially, a phototoxic dermatitis appears like an exaggerated sunburn-like reaction with an overheated painful erythema, mild to moderate swelling and, in severe cases, blistering. As the reaction is dependent on exposure to UV-light, areas exposed to direct sunlight are most commonly involved. Due to an increasing melanin production or from the deposition of the drug or one of its metabolites, phototoxic reactions may result in hyperpigmentation of the skin. Treatment options include withdrawal of the eliciting agent and avoidance of sunlight *via* UV-protective clothing and broadband sunscreens, especially with UVA filters.

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OP No. 11

HYDROXCHLOROTHIAZIDE DID NOT LEAD TO PHOTOTOXIC REACTIONS AND DNA DAMAGE IN HEALTHY VOLUNTEERS – THE "HCTOX-STUDY"

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Background/Aim: Pharmacoepidemiologic studies have shown that the use of hydrochlorothiazide (HCTZ) is associated with an increased risk of skin cancer (especially non-melanoma skin cancer), resulting in a decrease in HCTZ prescriptions, which in turn leads to a less effective blood pressure therapy in a significant proportion of patients. However, whether HCTZ causes skin cancer remains elusive. Hence, we aimed to examine the photosensitive potential of HCTZ *in vivo* in a randomized, placebo-controlled trial. Furthermore, we conducted a series of laboratory experiments to further elucidate the pathophysiologic mechanisms of carcinogenesis and phototoxicity caused by HCTZ *in vitro*. *Materials and Methods:* A randomized, double-blinded, placebo-controlled clinical trial to assess the phototoxic properties of HCTZ was conducted, assigning 30 healthy normotensive adult volunteers in a 2:1 ratio to either HCTZ 25 mg daily or placebo once

daily for 15 days. The skin photosensitivity by phototesting, office blood pressure, serum 25-hydroxyvitamin D (25(OH)D) status, and urinary excretion of thymidine-dimers, *i.e.*, cyclobutan-dimers by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) following whole-body irradiation were assessed. To further assess the pathophysiologic mechanisms of HCTZ-induced photosensitivity, human keratinocytes (HaCaT) were incubated with either dimethyl sulfoxide (DMSO), DMSO and HCTZ or DMSO and amiodarone for one hour, and then irradiated with UV-B radiation (311 nm one burst of 100 J/cm^2). Real-time polymerase-chain-reaction testing (RT-PCR-testing), western blots, and ELISA-testing were performed to analyze reactive oxygen species, inflammation, and carcinogenesis, as well as the formation of thymidine-dimers. *Results*: All 30 participants adhered to the protocol, which was confirmed by UHPLC-HRMS analysis of serum and plasma. Skin photosensitivity to UV-A and UV-B radiation exposure did not change in both groups (UVB-MED: HCTZ Δ=0.0 J/cm^2 *vs.* placebo Δ=0.2 J/cm^2; p=0.055). No thymidine-dimers were detected in either group. Systolic blood pressure (SBP) decreased in both groups (HCTZ Δ=–5.2 mmHg *vs.* placebo Δ=–5.4 mmHg; p=0.948), as did the diastolic blood pressure (DBP) (HCTZ Δ=–1.9 mmHg *vs.* placebo Δ=–4.3 mmHg; p=0.346). Serum 25(OH)D increased in both groups (HCTZ Δ=2.7 ng/ml *vs.* placebo Δ=0.9 ng/ml; p=0.566). HCTZ and DMSO alone did not increase the expression of inflammatory proteins (IL-6, TNFα) or tumor suppressor proteins (p53, p63, p73). However, HCTZ in combination with high-intensity bursts of UV-B radiation caused increased expression of inflammatory proteins and reactive oxygen species. No adverse events related to the study medication were reported. *Conclusion*: HCTZ did not appear to significantly increase photosensitivity to UV-A or UV-B radiation in healthy volunteers compared with placebo. Moreover, no relevant DNA-damages as measured by HPLC could be detected in either group. HCTZ alone did not lead to increased inflammation, formation of reactive oxygen species, or carcinogenesis in human keratinocytes. The combination of a UV-B burst and HCTZ however, did lead to an increase in inflammatory markers. A cumulative dose of 375 mg HCTZ appeared to be safe in healthy volunteers and did not lead to increased photosensitivity or DNA-damage *in vivo*.

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OP No. 12

SOLAR RADIATION FACTORS THAT AFFECT THE SEASONALITY OF DISEASES

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Many diseases have pronounced seasonality, with incidence and death rates much higher in winter than in summer (1). Several factors related to solar radiation have been proposed to explain the seasonality of diseases including temperature, vitamin D production from solar UVB exposure, and release of cutaneous photolabile nitric oxide from solar UVA exposure (2). Many diseases are linked to low 25-hydroxyvitamin D concentrations, including several with large seasonal variations such as cardiovascular disease and infectious diseases (3). This narrative review presents the evidence for seasonal variations in disease risk and activity and the extent to which temperature, UVB, vitamin D, UVA, and nitric oxide might explain the variations.

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2 Cherrie M, Clemens T, Colandrea C, Feng Z, Webb DJ, Weller RB and Dibben C: Ultraviolet A radiation and COVID-19 deaths in the USA with replication studies in England and Italy. Br J Dermatol *185(2)*: 363-370, 2021. PMID: 33834487. DOI: 10.1111/bjd.20093

3 Grant WB, Anouti FAI, Boucher BJ, Dursun E, Gezen-Ak D, Jude EB, Karanova T and Pludowski P: A narrative review of the evidence for variations in serum 25-Hydroxyvitamin D concentration thresholds for optimal health. Nutrients *14(3)*: 639, 2022. DOI: 10.3390/nu14030639

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OP No. 13

A NARRATIVE REVIEW OF OBSERVATIONAL STUDIES ON PERSONAL UVB EXPOSURE AND RISK OF CANCER

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There is strong evidence from geographical ecological studies that cancer incidence and mortality are related to solar UVB doses (1) and to 25-hydroxyvitamin D concentrations from observational studies (2). However, randomized clinical trials have largely failed to confirm that vitamin D supplementation reduces the risk of cancer (2). This raises the question whether ecological studies regarding cancer risk are correct, suggesting that ecological studies have overlooked important confounding factors. This is a narrative review of observational studies on personal UVB exposure and other cancer risk-modifying factors. This review shows that personal UVB exposure does, indeed, reduce the risk of many

types of cancer, although for some types of cancer, it increases risk, either by increasing concentrations of some viral causes of cancer or, in the case of prostate cancer, increasing absorption of calcium and phosphorus.

1 Moukayed M and Grant WB: Molecular link between vitamin D and cancer prevention. Nutrients *5(10)*: 3993-4021, 2013. PMID: 24084056. DOI: 10.3390/nu5103993
2 Grant WB: Review of recent advances in understanding the role of vitamin d in reducing cancer risk: breast, colorectal, prostate, and overall cancer. Anticancer Res *40(1)*: 491-499, 2020. PMID: 31892604. DOI: 10.21873/anticanres.13977

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OP No. 14
PHOTOSYNTHESIS IN PLANTS AND ALGAE

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Plants and algae use the energy of solar light to split water into oxygen (which is discarded), two electrons, and two protons. The photons are absorbed by a number of pigments, including chlorophylls, carotenoids, and phycobiliproteins. The excitation energy is transferred to a reaction center (P680), which is a chlorophyll dimer absorbing at 680 nm, in photosystem II. Upon excitation, the reaction center passes an electron to a primary acceptor (pheophytin) from where it is passed along a system of electron carriers to the reaction center (P700) in photosystem I where it is excited again. The energy is used to reduce $NADP^+$ to $NADPH+H^+$. The missing electron in P680 is replaced by one produced by the water splitting. The protons from this reaction accumulate in the thylakoid lumen and are transferred to the outside, driving an ATPase, which generates ATP. The resulting $NADPH+H^+$ and ATP from the light reaction are used in a dark reaction to reduce CO_2 and produce a C_6 sugar using the key enzyme rubisCO in the multistep Calvin cycle. 6 CO_2 + 12 H_2O ® $C_6H_{12}O_6$ + 6 O_2 + 6 H_2O DG°' + 2,872 kJ/mol. Mosses, ferns and higher plants use chlorophylls *a* and *b* and some carotenoids to harvest solar energy and transfer it to the reaction centers. Cyanobacteria possess only chlorophyll *a* and use phycobiliproteins to collect and funnel energy to the reaction centers. Eukaryotic algae utilize a number of other chlorophylls and carotenoids for the same purpose.

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OP No. 15
VITAMIN D STATUS IN INDIVIDUALS WITH ERYTHROPOIETIC PROTOPORPHYRIA

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Background/Aim: Due to photosensitivity, individuals with erythropoietic protoporphyria (EP) avoid sunlight. For decades, Danes avoiding sunlight have been advised daily oral vitamin D supplementation all year. In addition, our patients with EP were offered, serum 25-hydroxyvitamin D (S-25OHD) quantification, and counselling with prescription of daily oral vitamin D supplementation if S-25OHD was low. We conducted a retrospective cohort study (1) to examine the outcome of the general advise and counselling on S-25OHD level in patients in our clinic. *Patients and Methods:* Danish patients (n=46) had S-25OHD quantified in blood samples (n=721) collected from 2003 to 2021. Patients were followed by a median of 11 years. Individual counselling sessions were registered. S-25OHD of our EP patients were compared to former published data on UK EP patients not using vitamin D supplementation, and to those of the Danish general population (2, 3). *Results:* Our patients with EP had higher S-25OHD levels than those of UK patients not using vitamin D supplementation, but lower levels than those of the Danish general population. In total, 18% of the S-25OHD measurements in Danish EP patients were below 12 ng/ml, indicating deficiency, and 29% were between 12 ng/ml and 20 ng/ml, indicating insufficiency. Thirty-one patients had a total of 74 counselling sessions on vitamin D, giving a rise in S-25OHD levels of around 7 ng/ml the following year. Several patients, however, acquired insufficiency repeatedly. *Conclusion:* This investigation shows the positive effect of vitamin D advice on S-25OHD in individuals with EP. However, follow-up on vitamin D levels and advice is important to improve vitamin D status. The work was supported by the Alfred Benzon Foundation, Denmark.

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OP No. 16
SUNLIGHT, VITAMIN D AND THE COVID-19 DILEMMA: HISTORY REPEATING ITSELF

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The COVID-19 pandemic has had devastating health consequences for children and adults that are not dissimilar to the Spanish flu pandemic of 1918. Heliotherapy was popularized by Hippocrates around 400 BC. While at the same time Sniadecki was associating inadequate sun exposure with rickets, Florence Nightingal was encouraging direct sun exposure for a speedy hospital recovery. When sunlight exposure was found to have antirachitic and antituberculosis properties, it was reported that Spanish flu patients in 1918, who were treated with direct sunlight in the outside hospital at Camp Brooks in Massachusetts, fared better than those not exposed to direct sunlight. An analysis of the association between latitude and solar UV radiation with Spanish flu fatalities for those living in Northern latitudes compared to those in Southern latitudes in the US showed an increased risk of mortality by more than 50%. Hope Simpson has reported that there is a seasonal stimulus for the flu season reaching a peak in the winter, at a time when little, if any, vitamin D is produced from sun exposure. Vitamin D is now recognized as a major regulator of innate and adaptive immunity. Several studies have reported that blood levels of 25-hydroxyvitamin D [25(OH)D] above 30 ng/ml significantly reduced, by more than 50%, infectivity by this deadly virus compared to those with circulating levels of <20 ng/ml; the risk of infection continued to decline until the serum concentrations reached 55 ng/ml. Hospitalized patients with COVID 19 had decreased risk for morbidity, mortality, and ICU admission when their blood levels of 25(OH)D were >30 ng/ml. There needs to be programs to improve the vitamin D status of the world's population, including increased vitamin D food fortification and vitamin D supplementation and recommendations for sensible exposure to sun and artificial UVB emitting devices.

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OP No. 17
THE FUTURE OF VITAMIN D: NOVEL THERAPEUTIC APPROACHES FOR H EALTHY, OBESE, AND MALABSORPTION IN THE ERA OF COVID-19

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There is compelling scientific evidence that vitamin D is a potent and essential immune modulator that helps reduce the risk for autoimmune disorders and infectivity, and morbidity and mortality associated with infectious agents including COVID-19. However, before vitamin D can carry out these important immune functions, it has to be metabolized in the liver to 25-hydroxyvitamin D [25(OH)D]. 25(OH)D enters activated macrophages and dendritic cells and is transformed into its active form 1,25-dihydroxyvitamin D [1,25(OH)2D]. 1,25(OH)2D interacts with its receptor (VDR) to increase the production of cathelicidin. 1,25(OH)2D is also released locally to interact with activated B lymphocytes to reduce autoantibody production and activated T lymphocytes to modulate their functions including production of helpful and harmful cytokines. There are two sources of vitamin D: it can be produced in the skin during exposure to solar ultraviolet B radiation or ingested. Since time of day, season, latitude, and skin pigmentation dramatically affect the cutaneous production of vitamin D, this cannot be considered a dependable source at all times. Patients with fat malabsorption would benefit from a dependable artificial UVB source. Solius Inc. has developed a stand-up device that emits narrowband UVB radiation for producing cutaneous vitamin D. Clinical studies have shown that it is effective in increasing serum 25(OH)D concentrations. Obese and malabsorption patients have a variable response to vitamin D. An alternative is to give these patients 25(OH)D3, also known as calcifediol. 25(OH)D3 effectively and more rapidly improved vitamin D status in these patients similar to healthy adults. The ability to rapidly improve the vitamin D status with oral 25(OH)D3 has been effective in reducing morbidity and mortality in COVID-19 patients.

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OP No. 18
A NEW INSIGHT AS TO HOW VITAMIN D CAN HELP REDUCE MORBIDITY AND MORTALITY IN COVID-19 PATIENTS

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Numerous studies have reported that there is an inverse association between serum concentrations of 25(OH)D and COVID-19 infectivity, morbidity, and mortality. We had previously reported gene expression in peripheral blood mononuclear cells (PBMC) in healthy vitamin D deficient/insufficient adults who received either 600, 4,000 or 10,000 IUs vitamin D3 daily for 6 months. We performed a systematic literature search in the PubMed database for studies published until July 2021 identifying differentially

expressed genes (DEGs) in PBMC between severe and mild COVID-19 patients. The transcriptome of severe COVID-19 patients was compared with the data on the transcriptional changes observed in our six-month study. After filtering the DEGs for *p*-value <0.05 and fold change >1.5, folate receptor 3 (*FOLR3*) was found to be a gene whose expression was significantly altered both in severe and mild COVID-19 patients and in the healthy adults in our study. FOLR3 expression was down-regulated due to vitamin D supplementation in a dose-dependent manner (foldchange=–1.0, –1.7, and –2.7 for the 600, 4,000, and 10,000 IU/day supplementation groups, respectively). As FOLR3 is expressed in neutrophils as a secretory protein, a decreased FOLR3 transcript level could indicate that vitamin D supplementation decreases neutrophil count as has been recently reported in patients admitted with severe symptoms. Patients who were vitamin D sufficient had a significant decrease in the number of neutrophils compared to lymphocytes. This could be a potential mechanism through which a higher vitamin D status reduces COVID-19 severity, as a high neutrophil count and neutrophil-lymphocyte ratio (NLR) are biomarkers for predicting severe health consequences from COVID-19.

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OP No. 19

IMPACT OF VITAMIN D IN ALZHEIMER'S DISEASE

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Alzheimer´s disease (AD) is one of the most common neurodegenerative diseases affecting especially the older population. In the United States, it is estimated that over 500,000 new cases will be diagnosed this year. Several lines of evidence suggest a tight link between vitamin D hypovitaminosis and AD. However, very little is known about the molecular mechanisms linking AD with the vitamin D status. Utilizing neuroblastoma cells and a mouse model with mild vitamin D deficiency (20% reduction compared to wt mice) revealed that the secosteroid vitamin D affects several mechanisms involved in AD. Vitamin D affects directly the BACE gene expression and protein levels, which is in line with an altered BACE activity, leading to changed amyloid β (Aβ) levels. Aβ is the major component of senile plaques, one of the characteristic pathological hallmarks of AD. Beside the Aβ anabolism, the catabolism is also affected by the vitamin D status, further emphasizing the crucial role of vitamin D in AD pathology. Similar effects have been found for vitamin D_2 and D_3 analogues. Furthermore, vitamin D regulates the expression of genes involved in oxidative stress (*Park7*), inflammation (*Casp4*), lipid metabolism (*Abca1*), signal transduction (*Gnb5*), and neurogenesis (*Plat*) suggesting that vitamin D has not only a high impact on AD but also in other neurodegenerative diseases, which is also supported by current epidemiological studies.

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OP No. 20

SYNTHESIS OF ^{13}C-LABELLED VITAMIN D METABOLITES FOR THEIR USE IN LC-MS/MS APPLICATIONS

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Background/Aim: Simultaneous assessment of various vitamin D metabolites in human biofluids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) represents a new promising tool for the differential diagnosis of vitamin D-related diseases. Particularly, low-abundant medicinally-relevant vitamin D metabolites, such as 25(OH)$VD_{2/3}$, 24,25$(OH)_2VD_{2/3}$, 1,25$(OH)_2VD_{2/3}$, and 1,24,25$(OH)_3VD_{2/3}$, along with their 3-*epi*-derivatives have to be considered. *Materials and Methods*: The assessment of these metabolites requires the development of calibration and reference standards, that is, their labeling with multiple deuterium-, or even better, ^{13}C- atoms. *Results*: Some ^{13}C-labelled vitamin D metabolites have been chemically synthesized and obtained in good yield and high purity. *Conclusion*: Access to a wide variety of ^{13}C-labelled highly pure vitamin D metabolites enables the advancement of LC-MS/MS applications towards a better understanding of differential diagnosis of vitamin D-related diseases.

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OP No. 21

EFFICACY OF VITAMIN D_3 SUPPLEMENTATION ON CANCER MORTALITY IN THE GENERAL POPULATION AND THE PROGNOSIS OF PATIENTS WITH CANCER: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background/Aim: Vitamin D insufficiency is highly prevalent among cancer patients. However, there is an ongoing debate about the association between vitamin D_3 and cancer outcomes (*e.g.*, mortality). We aim to assess the efficacy of vitamin D_3 on cancer mortality and prognosis with special attention to potential effect modifiers, including baseline 25(OH)D levels, cancer at baseline, body mass index (BMI), and compliance. The main outcomes include: (I) cancer mortality in the general population, (II) cancer-specific survival, and (III) overall survival of cancer patients. *Materials and Methods:* A systematic review (SR) and individual patient data (IPD) meta-analysis (MA) of randomized controlled trials involving a vitamin D_3 intervention will be performed according to the PRISMA IPD guideline (CRD42020185566). MEDLINE, ISI Web of Science, CENTRAL, CDSR, KSR Evidence, and trial registers will be searched using predefined terms. Two independent reviewers will conduct the study selection and data extraction. Previous SRs will be updated by including recently completed trials and unpublished data on cancer mortality (*e.g.*, D2dCA, VIDAL). IPD meta-analyses will be performed on data from D-Health, FIND, VITAL, WHI, RECORD, ViDA, AMATERASU 5. Trial results will be (re-)analyzed using (un)adjusted Cox proportional hazard regression models. *Results*: The preliminary MA on cancer mortality led to a significant HR [95%CI] of 0.87 [0.79; 0.96]; p=0.006. *Conclusion:* This comprehensive SR and IPD MA will reveal the full potential of vitamin D_3 for reducing cancer mortality and improving cancer survival. It will help replace the one-dose-fits-all approach with targeted intervention tailored to individual needs and conditions (patient-centered medicine).

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OP No. 22

QUANTIFYING URINARY THYMINE DIMERS BASED ON LC-MS/MS

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Background/Aim: Solar ultraviolet radiation (UVR) is a carcinogen and exposure of the skin causes DNA damage, which may result in mutagenesis, immunosuppression, and skin cancer. Cyclobutane pyrimidine dimers (CPDs) including thymine dimers are among the most frequent DNA lesions. The aim of this study was to develop a mass spectrometry-based method to quantify thymine dimers repaired and excreted in the urine after UVR. *Materials and Methods:* Eight healthy volunteers were exposed to whole-body UVR 3 times on 3 consecutive days. Morning urine was collected on day 1 (before irradiation) and the following 7-9 days. A Waters Xevo TQ-XS tandem quadrupole mass spectrometer coupled to a Waters I-class ultra-performance liquid chromatography (UPLC) was used for quantitative analysis in multiple reactions monitoring (MRM) mode. The Danish Research Ethics Committee provided ethical approval (H-20076172). The study adhered to the Declaration of Helsinki; the volunteers gave written, informed consent. *Results*: The liquid chromatography tandem mass spectrometry (LC-MS/MS) results showed excretion of thymine dimers the morning after the first irradiation. With a maximum of excretion on days 5-6. The amount of thymine dimers decreased substantially after 7-9 days but was still higher than that before irradiation. *Conclusion:* A new LC-MS based method to quantify thymine dimers in the urine was developed. Our search indicated that thymidine dimers released following DNA repair are not degraded further and are excreted in urine as dimers. However, it is possible that dimers are present in urine in oligomeric forms.

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OP No. 23

SUN EXPOSURE – HAZARDS AND BENEFITS

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Background/Aim: There are carcinogenic effects of sun exposure, increasing the risk for skin cancer, especially for fair-skinned individuals. Therefore, there are recommendations to avoid sun exposure and to apply sun blockers. A more nuanced balanced message for Sunsafe is now advocated. *Materials and Methods/Results:* Despite an increased risk of death due to skin cancer, fair-skinned women seem to have a survival advantage. In addition, an inverse association between sun exposure and hypertension, thromboembolism, and type 2 diabetes mellitus has been shown. This is resulting in increased odds of cardiovascular diseases (CVD) and non-CVD/non-cancer mortality among women with low sun exposure habits. In addition, there are data to support that the prognosis of cancer is improved with increasing vitamin D/sun

exposure. *Conclusion:* In this narrative review, we will give a brief update of the hazards and benefits of sun exposure.

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OP No. 24
HIGH ORAL VITAMIN D INTAKE DOES NOT PROTECT AGAINST UVR-INDUCED SQUAMOUS CELL CARCINOMA IN HAIRLESS MICE

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Background/Aim: The role of vitamin D in skin carcinogenesis is unclear. Vitamin D compounds may play a role in protection against UVR-induced DNA damage, immune suppression and skin carcinogenesis. However, epidemiological studies have also shown an increased incidence of skin cancer associated with high levels of serum vitamin D. The aim was to investigate the influence of vitamin D supplementation on vitamin D levels in the serum, skin, and tumor and on skin cancer development in hairless immunocompetent mice. *Materials and Methods*: Female C3.Cg-Hrhr/TifBomTac immunocompetent mice (n=125) were randomly allocated into five groups. Two groups received diet enriched with vitamin D_3 corresponding to ~1,200 IU/day or 30 μg/day per mouse. One group received medium enriched vitamin D_3 (~600 IU/day or 15 μg/day per mouse). The last two groups received standard diet (~1.2 IU/day or 0.03 μg/day/mouse). Three standard erythema doses (SED) of UVR were given to three groups three times per week, one from each supplementation group. *Results:* Animals supplemented with high vitamin D_3 had about 150 times higher levels of serum vitamin D_3 (p=0.00031) and 3 times higher 25(OH)D_3 serum levels (p=0.00016) than standard diet. Serum vitamin D_3 levels in mice supplemented with a medium dose of vitamin D_3 were 18 times higher than those supplemented with standard diet, and 2.3 times higher for 25(OH)D_3 (p=0.00067). All UVR-exposed mice developed tumors. High and medium supplementation of vitamin D_3 did not influence the time to tumor development (p>0.05). *Conclusion:* This study showed that it was possible to raise vitamin D levels in the serum, skin, and tumors of mice by supplementation, but it did not affect the time to UVR-induced skin cancer.

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OP No. 25
VITAMIN D FOOD FORTIFICATION: TIME TO ACT

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Vitamin D deficiency may cause musculoskeletal diseases including rickets and osteomalacia, but vitamin D can also reduce mortality (premature deaths) and prevent extraskeletal diseases such as respiratory tract infections. Induced by exposure to ultraviolet-B (UVB) radiation (*i.e.*, sunlight or artificial UVB-emitting devices) vitamin D is mainly synthesized in the skin whereas nutritional intake is of minor importance. Several nutritional guidelines have recently increased the recommended dietary reference intakes for vitamin D. The recommended target levels for 25(OH)D serum concentration, that range from 25 to 50 nmol/l (10 to 20 ng/ml), can in general be achieved by a daily vitamin D intake of 10 to 20 μg (400 to 800 international units). However, in many countries, vitamin D intake falls below these recommendations. Notably, 25(OH)D serum concentrations <30 (12 ng/ml) and <50 nmol/l (20 ng/ml) occur in 13% and 40% of the general population in Europe, respectively. The obvious gap between the high prevalence of vitamin D deficiency and these officially recommended dietary intakes is of great concern with regard to public health and requires action from European health authorities. However, it has to be noted that although measures promoting a healthier lifestyle with *e.g.*, more outdoor activities and optimal nutrition are definitely warranted, these activities will not eliminate vitamin D deficiency. Moreover, these measures must, as in the case of solar UV exposure, be well balanced considering potential side effects. Efficacy and safety of vitamin D supplementation are limited by relatively poor treatment adherence and potential overdosing. In contrast, systematic food fortification with vitamin D has been shown to represent an effective and safe approach to optimize vitamin D status in the general population and, consequently, has already been implemented by many countries worldwide, including the USA, Canada, and Finland. We encourage health authorities and politics to implement vitamin D fortification of food. A solid starting

point for such an effort has already been provided by promising experiences with vitamin D fortification in several European countries such as Finland.

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OP No. 26

DEVELOPMENTS TOWARDS SYNTHESIS OF LABELED VITAMIN D METABOLITES

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Background/Aim: Development of hyphenated liquid chromatography – mass spectrometry (LCMS/MS)-based assays for detection of vitamin D metabolites at low concentration and their applications in clinical chemistry together with high-throughput analysis of main metabolites in serum, prompt us to synthesize stable isotope (^2H, ^{13}C) labeled metabolites to be used as internal calibration standards. *Materials and Methods/Results:* Labeled vitamin D_3 and D_2 metabolites were synthesized through a tandem Pd-catalyzed A-ring closure and Suzuki-Miyaura coupling with CD bi-cycle or through Horner-Washworth-Emmons reaction with an appropriate phosphine oxide. This methodology allowed the introduction of 6/9 deuteriums or 3/5 carbons-13 in vitamin D metabolites. Eight deuterium and six ^{13}C labeled metabolites were synthetized. *Conclusion*: Stable isotope (^2H, ^{13}C) labeled vitamin D metabolites were synthesized and can be used as internal standards in mass spectrometry

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OP No. 27

DAILY ORAL VITAMIN D_3 IN THE MANAGEMENT OF PSORIASIS: A CASE SERIES

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Background/Aim: Vitamin D, as an immune-modulator, exerts immunological activities on the innate and adaptive immune response. The role of vitamin D receptor resistance due to genetic polymorphisms is increasingly recognized in the pathology-prognosis of auto-immune disorders, warranting higher doses of vitamin D to combat resistance and achieve meaningful immunological and clinical effects. *Patients and Methods:* Six patients diagnosed with psoriasis were treated with daily oral vitamin D_3 in doses ranging from 30,000 IU to 60,000 IU daily for a period of 2 to 6 months and then followed by a lower daily maintenance dose. *Results:* The participants at baseline had an average psoriasis area and severity index (PASI) score of 29.75±15.75 and an itching score of 8.2±0.83 on a visual analog scale (VAS), where 0 signified no itching and 10 represented severe itching. The dose of vitamin D_3 was optimized for each patient based on periodic monitoring of the drop in parathyroid hormone levels. This serves as a biological indicator to monitor individual vitamin D response. Ionised Calcium levels were also checked to prevent hypercalcemia. Remission of the disease was observed within a span of 2-6 months in all of them. They were then kept on a lower daily maintenance dose ranging from 10,000 to 40,000 IU daily. The post measures of PASI and VAS scores among the participants were 0.4±0.32 and 0.5±0.5, respectively. None of the patients reported relapse or any adverse event throughout the study period. *Conclusion*: Supervised, daily, oral, in higher than usual dose, vitamin D_3 may be a safe and efficacious therapeutic modality in managing psoriasis.

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OP No. 28

MOLECULAR PATHOLOGY AND CLINICAL MANAGEMENT OF XERODERMA PIGMENTOSUM – AN UPDATE

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Xeroderma pigmentosum (XP) is a rare autosomal recessive DNA repair-defect syndrome with a worldwide prevalence of 1 in 1,000,000. It is associated with UV-sensitivity, premature skin aging and early onset of skin cancer, such as basal and squamous cell carcinoma as well as melanomas. Around 25% of XP patients develop neurological symptoms. The severity of the associated XP symptoms can vary between genotypes. The underlying genetic defects can be assigned to seven complementation groups – XP-A to XP-G – and one variant form (XPV). While the variant form affects the translesional DNA polymerase η, the affected genes of the seven complementation groups are all involved in the nucleotide excision repair (NER). The NER is essential for the repair of UV-associated DNA lesions such as cyclobutane pyrimidine dimers (CPD) and 6-pyrimidine-4-pyrimidone dimers (6-4 PP). It can recognize DNA damage during global

genome repair (GGR) and transcription-coupled repair (TCR). To prevent or delay symptoms of XP, an early diagnosis is essential, so that the patients can take protective measures such as systematic sun protection. As a Reference Center in the European Reference Network for rare skin diseases (ERN Skin), we specialize in DNA repair defect syndromes such as XP. We investigate gene functions of XP genes, but also offer genetic and functional testing of XP patients. Thus, produced genotype-phenotype correlations can not only help to further understand gene function, but also to provide better patient care. XP cannot be cured, only treated. Although surgical removal of skin lesions is still the therapy of choice for invasive cancers, new topical and systemic treatment options, especially immune checkpoint inhibitors, show promising results.

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OP No. 29

NICOTINAMIDE AND PHYTOCHEMICALS PHLOROGLUCINOL AND SYRINGIC ACID DELAY UVR-INDUCED SQUAMOUS CELL CARCINOMA ONSET IN HAIRLESS MICE

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Background/Aim: Ultraviolet radiation (UVR) is the primary risk factor for skin cancers such as squamous cell carcinomas (SCCs). Because of poor adherence to topical sun protection regimes, other prevention measures must be explored. Phytochemicals have demonstrated numerous protective effects and therefore, present a promising avenue for photoprevention. The aim of this study was to explore the protective effects of phytochemical compounds on UVR-induced carcinogenesis in hairless mice. *Materials and Methods:* A total of 125 female C3.Cg-Hrhr/TifBom Tac mice were randomized into five groups. Through the drinking water, the mice either received 100 mg/kg of hesperidin methyl chalcone (HMC), phloroglucinol (PG), or syringic acid (SA), 600 mg/kg of nicotinamide (NAM), or no supplementation (UV control). Thrice weekly, the mice were irradiated with three standard erythema doses (SED) of UVR to induce and promote carcinogenesis. *Results:* Oral supplementation with NAM, PG, and SA significantly ($p<0.05$) delayed the onset of SCCs in hairless mice. In the NAM and SA groups, this effect was associated with an increase in pigmentation following six months of UVR ($p<0.05$). In the HMC group, no effect on carcinogenesis was observed compared to the control group, and none of the four experimental groups exhibited any change in erythema following UVR. *Conclusion:* We have demonstrated that oral supplementation of NAM, PG, and SA to hairless mice can protect against the development of SCCs following UVR.

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OP No. 30

MODULATION OF THE SKIN MICROBIOTA BY OPTICAL RADIATION

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Background/Aim: Repeated short-term radiation with UV-B is considered a promising option to enhance vitamin D synthesis but may induce changes in the skin microbiota and thus, aggravate skin diseases associated with skin microbiota dysbiosis such as atopic dermatitis, acne vulgaris, and psoriasis. *Patients and Methods:* In order to test the impact of repeated UV-B treatment on the skin microbiota, a study group of 20 healthy volunteers was sampled twice on the cheek, neck, chest, elbow, and knee prior to UV-B treatment and after the last UV-light exposition. Swabs were either used to determine bacterial loads at the sampling sites by classical plate counting or subjected to DNA extraction and subsequent next-generation sequencing. *Results:* Cultivation of samples on blood agar plates and under aerobic conditions revealed larger person-to-person and sampling site variations (highest bacterial loads in cheek and neck samples > chest > knee and elbow), but no clear differences in bacterial numbers per swab site prior to or post UV-B treatment. In line with the variations in bacterial loads per person/sample site, larger differences in the amounts of total DNA per sample were noticed, which allowed only a subset of samples to be used for metagenomic shotgun sequencing in order to identify the genus/species compositions. *Conclusion:* DNA samples are currently processed, and results of this investigation will be presented.

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OP No. 31

MELANOMA RISK AND SOLARIUM USE: STILL MANY UNRESOLVED QUESTIONS, NOT THE TIME TO CLOSE THE DEBATE

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Background/Aim: During the last decades, there is a controversial debate among scientists whether risk for malignant melanoma (MM) may be increased by moderate sunbed use (1-6). However, some recent reports in the scientific literature demand this debate to be closed because a causal relationship between risk for MM and sunbed use would recently have been convincingly shown (6). It was the aim of this work to review the scientific literature to answer the question whether a causal relationship between risk for MM and moderate solarium use has been proven. *Materials and Methods:* We searched and systematically reviewed the scientific literature (PubMed; Web of Science) to update our previous publications on this topic (1-5). *Results:* There is no proof in our present scientific literature of a causal relationship between the risk to develop a MM and moderate sunbed use. Randomized controlled trials (RCTs) that could prove whether there is such a causal relationship, are lacking. The weak associations demonstrated by the cohort and case–control studies published to date fail to show causality. Notably, the observational studies on this topic are characterised by poor overall quality and, because of severe limitations that cause bias, by low resulting evidence levels and low grades of recommendation. In the majority of studies published to date, findings were not adequately and systematically recorded and adjusted for many of the confounding factors, including sun exposure, sunburn, and skin type. We previously reported in a meta-analysis (1) that included 2 cohorts and 29 case–control studies summary risk estimates that suggested a weak association (odds ratio=1.19, 95% confidence interval=1.04-1.35, p=0.009) for 'ever use' of a solarium with risk for MM. However, importantly, sensitivity analyses could not show an association for studies from Europe, studies with a low risk of bias, and studies performed after 1990. Additionally, resulting levels of evidence (3a–), and grades of recommendation (D) were low because of poor overall study quality including limitations such as confounding and lack of RCTs. Importantly, the same risk estimates (*e.g.*, odds ratios) as published in meta-analyses could well result from the following scenario: moderate sunbed use has no effect on risk of MM, but an 'unhealthy lifestyle' (*e.g.,* extensive sunbathing) causes an increased odds ratio of 1.2 in association with solarium use (it has been reported that 'sun worshippers' visit tanning salons more frequently as compared to control groups). Additionally, other recommendations of how to assess plausibility in a biological system, *e.g.,* the criteria defined by Hill in 1965 (7), do not support the hypothesis that moderate solarium use *per se* may increase melanoma risk. It must be emphasized that a large body of evidence from epidemiological and animal studies shows no increase in melanoma risk following chronic (moderate) UV exposure. In contrast, many studies indicate that chronic sub-erythemal exposure to solar radiation may even be protective and that outdoor workers may have a reduced risk of developing MM. *Conclusion:* To date, there is no convincing evidence that moderate/responsible sunbed use may increase risk to develop MM. Many unresolved questions remain, and this scientific debate should certainly not be closed.

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OP No. 32

LOW VITAMIN D STATUS IS ASSOCIATED WITH POOR CLINICAL OUTCOME IN ADVANCED MELANOMA TREATED WITH IMMUNE CHECKPOINT- OR BRAF/MEK-INHIBITORS: RESULTS FROM A PILOT STUDY

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Background/Aim: In malignant melanoma (MM) and other types of cancer, low vitamin D status is associated with increased risk and poor prognosis. However, data of the impact of vitamin D status, defined as 25(OH)D serum concentration (*s.c.*), on clinical outcome in advanced MM are limited. *Materials and Methods:* We performed a prospective pilot study to test the hypothesis whether vitamin D status can predict the efficacy and safety of BRAF-, MEK-, CTLA-4-, and/or PD-1-inhibitors in patients treated for metastasized MM. *Results:* In this pilot study, severe vitamin D deficiency (defined as 25(OH)D *s.c.* <10 ng/ml) was associated with markedly reduced overall (OS) and progression-free (PFS) survival, with higher tumor-load [TL, assessed as *s.c.* of S100 protein or lactate dehydrogenase (LDH)], and with a trend for a reduced number of adverse events (AEs). During the complete observation period (OP, 228 weeks/ mean 53.31 weeks), risk of death reduced by 82% (HR=0.179, p=0.000016) and risk for disease progression reduced by 76.3% (HR=0.237, p=0.000159) in patients with average 25(OH)D *s.c.* ≥10 ng/ml (mean 23.71 ng/ml), as compared with vitamin D deficient patients. Moreover, risk of death also reduced in patients with average 25(OH)D *s.c.* ≥10 ng/ml (mean 23.71 ng/ml), as compared with vitamin D deficient patients after adjusting for various parameters, including *BRAF*/*N-RAS* mutations (HR=0.138, p=0.000004) and markers of TL such as average *s.c.* of LDH (HR=0.306, p=0.026) and S100P (HR=0.225, p=0.003). An increase in average 25(OH)D *s.c.* of 1 ng/ml was associated with a 3.9% reduced risk for progressive disease (HR=0.961, p=0.044), with a reduction of LDH *s.c.* of 3.86 U/l (p=0.034, indicating a lower TL), and with a trend for fewer AEs (AE ratio –0.005; p=0.295). Individuals with average 25(OH)D *s.c.* ≥10 ng/ml and *BRAF*-mutant MM had a trend for more AEs, as compared to patients with *BRAF* wild type MM. *Conclusion:* Vitamin D deficiency is associated with poor clinical outcomes in patients treated for metastasized MM with BRAF/MEK inhibitors or immunotherapy, independently from other prognostic and predictive parameters. Although it needs to be confirmed in future interventional trials whether optimizing serum 25(OH)D improves clinical outcome in these MM patients, we recommend that 25(OH)D *s.c.* should be analyzed and vitamin D deficiency treated in all patients with advanced MM.

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OP No. 33

POTENTIAL HARMFUL EFFECTS ASSOCIATED WITH THE USE OF SUNSCREENS TO PREVENT SKIN CANCER – THE GOOD THE BAD AND THE UGLY OF SUN PROTECTION

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Background/Aim: The use of sunscreens is an integral part of most guidelines and campaigns to prevent skin cancer. In recent years, however, a constantly increasing body of evidence has raised several concerns in respect to their efficacy and safety. *Materials and Methods:* A systematic literature search was performed using Pubmed and web of science to identify publications that report on efficacy or safety of sunscreens. *Results:* This literature search identified many reports that question the efficacy of sunscreens and reveal potential risks that are associated with their use. Besides well known potential adverse events such as phototoxic or photoallergic dermatitis, additional risks for harmful effects include endocrine activities described as "endocrine disruption". Additionally, it has been shown that sunscreens are absorbed transcutaneously, can be detected in mother milk, human placental tissue, and the food chain. Additionally, they are now considered as a major thread for ocean life including coral reefs. *Conclusion*: When deciding whether or not to use sunscreens, recent reports indicating limited efficacy and potential risks should be considered. Well-designed future studies to investigate the efficacy and safety of sunscreens are urgently needed.

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OP No. 34

NOVEL STRUCTURAL ASPECTS OF VITAMIN D SIGNALING AND FUNCTION OF VDR

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The most active metabolite of vitamin D_3, 1,25D_3, acts *via* its binding to the vitamin D receptor (VDR, also termed NR1I1), a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily, which regulates the transcription of thousands of genes involved in calcium-phosphate homeostasis and various anti-proliferative and anti-inflammatory activities. 1,25D_3 binding triggers VDR conformational changes promoting its interaction with the retinoid X receptor (RXR) and co-regulators. To date, several thousand 1,25D_3 analogs have been synthetized and crystal structures of VDR ligand binding domain (LBD) have been solved in the presence of more than 150 analogs, providing valuable information about VDR interactions. Recent structural studies of VDR complexes with natural metabolites, synthetic ligands, and VDR-coregulator complexes have provided a new understanding of the VDR mechanisms. Established and emerging mechanisms of action of VDR from a structural perspective will be discussed.

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OP No. 35

REAL-WORLD EVIDENCE FOR THE EFFECTIVENESS OF VITAMIN D SUPPLEMENTATION IN THE REDUCTION OF TOTAL AND CAUSE-SPECIFIC MORTALITY. RESULTS FROM ALMOST HALF A MILLION PARTICIPANTS OF THE UK BIOBANK COHORT STUDY

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Background/Aim: Meta-analyses of randomized controlled trials (RCTs) demonstrated the efficacy of vitamin D supplementation to reduce cancer mortality, all-cause mortality, and respiratory tract infections. However, whether and to which extent this translates into effectiveness in the real world is unknown. This study aimed to investigate whether the intake of vitamin D supplements is associated with reduced all-cause mortality, cardiovascular, cancer, and respiratory mortality in real-world settings, and to validate the associations of 25(OH)D levels with mortality. *Materials and Methods*: This prospective cohort study employed a 445,601 UK Biobank population recruited between 2006 and 2010, using Cox proportional hazard regression to assess the associations of vitamin D supplements and vitamin D status with the mortality outcomes, adjusting for 48 identified independent determinants of vitamin D deficiency. *Results*: Overall, 4.3% of participants reported regularly taking vitamin D supplements and the majority had either vitamin D deficiency (21.0%) or insufficiency (34.3%). A total of 29,107 (6.5%) participants died during a median follow-up of 11.8 years. Participants with self-reported, regular vitamin D supplement use had 6% lower all-cause mortality, and 24% decreased respiratory disease mortality. Vitamin D deficiency and insufficiency were strongly associated with all-cause, cardiovascular disease, cancer, and respiratory disease mortality. *Conclusion*: This large, population-based study confirmed that the efficacy of vitamin D supplements for all-cause mortality shown in RCTs is similar to real-world data. The RCT-based evidence for a protective effect from respiratory tract infections may translate into lower respiratory mortality.

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OP No. 36

EFFICACY AND SAFETY OF A PERSONALIZED VITAMIN D_3 LOADING DOSE FOLLOWED BY 2,000 IU PER DAY TO RAISE SERUM 25-HYDROXYVITAMIN D IN COLORECTAL CANCER PATIENTS WITH VITAMIN D INSUFFICIENCY. INTERIM ANALYSIS OF A RCT

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Background/Aim: Vitamin D insufficiency, defined by 25-hydroxyvitamin D (25(OH)D) levels <50 nmol/l, is common among colorectal cancer patients, especially shortly after tumor surgery. An approach using a personalized vitamin D_3 loading dose to rapidly increase 25(OH)D has not yet been tested in cancer patients. The objectives of the interim analysis were to test whether there are differences in serum 25(OH)D levels, the prevalence of adequate vitamin D status (25(OH)D ≥50 nmol/l), and frequencies of the safety parameters of hypervitaminosis D, hypercalcemia, hypercalciuria, and severe renal impairment in the vitamin D_3 and placebo group at visit 1 (trial day 12-21, end of loading dose) and visit 2 (trial week 13-16, end of maintenance dose). *Materials and Methods:* The VICTORIA trial is a randomized, double blind, controlled trial. Overall, 456 colorectal cancer patients will be recruited in up to nine German rehabilitation clinics. Study inclusion requires 25(OH)D levels <50 nmol/l. The personalized loading dose is calculated from baseline 25(OH)D level and BMI and is followed by a maintenance dose of 2,000 IU vitamin D_3 daily until the end of trial after 12 weeks. *Results:* This abstract shows the results of the first n=70 unblinded study participants of whom n=64 (n=34 verum, n=30 placebo) could be included in the intention-to-treat analyses at visit 1 and n=48 at visit 2. In the placebo group, the mean 25(OH)D

levels [95% confidence interval (CI)] at screening [28.5 (24.1-32.9) nmol/l] did not change much until visit 1 [31.7 (27.2-36.2) nmol/l] and visit 2 [35.9 (28.8-43.0) nmol/l]. In the verum group, the mean 25(OH)D levels at screening [25.8 (22.6-28.9) nmol/l] increased until visit 1 [63.2 (58.1-68.3) nmol/l] and visit 2 [72.2 (65.5-79.0) nmol/l]. The prevalence of adequate vitamin D status in the placebo and verum group was 6.7% and 79.4% at visit 1 and 20% and 95.7% at visit 2, respectively. All comparisons between the trial arms were highly statistically significant (p<0.0001). No events for the safety parameters were observed, except for 5 cases of hypercalciuria in the verum and 1 case in the placebo group at visit 1 (p=0.202). The study medication was discontinued, and a normalization of urinary calcium levels was observed at visit 2. *Conclusion:* The personalized vitamin D_3 loading dose was substantially more effective than placebo in increasing 25(OH)D levels, and the maintenance dose of 2,000 IU vitamin D_3 per day successfully maintained the achieved levels. Hypercalciuria may occur but is reversible.

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OP No. 37

ALTERNATIVE NUCLEAR RECEPTORS FOR VITAMIN D HYDROXYDERIVATIVES

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Background/Aim: The effects of canonical active forms of vitamin D_3, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) are mediated *via* interaction with the vitamin D receptor (VDR), which functions as an agonist-activated transcription factor that binds to VDR responsive elements (VDRE) to influence the expression of target genes. *Materials and Methods:* We have established a non-canonical pathway of vitamin D metabolism that is initiated by the action of a rate-limiting enzyme of steroidogenesis, CYP11A1, followed by modifications by other CYP enzymes, including CYP27B1: $D_3 \rightarrow 20(OH)D_3 \rightarrow 20,23(OH)_2D_3 \rightarrow 17,20,23(OH)_3D_3$ + $(OH)nD_3$. *Results:* Functional studies, bioinformatics analyses, and molecular modeling demonstrated that CYP11A1-derived D_3-hydroxyderivatives can act as agonists on VDR and that the activity on this receptor is enhanced by the addition of C1α(OH). These D_3 derivatives also function as inverse agonists on retinoic acid orphan receptors α and γ (ROR and RORγ) and as agonists on the aryl hydrocarbon (AhR) and liver X receptors (LXR) α and β. This action also includes $1,25(OH)_2D_3$. The receptor selectivity for D_3-hydroxyderivatives appears to be defined by the localization of hydroxyl groups on the side chain and C1α. *Conclusion:* These findings open new areas for studies on the role for active derivatives of vitamin D_3 that is context-dependent and defined by their structure.

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OP No. 38

CYP11A1-DERIVED VITAMIN D HYDROXYDERIVATIVES AS THERAPEUTICS AGAINST SKIN CANCER

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Background/Aim: Derivatives of newly described pathways of vitamin D_3 activation by CYP11A1 show biological activity against skin and immune cells. They also show anti-melanoma activity both *in vivo* (mediated by 20-hydroxyvitamin D_3) and *in vitro*. It was the aim of this study to further characterize the anti-skin cancer activity of these compounds. *Materials and Methods/Results:* Vitamin D receptor (VDR) silencing strongly inhibited the *in vitro* anti-melanoma effects of CYP11A1 D_3-hydroxyderivatives. $VDR^{-/-}$ melanoma cells showed a higher proliferative rate and increased capability for anchorage independent growth. We have also tested the anti-cancer activity of CYP11A1-derived D_3-hydroxyderivatives using a panel of *in vitro* assays. These compounds showed anti-tumor activity against human oral and epidermal squamous cell carcinomas, and murine basal cell carcinoma. Possible mechanisms of action included the down-regulation of hedgehog and WNT/β-catenin pathways. Preliminary *in vivo* studies have shown that $20(OH)D_3$ can inhibit UVB-induced skin carcinogenesis in Ptch1+/–/SKH-1 mice. The latter is consistent with the radioprotective properties of active D_3-hydroxyderivatives.

Conclusion: CYP11A1-derived D_3-hydroxyderivatives are excellent candidates for prevention or therapy of skin cancer.

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OP No. 39

ULTRAVIOLET B (UVB) REGULATION OF THE CUTANEOUS AND SYSTEMIC IMMUNE SYSTEM

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The skin operates as a fully functional peripheral neuro-immuno-endocrine organ, which modulates both local and systemic homeostasis. It uses the same mediators and signal transduction pathways functioning in the central nervous (CNS), endocrine, and immune systems. These include corticotropin releasing hormone (CRH), urocortins, POMC-peptides, cytokines, enkephalins, biogenic amines, melatonin, steroids, secosteroids, lumisterols, and others. Cutaneous production of these molecules can be stimulated by UVB and are released to the circulation. The cutaneous messengers modulate local and systemic homeostasis through highly organized pathways. These include activation of the central hypothalamic-pituitary adrenal axis (HPA), which stimulates the release of corticosteroids to inhibit immune activity and counteract stress through a feedback mechanism. Thus, released from the skin, CRH and urocortin activate the pituitary, and cytokines (including IL-1, IL-6 and TNFα) activate both the hypothalamus and pituitary, all in order to activate the adrenal cortex. In addition, UVB activation of local nerves can generate rapid responses transmitted by ascending nerves to the CNS, which translates them into descending signaling leading to the inhibition of systemic and local immune activity. These responses can be observed 30-60 min after UVB exposure. Specific for the skin is UVB-induced generation of secosteroids/sterols that after metabolic activation by CYP enzymes would exert immunomodulatory effects at the local and systemic levels. These UVB-modulated systems, separately or in concert, would not only defend the skin's integrity, but also reset the body homeostasis and systemic immune activity to the environmentally most desirable mode.

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OP No. 40

VITAMIN D AND IMMUNE RESPONSE: $1,25(OH)_2D_3$ REGULATES HOST DEFENCE-RELEVANT GENES BY TRANSCRIPT INITIATION AND ELONGATION

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Background/Aim: Vitamin D suppresses adaptive immune responses but stimulates the innate immune system. Vitamin D targets cell types that belong to the immune system, such as monocytes/macrophages, dendritic cells (DCs), as well as B-and T cells. 5-Lipoxygenase (5-LO, *ALOX5* gene) is a component of the innate immune system that is strongly induced by TGFβ and $1,25(OH)_2D_3$, which is partially promoter-independent. *Materials and Methods/Results*: In an activity-guided approach using reporter gene assays, the distal part of the *ALOX5* gene was included in the reporter gene plasmid and by data from VDR ChIP-Seq analyses, we localized vitamin D response elements (VDREs) in the distal part of the *ALOX5* gene. When we analyzed the effects of 1,25(OH)2D3 plus TGFβ on chromatin modifications in these regions of the *ALOX5* gene in the human monocytic cell line Mono Mac 6 cells by ChIP, we found an increase in histone H4 K20 monomethylation (H4K20me) and a prominent presence of H3K36me3 by $1,25(OH)_2D_3$. Combined treatment with $1,25(OH)_2D_3$ and TGFβ also increased histone H4 acetylation, a marker for open chromatin, and the elongation form of RNA polymerase II. Furthermore, we found that elongation of ALOX5 transcripts by 1,25(OH)2D3 and VDR was enhanced by AF4, a component of the elongation complex, and inhibited by class I HDAC inhibitors and the cdk9 inhibitor flavopiridol. *Conclusion:* Calcitriol induces the immune-relevant *ALOX5* target gene *via* VDR-dependent transcript elongation in an AF4- and HDAC class I-dependent manner.

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OP No. 41

ULTRAVIOLET B PHOTOTHERAPY OF SKIN DISEASES - WHAT'S VITAMIN D GOT TO DO WITH IT?

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Phototherapies with medium wave ultraviolet light (broad band and narrow band UVB) represent a valuable part of the therapeutic armamentarium in dermatology. A broad range of dermatological disorders are amenable to UVB phototherapy and can be cured or substantially improved by a course of UVB phototherapy. The most common UVB-responsive dermatoses are psoriasis, eczema, cutaneous T-cell lymphoma, lichen planus, pruritic dermatoses, and vitiligo. In addition, in some photodermatoses, such as polymorphic light eruption, patients can be subjected to a series of UVB exposures to photodesensitize their skin and thus to prevent a sun-induced

rash during the following summer period. UVB phototherapies are widely available, easy to perform, and have the advantage of a rapid onset of action, high efficacy, and an excellent short- and long-term safety. Interesting advancements include targeted UVB phototherapies such as the excimer laser or excimer light that employ highly intensive narrowband UVB radiation with a peak emission at 308 nm. Targeted UVB phototherapies are particularly suited for the treatment of stable localized skin disease since the light is exclusively directed to lesional skin whereas healthy unaffected skin is spared from unwanted UV exposure. Besides exerting numerous immunomodulatory effects and stimulating melanogenesis, UVB radiation is also essential for cutaneous vitamin D synthesis by converting 7-dehydrocholesterol to previtamin D_3. Consequently, numerous studies have shown that UVB phototherapy effectively increases serum concentrations of vitamin D; however, no clear correlation has been found so far between UV-induced improvement of skin diseases and changes in serum vitamin D levels. Further studies are required to disentangle the direct effect of UVB radiation on diseased skin from indirect effects *via* UVB-induced alterations in vitamin D homeostasis and to clarify whether induction of vitamin D is just an incidental byproduct of UVB phototherapy or adds to the therapeutic action of UVB radiation in dermatological skin diseases.

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OP No. 42

EXPLORING THE ENHANCEMENT OF DIETARY VITAMIN D IN CLIMATES WHERE CUTANEOUS SYNTHESIS IS SEASONALLY RESTRICTED

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The concept of a 'vitamin D winter', that is a period of the year when there is insufficient UVB radiation available in sunlight to enable any appreciable vitamin D production in the skin, is well known and applies at middle to high latitudes. In the UK, the vitamin D winter lasts from approximately October to February, resulting in a natural seasonal cycle in vitamin D status. Modern diets do not contain sufficient vitamin D to prevent the marked decline in vitamin D status by the end of winter. Thus, maintaining an acceptable vitamin D status all year round requires either enhancement of dietary vitamin D through food fortification or supplementation, achievement of higher end-summer vitamin D status, or a UVB substitute for sunlight during the winter months. The public health imperative for such interventions depends on what is considered an acceptable vitamin D status, underlying climate, and the personal characteristics of the population considered – most specifically their skin type. Here, we present examples from both a model of dietary intervention and an experimental UV substitution. The model accounts for a detailed local climatology, estimated exposure patterns, skin type, and dietary intake, allowing for exploration of the population's vitamin D status under different dietary interventions. The model can be applied to white-skinned or black and minority ethnic (BAME) populations, or a national population with representative white and BAME percentages. The artificial UV radiation was applied in a work environment through the winter months, in a blinded cross-over study, to determine whether the low UV doses thus achieved could maintain end-summer levels of vitamin D in healthy, working age volunteers. The relative merits of these two approaches to addressing the seasonal cycle in vitamin D status will be discussed.

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OP No. 43

SIMPLE PROCEDURE TO DETERMINE THE MINIMAL ERYTHEMA DOSE (MED)

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Background/Aim: People sensitive to sunlight may just be fair-skinned or may suffer from photodermatosis. To test a person's sensitivity to sunlight, UVB or UVA, we have developed a simplified test procedure and equipment. Our primary purpose was the development of a soft label printed with areas to allow penetration of different UVR doses. *Materials and Methods:* The label allows us to administer 6 different UVR doses to the skin with 25% incremental doses. Two lamps were developed to adhere precisely to the label to avoid any light scatter to the surrounding skin, so the patient does not need a protective cover. One light source is a solar simulator with a xenon lamp and reflector for even light distribution. The other is a lightweight LED-based light source, which mounts directly on the label. *Results:* The exposure time to obtain 3 SED (the sensitivity of the average Dane) was 4.5 min for the solar simulator and about 18 min for the UVA1 source. The erythema weighted doses in the UVB range for the solar simulator was 98% of the total dose and 2% in the UVA range. For the UVA1 light source, 99% of the erythema weighted radiation was in the 353-396 nm range. In very sensitive persons with chronic actinic dermatitis, several test doses (up to 24 test areas) may be needed to determine the MED level. *Conclusion:* We have established the relation between just perceptible erythema (+), stronger erythema with sharp borders +, and the even stronger reactions ++ and +++ to predict the (+) from these stronger reactions in order to limit the number of tests.

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OP No. 44

REMOVAL OF SIDE EFFECTS IN PHOTODYNAMIC THERAPY OF ACTINIC KERATOSES

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Classic photodynamic therapy (PDT) involves superficial curettage, application of 5-amino-levulinic acid (ALA) or methyl aminolevulinate (MAL), and occlusion for 3 hours followed by illumination with red LED 37 J/cm^2. Side effects include the following: unpleasant or painful pre-treatment with curettage (bleeding and oozing), stinging during ALA/MAL application, patients crowding the clinic during the wait, severe pain during illumination, and inflammation lasting 1-2 weeks. Adjustments to avoid side effects when treating AK of the face and scalp showed that curettage can be omitted with no loss of efficacy, eliminating the bleeding and oozing (very important in patients on anticoagulants) and the stinging from ALA/MAL application. Occlusion is redundant when skin does not need covering during the wait. Pain is avoided by illuminating protoporphyrin IX (PpIX) during its formation, either outdoors by daylight or indoors by lamps of appropriate wavelengths. Areas to be treated are illuminated from 30 min to 2.5 hours after ALA/MAL application. The daylight illumination may take place at home in the garden or similar, preventing the patients from crowding the clinic. Daylight PDT reduces the risk of post-treatment inflammation and can be further reduced by shortening the ALA/MAL incubation time and by use of topical corticosteroids. We will present all these possibilities of simplifying the PDT procedure.

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OP NO. 45

A REVISED ACTION SPECTRUM FOR VITAMIN D SYNTHESIS BY SUB-ERYTHEMAL UV RADIATION EXPOSURE IN HUMANS *IN VIVO*

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Background/Aim: Action spectra are used to determine risk/benefit relationships from ultraviolet radiation (UVR) exposure. Terrestrial UVB radiation (~295-315 nm) triggers vitamin D_3 synthesis by photoconversion of skin 7-dehydrocholesterol to pre-vitamin D_3. An action spectrum for this reaction has been determined from *ex vivo* skin data, but its reliability has been debated. We tested the validity of this action spectrum by irradiating 75 healthy young volunteers with five broadband UVR spectra. *Materials and Methods:* Serum 25-hydroxyvitamin D_3 [25(OH)D_3] levels were measured before, during, and after five serial whole- or partial-body sub-erythemal UVR exposures. Dose response curves (UVR dose *vs*. 25(OH)D_3) were generated by linear regression for each spectrum. *Results/Conclusion:* Weighting the regression lines with the pre-vitamin D action spectrum did not result in a common line, as would be expected, unless this spectrum was subjected to a 5 nm blue-shift. This shift better matches published *in vitro* action spectrum data for vitamin D_3. Thus, risk (typically erythema)/benefit calculations for solar UVR exposure determined by the *ex vivo* pre-vitamin D_3 action spectrum require re-evaluation.

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OP No. 46

MASS SPECTROMETRIC DETERMINATION OF VITAMIN D METABOLITES IN SERUM, A CONVENIENT TOOL TO IDENTIFY INDIVIDUALS WITH 24-HYDROXYLASE DEFICIENCY

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Background/Aim: Liquid-chromatography-tandem-mass-spectrometry (LC-MS/MS) is a highly sensitive and selective analytical technique that allows the simultaneous measurement of multiple vitamin D metabolites including 25-hydroxyvitamin D_3 (25(OH)D_3), 25-hydroxyvitamin D_2 (25(OH)D_2), 24,25-dihydroxyvitamin D (24,25(OH)$_2D_3$), and 25,26-dihydroxyvitamin D (25,26(OH)$_2D_3$). Previous studies suggest that parallel measurement of these metabolites may help to identify abnormalities in vitamin D metabolism, such as 24-hydroxylase (CYP24A1) deficiency or functional 25(OH)D deficiency. Variants of the *CYP24A1* gene with reduced enzyme activity have been reported to cause hypercalcemia in children (idiopathic infantile hypercalcemia; IIH) and adults due to impaired catabolism of 25(OH)D and 1,25-dihydroxyvitamin D (1,25(OH)$_2$D). Depending on the type of enzyme defect and the supply with 25(OH)D, this can lead to nephrocalcinosis and endogenous vitamin D intoxication, especially when high doses

of vitamin D are supplemented. The present study aimed to investigate the prevalence of abnormalities in vitamin D metabolism amongst healthy blood donors and patients with unclear hypercalcaemia. *Materials and Methods:* $25(OH)D_3$, $25(OH)D_2$, $24,25(OH)_2D_3$, and $25,26(OH)_2D_3$ were measured with a fully validated LC-MS/MS method in serum samples from 2,010 participants of the DESIRE cohort (EK Nr: 1170/2019) consisting of 701 female and 1,309 male blood donors (mean age of 43.4 years). In addition, four serum samples from patients with unclear hypercalcaemia were also analysed. *Results:* The individuals of the DESIRE cohort had the following vitamin D metabolite mean (min-max) concentrations: 69.8 nmol/l (min-max: 14.7-267 nmol/l) for $25(OH)D_3$, 1.9 nmol/l (0.3-38.4 nmol/l) for $25(OH)D_2$, 5.5 nmol/l (0.14-36.4 nmol/l) for $24,25(OH)_2D_3$, and 1.5 nmol/l (0.3-11.3 nmol/l) for $25,26(OH)_2D_3$. The calculated vitamin D metabolite ratio (VMR) ($24,25(OH)_2D_3/25(OH)D_3 \times 100$) was 7.4% (min-max: 0.8-32.7%). A suspicious VMR was only found in one participant (0.05%). The four patients with hypercalcaemia showed $25(OH)D_3$ concentrations of 88.7-166 nmol/l, $24,25(OH)_2D_3$ of 0.16-0.67 nmol/l and VMR of 0.10-0.73%. These suggest a metabolic disorder. Confirmatory genetic testing revealed a homozygous *CYP24A1* mutation (R396W) in all hypercalcaemic patients. *Conclusion:* LC-MS/MS can identify patients with 24-hydroxylase deficiency, and thus it has clinical utility. Further analysis of potential 24-hydroxylase polymorphisms in the DESIRE cohort will provide novel insights into the prevalence and relevance of such enzyme variants.

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OP No. 47

VITAMIN D-BINDING PROTEIN, TOTAL, "NON-BIOAVAILABLE", BIOAVAILABLE, AND FREE 25-HYDROXYVITAMIN D, AND MORTALITY IN A LARGE POPULATION-BASED COHORT OF OLDER ADULTS

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Background/Aim: Epidemiological studies consistently find low concentrations of 25-hydroxyvitamin D (25(OH)D) in blood to be associated with increased mortality. A recent large-scale Mendelian randomization study strongly supports suggestions of a causal relationship among individuals with low vitamin D status. Evolving evidence has suggested that "bioavailable" or free 25(OH)D may better predict mortality. We aimed to compare the prognostic values of vitamin D-binding protein (VDBP), total, bioavailable, complementary "non-bioavailable", and free 25(OH)D for total and cause-specific mortality in a large population-based cohort study of older adults from Germany. *Patients and Methods:* Bioavailable, complementary "non-bioavailable", and free 25(OH)D concentrations were calculated among 5,899 participants aged 50-75 years, based on serum concentrations of total 25(OH)D, VDBP, and albumin. The cohort was followed with respect to all-cause and cause-specific mortality from recruitment in 2001-2002 up to the end of 2018. Multivariable Cox proportional hazards regression models were used to assess the associations between various vitamin D biomarkers and mortality, and further stratified by vitamin D status. *Results*: During a median follow-up of 17.1 years, 1,739 participants died, of whom 575, 584, and 94 died of cardiovascular diseases, cancer, and respiratory diseases, respectively. Very similar inverse associations with total mortality [hazard ratio (HR) per standard deviation decrease: 1.17, 95%CI=1.11, 1.24 for total 25(OH)D; HR=1.14, 95%CI=1.08, 1.21 for bioavailable 25(OH)D; HR=1.12, 95%CI=1.06, 1.18 for free 25(OH)D] and cause-specific mortalities were seen for all biomarkers of vitamin D status. The strongest associations were consistently seen for respiratory mortality. These inverse associations were strongest among participants with low vitamin D levels (<50 nmol/l). No significant associations were seen between VDBP and mortality. *Conclusion:* Total, non-bioavailable, bioavailable, and free 25(OH)D showed very similar inverse associations with total and cause-specific mortality in this large population-based cohort, which were strongest among those with low vitamin D status.

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OP No. 48

THE IMPACT OF VITAMIN D ON CARDIOVASCULAR DISEASE, AND BEYOND

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Various studies have investigated potential effects of vitamin D on the cardiovascular system. Data in experimental animals demonstrate adverse effects on the cardiovascular system of both vitamin D deficiency and excess, indicating a U-shaped association of vitamin D status with cardiovascular disease (CVD) risk. Large observational studies and two very large Mendelian randomization analyses support the association of vitamin D deficiency, *i.e.*, circulating 25-hydroxyvitamin D concentrations <30 nmol/l, with poor CVD outcome, and some studies also indicate an inverse J-shaped association of vitamin D status with CVD risk. Although vitamin D supplementation trials largely failed to demonstrate beneficial vitamin D effects on CVD risk, most of them did not focus on individuals with vitamin D deficiency. With respect to other nonclassical vitamin D-related diseases such as chronic obstructive pulmonary disease, asthma, and infections, meta-analyses of randomized trials have reported beneficial vitamin D effects mainly in individuals with vitamin D deficiency and/or at low daily vitamin D doses. Altogether, data indicate that individuals with deficient circulating 25-hydroxyvitamin D should be the target population for the prevention of any vitamin D deficiency-related risk. Future studies may clarify the importance of gene variants affecting vitamin D metabolism with regard to nonclassical vitamin D-related diseases.

Poster presentations (P)

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P No. 1

CROSS TALK OF ARYL-HYDROCARBON-RECEPTOR (AHR) – AND VITAMIN D RECEPTOR (VDR) – SIGNALING IN HUMAN KERATINOCYTES

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Background/Aim: Ultraviolet (UV)-B radiation activates the aryl hydrocarbon receptor (AhR) and induces its downstream target gene *CYP1A1*. CYP1A1 is involved in xenobiotic metabolism and skin cancer development. The classical biologically active vitamin D metabolite 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] binds to the vitamin D receptor (VDR), exerts multidimensional functions that include protecting the skin against inflammation and cancer, and is metabolized by the VDR target gene *CYP24A1*. CYP24A1 over-expression has been identified in many types of cancer and has been suggested to exert pro-oncogenic effects. Recent studies reported interactions of the AhR and VDR in various organs. *Materials and Methods:* In this study, we investigated the crosstalk between AhR and VDR signaling in immortalized human keratinocytes (HaCaT) and in a cutaneous squamous cell carcinoma cell line (SCL-1) over 24 hours. Transcriptional activity of AhR, CYP1A1, COX-2, VDR, and CYP24A1 was measured and compared using real-time quantitative polymerase chain reaction (RTqPCR). *Results:* Comparing HaCaT with SCL-1 cells, differential expression of many genes was found. In HaCaT keratinocytes no pathway interconnections were found. As expected, UV-B mainly increased AhR and CYP1A1 mRNA while $1,25(OH)_2D_3$ increased COX-2 and CYP24A1 but did not affect VDR expression. In SCL-1 cells, UV-B induced AhR and COX-2 mRNA but did not affect CYP1A1. Interestingly, $1,25(OH)_2D_3$ alone was found to strongly augment CYP1A1 mRNA, whereas this effect became even stronger in combination with UVB. Treatment with its antagonist CH-223191 indicated no involvement of AhR, suggesting that other mechanisms may be responsible for this observation. Moreover, $1,25(OH)_2D_3$ inversely regulated VDR and CYP24A1 mRNA and $1,25(OH)_2D_3$ plus UV-B increased CYP24A1 stronger than any other condition. *Conclusion:* $1,25(OH)_2D_3$, alone or in combination with UV-B, may interact with CYP to modulate non-melanoma skin cancer development. However, further studies are needed to increase our understanding of the mechanisms that underly the observations that we report in this study.

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P No. 2

REGULATION OF CIRCADIAN CLOCK GENES BY UVB RADIATION AND VITAMIN D: A PILOT STUDY IN HUMAN EPIDERMAL KERATINOCYTES DURING DIFFERENT STAGES OF SKIN PHOTOCARCINOGENIESIS

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Background/Aim: Many important cellular processes are constantly under regulation of a timekeeping system, known as the circadian clock (CC), with the main regulatory genes involved referred to as the circadian clock genes (CCGs). In spite of a master pacemaker existing in the suprachiasmatic nucleus (SCN) of the hypothalamus, several clock systems have been shown to operate independently in peripheral tissues, including the skin. CCGs interact with cellular processes by both regulating them and being regulated by them, a prime example of this being the bilateral relationship between CC and ultraviolet B radiation (UV-B): On one hand, UV-radiation

regulates expression of CCGs in many cell types, and on the other, it has recently been shown that expression of CCGs modulates susceptibility for UV-B-induced cellular damage, including the formation of pyrimidine dimers and other DNA-lesions that are a hallmark of photocarcinogenesis. However, the molecular mechanisms that regulate the interplay of CCGs and UV-B are not fully understood. It was the goal of this study to gain further insights into this topic. In particular, we aimed to investigate whether UV-effects on CC may at least in part be mediated by $1,25(OH)_2D_3$ (D_3), the active form of vitamin D, that both depends on UV-B for its synthesis and is known to protect the skin from UV-B's damaging properties. *Materials and Methods*: We treated cells with D_3, UV-B, alone and in combination, and measured the expression of two core clock genes, brain, and muscle ARNT-like 1 (BMAL1) and Period-2 (Per2), both over several time points and in cells representing: normal (normal human epidermal keratinocytes – NHEK; p53 wild type), precancerous (HaCaT keratinocytes; mutated p53 status) and cancerous keratinocytes (squamous cell carcinoma SCL-1; p53 null status). We also assessed the role of vitamin D receptor (VDR) and aryl hydrocarbon receptor (AhR) pathways by measuring UVB-induced damage, repair, and cellular toxicity after treatment with D_3 and UVB and under chemical antagonization of either/both VDR and/or AhR. While UV-B and D_3 were individually showcasing suppression of CCGs, their combined action reversed these effects for both BMAL1 and Per2 ($p<0.001$). Moreover, only UV-B showed a significant influence on cellular toxicity ($p<0.05$). At the same time, overall expression of CCGs and in reaction to UV-B significantly differed between NHEK/HaCaT and SCL-1 cells, with cancerous cells being associated with a lower general expression of CCGs and a higher vulnerability against UV-B's insulting effects. *Conclusion:* In regard to direct effects and interaction of UVB and D_3 and the roles of VDR and AhR on DNA damage and repair, no definite conclusions can be drawn from this pilot study. UV-B differentially regulates the expression of BMAL1 and Per2 in human keratinocytes during different stages of skin photocarcinogenesis. Interestingly, D_3 (UVB*D_3) reversed these effects when applied immediately post-radiation. With disruption of circadian rhythms being directly linked to many forms of cancer, we propose that regulation of circadian rhythms may represent a potentially new cancer protecting role of the hormone (D_3).

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P No. 3
LINK BETWEEN VITAMIN D METABOLISM AND ALZHEIMER'S DISEASE-RELATED PROCESSES

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Background/Aim: Several epidemiological studies have suggested a link between vitamin D hypovitaminosis and the pathological mechanisms involved in the most common neurodegenerative disorder, Alzheimer´s disease (AD). However, the mechanistical impact of the vitamin D metabolism in the brain under pathological conditions like AD still need to be clarified. *Materials and Methods:* One aim of this project was to analyze, how vitamin D deficiency influences AD-related pathways *in vivo* using a mild hypovitaminosis mouse model by investigating 120 AD-related genes. *Results:* We found that the expression of several genes involved in different pathways associated with AD are altered including neurogenesis (*Plat*), inflammation (*Casp4*), lipid- and energy-metabolism (*Abca1* and *Acat1*), APP homeostasis (*Snca*, *Nep*, *Psmb5*), oxidative stress (*Park 7*) and signal transduction (*Gnb5*). Taking into consideration, that the APP processing leading to Aβ is a membrane-associated process and several genes identified in the vitamin D transcriptome are lipid-associated, we also aimed to clarify the effect of vitamin D and its analogues on lipid metabolism utilizing a shotgun mass spectrometry approach after vitamin D treatment. Interestingly, polyunsaturated fatty acids (PUFAs), playing also a crucial role in AD, were found to be altered after vitamin D incubation. *Conclusion*: Vitamin D metabolism is able to influence the pathological mechanisms of AD by affecting different pathways.

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P No. 4
DETECTION OF VITAMIN D HYDROXYDERIVATIVES IN THE BLOOD OF HEALTHY VOLUNTEERS FOLLOWING UVB NARROW-BAND PHOTOTHERAPY: A PILOT STUDY

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Background/Aim: The skin represents a key tissue of the human body's vitamin D endocrine system, representing both the site of UV-B-induced vitamin D synthesis and a target tissue for biologically active vitamin D derivatives. While vitamin D deficiency, defined as low serum concentrations of 25(OH)D, has now been identified as a major health issue, little is known about the UV-B-induced synthesis of other vitamin D metabolites present in the skin, including $24,25(OH)_2D_3$, $20(OH)D_3$ and 3-epi-25-OH-VitD_3, and their physiological relevance. *Materials and Methods:* In this prospective pilot study, we assessed the effect of UVB

narrow band (UVBnb, 311 nm) phototherapy on serum concentrations of some vitamin D hydroxyderivatives, namely $25(OH)D_3$. $24,25(OH)_2D_3$, $1,25(OH)_2D_3$, $1,25-(OH)_2D_2$, $20(OH)D_3$ and 3-epi-$25(OH)D_3$ in healthy volunteers. *Results:* Two weeks of UVBnb treatment resulted in an increase in the serum concentration of $25(OH)D_3$ that was associated with marked changes in the serum concentration of other vitamin D hydroxyderivatives. *Conclusion:* We here report a "fingerprint" of vitamin D derivatives in the serum following UV phototherapy (UVBnb) in healthy volunteers. Considering the growing interest in the binding and activation of several of these hydroxyderivatives to alternate receptors, including aryl hydrocarbon receptor (AhR), retinoid orohan receptors (RORs), and liver X receptor (LXR), the investigation of the functional relevance of these vitamin D hydroxyderivatives and their serum concentrations in response to UV-B-induced vitamin D synthesis as compared to oral uptake of vitamin D (*e.g.*, *via* supplements) deserves systematic analysis.

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P No. 5

VITAMIN D STATUS AND ALOPECIA AREATA (AA) - A META-ANALYSIS

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Background/Aim: Recent laboratory and clinical investigations underline the important role of the vitamin D status in the human immune system. Notably, only a few studies that investigate the impact of the vitamin D status on alopecia areata (AA), have been published to date. AA represents a widespread autoimmune disease affecting the hair follicle that is characterized by a multifactorial and only partly understood pathogenesis. It was the aim of this study to perform a meta-analysis to investigate the impact of the vitamin D status on AA in humans. *Materials and Methods:* We performed a literature search (PubMed, Google Scholar, Cochrane Library, and Web of Science), and for statistical analysis, we applied Stats Direct 3.0.150. To assess the quality of all studies identified, we applied a modified New Castle Ottawa Scale. For the meta-analysis, we used three separate approaches, that assessed 1) the difference of the serum 25-hydroxyvitamin D values, 2) the prevalence of 25-hydroxyvitamin D serum values <20 ng/ml, and 3) the prevalence of 25-hydroxyvitamin D serum values <30 ng/ml, comparing patients with AA and controls. *Results:* We included case-control studies (n=15) but we could not identify interventional or cohort studies. Remarkably, our approach to compare mean 25-hydroxyvitamin D serum values (11 studies, 916 cases) revealed a mean difference of 10.09 ng/ml (95%CI=6.87, 13.31, *p*-value <0.0001), the analysis of vitamin D status <20 ng/ml (6 studies, 744 cases) showed an odds ratio of 7.66 (95%CI=1.91, 30.73, *p*-value=0.0041) and analysis of vitamin D status <30 ng/ml (6 studies, 471 cases) showed an odds ratio of 2.69 (95%CI=0.51, 14.29, *p*-value=0.2446). *Conclusion:* Serum 25-hydroxyvitamin D concentration was markedly reduced in AA patients as compared with controls. However, because of the limitations of the studies suitable for this meta-analysis, including the lack of interventional studies and difficulties in adjusting for confounding factors, findings of this study do not allow any conclusion on a causal relationship between vitamin D status and AA risk. To answer the question whether the association between increased risk for AA and low serum 25-hydroxyvitamin D concentrations may result from a causal relationship or may be of importance for the clinical outcome of this disease, large-scale prospective interventional studies are urgently needed in the future.

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P No. 6

META-ANALYSIS AND SYSTEMATIC REVIEW OF THE ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF THE VITAMIN D RECEPTOR GENE AND RISK FOR MALIGNANT MELANOMA (MM)

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Background/Aim: During the last decades, a constantly growing body of evidence has convincingly shown the importance of the vitamin D endocrine system for pathogenesis and clinical outcome of malignant melanoma (MM). We performed a systematic review and meta-analysis to analyse the association between single nucleotide polymorphisms (SNPs) of the gene encoding for the vitamin D receptor (VDR) and risk for MM. *Materials and Methods*: We performed a systematic literature search (ISI Web of Science, Medline). After careful analysis, a total of 14 studies, analysing seven VDR SNPs, namely rs2228570 (*FokI*), rs731236 (*TaqI*), rs11568820 (*Cdx2*), rs1544410 (*BsmI*), rs4516035 (A-1012G), rs7975232 (*ApaI*) and rs739837 (*BglI*), were included in the meta-analysis. In our statistical analyses, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for dominant and recessive models and the results were demonstrated in Forest Plots. Moreover, we analysed potential publication bias using Funnel

Plots and the Egger-Test. *Results*: Our meta-analysis demonstrated a significant risk reduction of 15% in MM incidence for the rarer allele B in the dominant model (Bb + BB *vs*. bb). The dominant model (Ff + ff *vs*. FF) of the SNP rs2228570 (*FokI*) demonstrated that carriers of the rarer allele f were 22% more likely to develop MM. For the SNP rs7975232 (*ApaI*), there was a 20% higher risk of MM for carriers of the rarer a allele (Aa + aa *vs*. AA). Notably, the findings of the meta-analysis demonstrated no significant association between the other SNPs that were investigated. *Conclusion*: The VDR SNPs *FokI*, *ApaI,* and *BsmI* may modulate the susceptibility to develop MM. We demonstrated an increased risk of developing a MM for carriers of the rarer allele f of rs2228570 (FokI) and the rarer allele a of SNP rs7975232 (ApaI). In contrast, carriers of the less common allele B of the SNP rs1544410 (BsmI), demonstrated a protective effect and thus, reduced disease risk was observed.

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P No. 7
DISTRIBUTION AND DETERMINANTS OF VITAMIN D-BINDING PROTEIN, TOTAL, "NON-BIOAVAILABLE" BIOAVAILABLE, AND FREE 25-HYDROXYVITAMIN D CONCENTRATIONS IN A LARGE POPULATION-BASED COHORT OF OLDER ADULTS

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Background/Aim: Serum 25-hydroxyvitamin D (25(OH)D) [total 25(OH)D] has been widely used to indicate vitamin D status. About 85-90% and 10-15% of serum 25(OH)D are bound to vitamin D-binding protein (VDBP) and albumin, respectively. Less than 1% circulates in a free form, known as free 25(OH)D. Evidence indicated that bioavailable or free 25(OH)D may have better prognostic values for vitamin D-related health outcomes. The objective was to assess and compare distributions and determinants of VDBP, total, bioavailable, "non-bioavailable", and free 25(OH)D. *Materials and Methods:* We included 5,899 older adults, aged 50-75 years, from a large population-based cohort in Germany. We calculated bioavailable (and complementary "non-bioavailable") and free 25(OH)D concentrations based on total 25(OH)D, VDBP, and albumin concentrations. We applied linear regression models to assess determinants of vitamin D biomarkers. *Results:* There were seasonal variations among vitamin D biomarkers. The highest VDBP levels were observed in spring, whereas the highest levels of total, non-bioavailable, bioavailable, and free 25(OH)D were observed in summer. Older age and higher body mass index were associated with lower levels of all markers. Their associations with C-reactive protein varied. Bioavailable and free 25(OH)D levels strongly varied by VDBP genotypes, which had opposite influences on non-bioavailable 25(OH)D. *Conclusion:* Concentrations of vitamin D biomarkers were influenced by numerous genetic and non-genetic determinants, which may contribute to better understanding their associations with vitamin D-related health outcomes.

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P No. 8
SUNSCREEN USE FOR PREVENTION OF SKIN CANCER: ONLY BENEFITS OR IS THERE A RISK OF HARMFUL EFFECTS?

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Background/Aim: The use of sunscreens is an important part of many guidelines and campaigns to prevent skin cancer. However, increasing evidence has recently raised concerns concerning their efficacy and safety. *Materials and Methods:* A systematic literature search was performed (until 12/2019) using Pubmed and web of science to identify publications that analyse the efficacy or safety of sunscreens. *Results:* This literature search identified many reports that question the efficacy of sunscreens and reveal potential risks that are associated with their use. Besides well known potential adverse events such as phototoxic or photoallergic dermatitis, these risks include endocrine activities described as "endocrine disruption". Additionally, it has been shown that sunscreens are absorbed transcutaneously, can be detected in mother milk, human placental tissue, and the food chain. Additionally, they are now considered as a major threat for ocean life including coral reefs. *Conclusion*: When

deciding whether or not to use sunscreens, recent reports indicating limited efficacy and potential risks should be considered. Well-designed future studies to investigate the efficacy and safety of sunscreen are urgently needed.

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P No. 9
SUNBEDS AND MELANOMA RISK: WHAT IS THE EVIDENCE?

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Background/Aim: For decades, there is a continuous scientific debate whether moderate sunbed use may increase the risk to develop malignant melanoma (MM). However, some recent reports in the scientific literature demand the debate to be closed now because a causal relationship between sunbed use and MM risk would now have been convincingly shown. *Materials and Methods:* In this work, we analyzed our present scientific knowledge in this research area. *Results:* We resume that there is no proof for a causal relationship between MM risk and moderate sunbed use. *S*uch a causal relationship could be proven by interventional studies (randomized controlled trials, RCTs), but these are lacking. The weak associations reported to date in cohort and case–control studies fail to prove causality. Notably, because of severe limitations which cause bias, the observational studies published in this research area are characterized by poor overall quality and low levels of the resulting evidence. To date, the majority of studies report risk estimates (*e.g.*, odds ratios, ORs), that were not systematically and adequately adjusted and recorded for many confounding factors, including skin type, sun exposure, sunburn, and others. Notably, summary risk estimates of a meta-analysis that we published in 2018 (1) pointed at a weak association [OR=1.19, 95% confidence interval=1.04-1.35, p=0.009) for 'ever use' of a solarium with MM risk. However, sensitivity analyses of this meta-analysis, that included two cohort and twenty-nine case–control studies did not show an association for studies with a low risk of bias, studies performed after 1990, and studies from Europe. In addition, because of severe limitations including confounding and lack of RCTs, overall study quality, resulting levels of evidence (3a–) and grades of recommendation (D) were poor. It has to be noted that the identical risk estimates (*e.g.*, ORs) as to date published in meta-analyses may well be the result of the following scenario: moderate solarium use has no effect on MM risk, but an 'unhealthy lifestyle' (*e.g.,* extensive sunbathing) causes an inflated OR of 1.2 in association with solarium use (it was shown that 'sun worshippers' visit tanning salons more frequently). It has to be noted that other classifications, *e.g.,* the criteria for plausibility in a biological system defined by Hill in 1965 (2), fail as well to support causality for the inference that moderate sunbed use may increase MM risk *per se*. It must be emphasized that a convincing body of evidence from epidemiological and animal studies reports no increase in MM risk following moderate (chronic) exposure with solar or artificial UV radiation. Moreover, some reports indicate that outdoor workers are at reduced risk to develop MM and that sub-erythemal chronic exposure to solar radiation may protect against MM. *Conclusion:* At present, the scientific literature provides no convincing evidence that responsible/moderate solarium use increases MM risk. Many unresolved questions still remain and the debate cannot be closed.

1 Burgard B, Schöpe J, Holzschuh I, Schiekofer C, Reichrath S, Stefan W, Pilz S, Ordonez-Mena J, März W, Vogt T and Reichrath J: Solarium use and risk for malignant melanoma: Meta-analysis and evidence-based medicine systematic review. Anticancer Res *38(2)*: 1187-1199, 2018. PMID: 29374757.

2 Hill AB: The environment and disease: Association or causation? Proc R Soc Med *58*: 295-300, 1965. PMID: 14283879.

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P No. 10
IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS [SNPs] OF GENES INVOLVED IN SKIN PIGMENTATION FOR VITAMIN D STATUS: WHAT CAN WE LEARN FROM EVOLUTION?

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Background/Aim: In Caucasian populations, vitamin D deficiency is very common. In addition to increased overall mortality, low vitamin D status was reported to be associated with increased incidence and poor outcome of many diseases, such as various malignancies, infectious, autoimmune, and cardiovascular diseases. Interestingly, although a broad variety of individual factors that influence a person's vitamin D status, including skin type, have been reported, to date only a few genetic determinants of serum 25-hydroxyvitamin D (25[OH]D) concentration have been identified. *Materials and Methods:* In a large cohort of mostly Caucasians (participants of the Ludwigshafen Risk and Cardiovascular Health Study; n=2970), we investigated whether single nucleotide polymorphisms

(SNPs) of genes that contribute to skin pigmentation may predict a person's vitamin D status (defined as serum 25(OH)D levels). Serum 25(OH)D and SNPs (n=960) related to genes that contribute to skin pigmentation including agouti signalling peptide (*ASIP*), cAMP-dependent protein kinase type I-alpha regulatory subunit (*PRKAR1A*), cAMP-dependent protein kinase type II-alpha regulatory subunit (*PRKAR2A*), cAMP-dependent protein kinase type II-beta regulatory subunit (*PRKAR2B*), cyclic AMP-dependent transcription factor (*ATF1*), microphthalmia-associated transcription factor (*MITF*), dopachrome tautomerase (*DCT*), tyrosinase (*TYR*), TYR-related protein 1 (*TYRP1*), oculocutaneous albinism II (*OCA2*), two pore segment channel 2 (*TPCN2*), proopiomelanocortin (*POMC*), cAMP-dependent protein kinase catalytic subunit beta (*PRKACB*), cAMP-dependent protein kinase catalytic subunit gamma (*PRKACG*), tubulin beta-3 chain/melanocortin receptor 1 (TUBB3/*MC1R*), Cadherin-1 (*CDH1*), catenin beta 1 (*CTNNB1*), Endothelin 1 (*EDN1*), endothelin 3 (*EDN3*), endothelin receptor type B (*EDNRB*), fibroblast growth factor 2 (*FGF2*), KIT, KIT ligand (*KITLG*), nerve growth factor (*NGF*), interferon regulatory factor 4 (*IRF4*), exocyst complex component 2 (*EXOC2*), solute carrier family 24 A4 (*SLC24A4*), solute carrier family 45 A2 (*SLC45A2*), and tumor protein 53 (*TP53*)] were analyzed. *Results:* In comparison to the serum 25(OH)D level of the total cohort (median, 15.5 ng/ml), a total of 46 SNPs were associated ($p<0.05$) with reduced or increased serum 25(OH)D levels. However, only one SNP located in the *EXOC2* gene reached the aimed significance level after correction for multiple comparisons (false discovery rate) and was associated with a Δ25(OH)D value of more than 5.00 ng/ml. Eleven SNPs located in the genes encoding for *TYR* (n=4), *EDN1* (n=3), *MITF* (n=2), *TYRP1* (n=1), and *PRKACG* (n=1) fulfilled after correction for multiple comparisons (false discovery rate) the aimed significance level, but were not associated with a Δ25(OH)D value greater than 5.00 ng/ml. *Conclusion:* SNPs of genes that contribute to skin pigmentation are predictive of vitamin D status [measured as serum 25(OH)D concentration] in the Caucasian population. Here, we show that out of the SNPs in the 29 different genes analyzed, SNPs of 11 genes, including *EXOC2*, *TYR*, and *TYRP1*, have the greatest association with vitamin D status. These findings have important implications for a better understanding of the role of skin pigmentation, solar UV radiation, and vitamin D in human evolution.

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Po No. 11

PROMISCUOUS LIAISONS: DISRUPTED P53 -, NOTCH - AND VITAMIN D – PATHWAYS CONTRIBUTE TO EVASION OF ANTI-GROWTH SIGNALING DURING CUTANEOUS PHOTOCARCINOGENESIS

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Background/Aim: Evasion of anti-growth signaling is an important feature of non-melanoma skin cancer (NMSC), with disrupted p53-signaling holding an outstanding position as the principal underlying driver mutation. *Materials and Methods:* We developed an organotypic culture model that enables the analysis of different stages of photocarcinogenesis (PC) by comparing normal human keratinocytes (NHK/p53wt), HaCaT (p53mut – representing initiated cells at early stage), and SCL-1 (p53null – malignant cells at late stage) cells. *Results:* Applying automated analysis of cellular growth, immunohistochemistry, western analysis, RT-PCR, and microarray profiling, we showed that during PC, stage-dependent dysregulation of growth is associated with alterations affecting a broad variety of different pathways implicated in cancer. Comparing untreated NHK, HaCaT, and SCL-1 cells, 379 out of 660 miRNAs and 17,624 out of 19,453 protein coding gene transcripts were differentially expressed (as assessed by ANOVA), with a subset of these miRNAs (including miR-144-3p, miR-483-5p) and mRNAs having been previously implicated in cancer and/or p53, vitamin D or Notch signaling. Moreover, we report a differential cellular response to two pro- and anti-carcinogenic agents, the complete carcinogen UV-radiation and/or the endogeneous anti-growth factor 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), respectively. As a major result, we postulate that loss of the ability to respond to UV-B treatment with induction of expression of DNp73 and JAG2, may represent a new key mechanism involved in PC. Considering the important function of D Np73 and JAG2 for many pathophysiological processes that contribute to PC, including evasion of anti-growth signaling, apoptosis, DNA repair, stemness, and senescence, it can be speculated that this mechanism is of high relevance for NMSC. *Conclusion:* In NMSC, evasion of anti-growth signaling is associated with disrupted p53 signaling and a consecutive "chain reaction" resulting stage-dependently in multiple other dysregulated pathways implicated in cancer. In addition, we here postulate a new key mechanism that is of importance for the evasion of anti-growth signaling in NMSC, involving loss of induction of DNp73 and JAG2; although the causal relationship and functional significance of this observation need to be confirmed in future studies.

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