ABSTRACTS OF THE 4TH INTERNATIONAL SYMPOSIUM ON VITAMIN D AND ANALOGS IN CANCER PREVENTION AND THERAPY

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1
D-LIGHTFUL VITAMIN D FOR HEALTH

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Vitamin D is the sunshine vitamin. During exposure to sunlight, 7-dehydrocholesterol absorbs ultraviolet B radiation resulting in the cutaneous production of previtamin D_3. Once formed, previtamin D_3 undergoes an internal isomerization resulting in the production of vitamin D_3. During prolonged exposure to sunlight, pre-vitamin D_3 and vitamin D_3 absorb UVB radiation resulting in their conversion to a variety of biologically inert (on calcium metabolism) products. A variety of factors markedly influence the production of vitamin D in the skin including skin pigmentation, sunscreen use, time of day, season of the year, latitude and aging. Once formed, vitamin D undergoes sequential hydroxylations in the liver and kidneys to form 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], respectively. 1,25$(OH)_2$D plays an important role in maintaining calcium homeostasis and maximizing bone health. There is mounting evidence that increasing blood levels of 25(OH)D reduces risk of many chronic illnesses including autoimmune diseases, cancer, heart disease and type II diabetes. There is also evidence for its reducing the risk of infectious diseases, preeclampsia and requiring a primary Cesarean section. Improving vitamin D status has also been associated with improvement in neurocognitive function and in muscle strength. Serum 25(OH)D>30 ng/m can be achieved in most children by ingesting 1,000 IU of vitamin D a day and for teenagers and adults 2,000 IU of vitamin D a day.

2
NEW PARADIGMS FOR GENE REGULATION BY 1,25-DIHYDROXYVITAMIN D_3

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1,25-Dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) exerts biological activity in target tissues by regulating the expression of genes involved in cellular differentiation and function. These activities are mediated by VDR which binds to DNA sequences at target genes and functions to nucleate coregulatory complexes essential for gene modulation. To examine these concepts, we have used both gene expression studies and chromatin immunoprecipitation (ChIP) linked to deep sequencing techniques (ChIP-seq) to explore sites of action of VDR and its partner RXR in bone and intestinal cells. We have also used these techniques to examine colocalization with other DNA binding proteins, lineage-specific factors and coregulators. VDR binds to several thousand sites on mouse and human genomes in osteoblast and intestinal/colon cells upon activation by 1,25$(OH)_2D_3$. RXR co-binds to the majority of these sites as well, as do numerous coregulatory factors. Surprisingly, most of these sites are located distal to regulated gene promoters. In bone cells, many of these sites overlap with those of the master regulator RUNX2 and the chromatin remodeler C/EBPβ, whereas in intestinal cells, these sites frequently contain both C/EBPβ and the homeobox factor Cdx2. These regulatory regions modulate the expression of genes for *CYP24A1, VDR, SPP1, RANKL, CBS* and others in osteoblasts, and *CYP24A1, CYP3A4, CYP2B6, ABCB1*, and *PADI1* in intestinal cells. Interestingly, ChIP-seq analysis of TCF4/β-catenin binding revealed numerous sites of action on target genes for the Wnt activation pathway in colorectal cancer cells. A small but signficant colocalization of both VDR/RXR and TFC4/β-catenin sites highlighted a number of growth regulating genes that included *c-FOS* and *c-MYC*. The funtional activity of 1,25$(OH)_2D_3$ on these latter genes has been explored. These new approaches to the study of gene expression have revealed an important set of overarching principles of 1,25$(OH)_2D_3$ action in cells.

3
A GENOME-WIDE PERSPECTIVE ON VITAMIN D SIGNALING

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As a model system for a genome- and transcriptome-wide understanding of the primary actions of the nuclear hormone 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) and its receptor VDR, we selected THP-1 human monocytic leukemia cells. Mircoarray time course experiments over a period of 4 hours after 1α,25$(OH)_2D_3$ treatment demonstrated that the mRNA expression of close to 2,000 of the 17,000 expressed genes in THP-1 cells is statistical significantly affected, although many of these effects are only transient and of lower magnitude. ChIP-Seq analysis at time point 40 min after 1α,25$(OH)_2D_3$ treatment identified 2340 genomic VDR binding locations, 520 of which occur already in absence of ligand, but most of them do not contain a DR3-type response element (RE), while many of the 1171 sites that light up specifically after VDR activation carry at least one DR3-type RE. Interestingly, most of the up-regulated 1α,25$(OH)_2D_3$ target genes show VDR binding within 400 kb of their transcription start site (TSS), while this applied only for some less than half of the down-regulated genes. The genomic VDR loci showed quite a variation in their gene regulatory scenarios ranging from a single VDR location close to the target gene TSS, more complex ones with one VDR location more distal to the TSSs of two target genes and very complex ones with many VDR

locations in a cluster of target genes. In conclusion, VDR has far more than expected target genes in THP-1 cells and ligand binding shifts genome-wide VDR locations to distal regions of its primary target genes carrying DR3-type REs that occur in a large variation of regulatory constellations.

4
SOLUTION STRUCTURES OF NUCLEAR RECEPTOR HETERODIMERS

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Nuclear receptors (NRs) control numerous physiological processes through the regulation of gene expression. We have determined the solution structures of three functional heterodimers RXR/RAR, RXR/PPAR and RXR/VDR bound to natural hormone response elements in presence or absence of coactivator, using Small Angle X-ray Scattering and Fluorescence Resonance Energy Transfer techniques. In contrast to the reported crystal structure of RXR/PPAR, the structures in solution exhibit an extended asymmetric shape with distinct modules containing DNA bound to DNA binding domains and heterodimers of ligand binding domains respectively. A structural basis for understanding the role of DNA in the spatial organization of NRs heterodimers into functional transcription complexes can be inferred.

5
THE VITAMIN D RECEPTOR: THE FOUNTAIN OF YOUTH IN CANCER PREVENTION

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The nuclear vitamin D receptor (VDR) binds 1,25-dihydroxyvitamin D_3 (1,25D), its high affinity endocrine ligand, to signal intestinal calcium and phosphate absorption plus bone remodeling to prevent osteoporotic fractures. 1,25D/VDR also controls gene expression to delay aging and chronic diseases such as cancer, arteriosclerosis, stroke, and infection. 1,25D/VDR regulates the transcription of genes relevant to cancer, *e.g.*, *CYP27B1, CYP24A1,* and *FGF23*. Circulating 25-hydroxyvitamin D_3 is converted to 1,25D locally by extrarenal CYP27B1, and binds VDR to promote anticancer actions. Moreover, vitamin D affects the expression of a host of oncogenes, their receptors, tumor suppressors, and DNA repair systems. VDR also affects Wnt signaling through direct interaction with β-catenin, and blunts β-catenin mediated transcription in colon cancer cells to attenuate their growth. Additionally, novel VDR ligands, such as curcumin, can activate VDR signaling, with a differential gene expression profile to synergize with vitamin D in lowering the risk of colon tumorigenesis. VDR also binds the carcinogenic secondary bile acid, lithocholic acid, to induce its CYP3A4-mediated detoxification. In conclusion, we hypothesize that the prevention of colon cancer by VDR activation serves as a paradigm for understanding the mechanisms whereby VDR attenuates aging and chronic disease in the cardiovascular, immune, and musculoskeletal systems.

6
VITAMIN D AND COLON CANCER: REGULATION AND EFFECTS OF SPROUTY-2 AND DICKKOPF-1 GENES AND PROTEOMIC ANALYSIS

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1Alpha,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) inhibits proliferation and promotes differentiation of human colon cancer cells *via* regulation of a number of genes and the antagonism of the Wnt/beta-catenin pathway. We have shown that $1,25(OH)_2D_3$ induces DICKKOPF (DKK)-1, a secreted Wnt antagonist, while it represses SPROUTY (SPRY)-2, a regulator of tyrosine kinase receptor signalling. Our results indicate that *DKK-1* and *SPRY-2* genes have unexpected biological activities that may contribute to explain the protective action of $1,25(OH)_2D_3$ against colon cancer. *DKK-1* has antiproliferative action in cultured cells and xenografted animals and its expression is lost in 25% of advanced tumors by epigenetic silencing. Recent data suggest that DKK-1 has additional Wnt-unrelated antitumor activities that may further contribute to its tumour suppressive activity. *SPRY-2* is up-regulated in colon tumors and promotes an invasive

phenotype at least in part by the inhibition of E-cadherin and the adhesive phenotype. SPRY-2 and E-cadherin are reciprocally regulated and have opposite patterns of expression *in vivo*. $1,25(OH)_2D_3$ represses *SPRY-2* by adhesion-dependent and –independent mechanisms. Finally, by comparative proteomic analysis of nuclear fractions of human SW480-ADH cells treated with $1,25(OH)_2D_3$ or vehicle, we have identified novel proteins whose expression is regulated by this hormone. Interestingly, several of these proteins are components of the spliceosome, suggesting a role of $1,25(OH)_2D_3$ in the control of the splicing process.

7
NEW INSIGHTS INTO THE ROLE OF CYP24A1 IN VITAMIN D METABOLISM

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25-Hydroxyvitamin D-24-hydroxylase (CYP24A1) is the kidney and target-cell enzyme responsible for the 5-step catabolic sequence known as the 24-oxidation pathway from $1,25\text{-}(OH)_2D_3$ to calcitroic acid, a known biliary catabolite, as well as a similar pathway which starts with 23-hydroxylation and culminates in the $1,25\text{-}(OH)_2D_3$-26,23-lactone. CYP24A1 is strongly induced/feedback regulated by its substrate $1,25\text{-}(OH)_2D_3$. Although some recent evidence suggests that 24-hydroxylated metabolites of CYP24A1 play a role in bone fracture repair, the overwhelming body of evidence suggests that CYP24A1 exists to breakdown the precursor, 25-OH-D, or the hormone, $1,25\text{-}(OH)_2D$, into inactive products. Recently a genome-wide association study of the genetic determinants of serum 25-OH-D concentrations identified CYP24A1, along with DBP, CYP2R1 and DHR7 as the 4 major proteins involved. Currently, no human disease has been identified with coding sequence mutations of *CYP24A1*, but human databases are now reporting several polymorphisms of the protein. *CYP24A1*-null mice have 50% lethality at weaning and surviving animals exhibit difficulty clearing a bolus of $[1\beta\text{-}^3H]1,25\text{-}(OH)_2D$ and appear to control serum $1,25\text{-}(OH)_2D$ production by down-regulating *CYP27B1*. Over the past 5 years, our laboratory has created *in vitro* and tested the enzyme activity of over 50 mutations of h*CYP24A1,* mostly involving active site residues. As might be expected, some mutations abolish activity and some have no effect. But one interesting mutation (*e.g.* A326G) changes the site of hydroxylation from C-24 to C-23 and results in $1,25\text{-}(OH)_2D_3$-26,23-lactone rather than calcitroic acid; while another mutation V391L causes the enzyme to gain $1\alpha\text{-OH-}D_3$-25-hydroxylase activity. Recent work has suggested that *CYP24A1* is up-regulated by FGF-23 and this process may contribute to the low serum 25-OH-D and $1,25\text{-}(OH)_2D$ levels arising in chronic kidney disease (CKD) patients. Specific hCYP24A1 inhibitors have emerged that may have utility in disease states with overexpression of CYP24A1, including CKD, certain types of cancer and dermatological conditions. Accordingly, CYP24A1 is being recognized as a major component of the vitamin D signal transduction system, one that can be defective or be overexpressed and thus represent a major drug target. This presentation will review some of the latest findings in each area of CYP24A1 research.

8
CALCITRIOL ACTIONS IN BREAST CANCER

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Calcitriol (1,25-dihydroxyvitamin D_3), the hormonally active form of vitamin D, exerts antiproliferative, anti-inflammatory and proapoptotic effects in many malignant cells including breast cancer (BCa) cells. These actions raise the possibility of its use in BCa prevention and therapy. We previously showed that calcitriol inhibits estrogen synthesis and signaling in cultured BCa cells. Calcitriol's effect on aromatase, the enzyme that is critical for estradiol synthesis, was tissue specific, decreasing aromatase expression in BCa cells and adipocytes while increasing it in osteosarcoma cells. Thus calcitriol is a selective aromatase modulator (SAM). More recently we examined calcitriol actions *in vivo* in immunocompromised mice bearing MCF-7 tumor xenografts. We studied aromatase expression, estrogen signaling and tumor growth when calcitriol or the aromatase inhibitors (AIs) anastrazole and letrozole were given to the mice alone or in combination at sub-optimal doses. Calcitriol and the AIs administered alone exhibited significant tumor inhibitory activity (>50% shrinkage). Maximal tumor growth inhibition was seen with combinations of calcitriol and either AI. Calcitriol also acted as a SAM *in vivo* decreasing aromatase expression in xenograft tumors and the surrounding breast adipose tissue while increasing aromatase expression in bone marrow cells. In addition, calcitriol caused a significant reduction in estrogen levels in BCa tumor tissue and breast adipose tissue. Changes in the expression levels of various target genes demonstrated the suppressive effects of calcitriol on estrogen signaling and other inflammatory and growth stimulatory pathways. We hypothesize that cumulatively these actions will enhance the beneficial effect of combining calcitriol with an AI in the treatment of women with BCa. The actions of calcitriol would improve the anti-proliferative efficacy of AIs, and because of its tissue selective activity to stimulate aromatase in bone, calcitriol might also potentially protect bone from estrogen deprivation and reduce the AI side-effect of causing bone loss.

9
VITAMIN D AND CALCIUM SIGNALING IN CANCER

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$1,25(OH)_2D_3$ (1,25D) regulates a variety of signaling pathways *via* intracellular Ca^{2+}. Modulating apoptosis is emerging as a strategy for treatment and prevention of cancer. Cellular Ca^{2+} has been implicated in triggering apoptosis, however, vitamin D/Ca^{2+}-dependent targets involved in apoptotic signaling have not been identified. We investigated mechanisms of 1,25D-induced Ca^{2+} signaling and Ca^{2+}-mediated apoptosis in breast cancer cells. The results obtained demonstrate that 1,25D regulates Ca^{2+} entry from the extracellular space, Ca^{2+} mobilization from intracellular stores and intracellular Ca^{2+} buffering. We have also shown that the apoptotic Ca^{2+} signal represents a sustained increase in $[Ca^{2+}]_i$ reaching elevated, but not cytotoxic levels. The apoptotic Ca^{2+} signal induced by 1,25D in breast cancer cells is associated with activation of Ca^{2+}-dependent μ-calpain and Ca^{2+}/calpain-dependent caspase-12. Activation of these proteases appears to be sufficient for execution of apoptosis. Normal mammary epithelial cells are resistant to induction of apoptosis with 1,25D due to their large Ca^{2+} buffering capacity. These results indicate that 1,25D-induced cellular Ca^{2+} signal can act as an apoptotic initiator that directly recruits Ca^{2+}-dependent apoptotic effectors capable of executing apoptosis.

10
EXPRESSION OF VITAMIN D RECEPTOR IN SQUAMOUS EPITHELIAL VULVAR CANCER AND VULVAR INTRAEPITHELIAL NEOPLASIA

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Introduction: The antiproliferative effects of calcitriol is mediated *via* the vitamin D receptor. The aim of this study is to evaluate whether vulvar cancer express the vitamin D receptor and when the vitamin D receptor is expressed whether it is upregulated compared to benign vulvar lesions. Furthermore the expression of VDR in precursor lesion is examined. *Materials and Methods:* The expression of VDR in benign vulvar lesions (n=20), vulvar intraepithelial neoplasias (n=20) and vulvar cancer (n=20) was determined by immunohistochemistry using the Remmele score and by western blot. *Results:* The vitamin D receptor is expressed in benign vulvar lesions and in vulvar cancer. Comparing benign lesions with malignant lesions the expression of VDR is upregulated in vulvar cancer. *Conclusion:* Vulvar cancer and vulvar intraepithelial neoplasias may be a target for antiproliferative treatment with vitamin D analogs.

11
EVALUATION OF $25(OH)D_3$ IN SERUM OF PATIENTS WITH VULVAR CANCER AND BENIGN VULVAR LESIONS

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Introduction: The antiproliferative effects of calcitriol is mediated *via* the vitamin D receptor. In previous studies we showed that the VDR was expressed in vulvar cancer and seemed to be up-regulated. The aim of this study is to evaluate whether the serum levels of $25(OH)D_3$ in patients with vulvar cancer and in patients with benign vulvar lesions are similar or whether they are different. Low serum levels of $25(OH)D_3$ in patients with vulvar cancer could indicate a role of $25(OH)D_3$ in carcinogenesis of vulvar cancer. *Patients and Methods:* The level of $25(OH)D_3$ in serum was determined in patients with vulvar cancer (n=20) matched with patients with benign vulvar lesions (n=20). *Results:* The level of $25(OH)D_3$ in serum was significantly lower in patients with vulvar cancer compared with patients with benign vulvar lesions. *Conclusion:* Low serum levels of $25(OH)D_3$ are involved in vulvar cancer.

12
$1\alpha,25$-DIHYDROXYVITAMIN D_3 INDUCES THE *DE NOVO* EXPRESSION OF E-CADHERIN IN MDA-MB-231 BREAST CANCER CELLS

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Breast cancer is the main cause of death by cancer in women worldwide. In several cancer models, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) was shown to participate in growth inhibition and induction of differentiation, being, therefore, a potential anticancer agent for breast tumours. We demonstrated that $1,25(OH)_2D_3$ promotes the differentiation of highly metastatic MDA-MB-231 breast cancer cells by inducing the *de novo* expression of E-cadherin. This effect was shown to be time- and dose-dependent. Furthermore, the level of expression induced by $1,25(OH)_2D_3$ was almost 3 times higher than that induced by the demethylating agent 5-aza-dC. When combined together, $1,25(OH)_2D_3$ and 5-aza-dC displayed an additive effect in the activation of *CDH1* gene expression. Additionally, MDA-MB-231 cells exhibited E-cadherin expression at the plasma membrane upon $1,25(OH)_2D_3$ treatment, indicating the up-regulation of a functional adhesion molecule; in contrast, its expression induced by 5-aza-dC was granular and dispersed throughout the cytoplasm, suggestive of a non-functional protein. When these cells were treated with both agents, there was a rescue of E-cadherin expression back to the membrane, hinting that $1,25(OH)_2D_3$ is indeed inducing not only the expression of E-cadherin, but, apparently, is also important for the correct membrane localization of the protein as a cell–cell adhesion molecule.

13

INFLUENCE OF CALCITRIOL ON PG- AND VITAMIN D-METABOLISING ENZYMES IN BENIGN AND MALIGNANT BREAST CANCER CELL LINES

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Cyclooxygenase-2 (COX-2) is a potential molecular prognostic factor for breast cancer and calcitriol ($1,25(OH)_2D_3$), the biologically active form of vitamin D a promising target in breast cancer therapy. The expression of PG-metabolising enzymes, PG-receptors, vitamin D metabolising enzymes and the VDR were determined in benign and malignant breast cell lines. Moreover, the influence of calcitriol on cell proliferation was determined and the effect of calcitriol on the enzyme expression was examined. We detected a dysregulated vitamin D-metabolism, especially in the invasive breast cancer cell line. Calcitriol showed an antiproliferative effect only in the benign but not in the malignant cell lines, and the expression of COX-2 and 15-PGDH was influenced by calcitriol only in the benign breast cell line. These results suggest a link between the two metabolisms and therefore a possible synergism between COX-2 inhibition and calcitriol in breast cancer cells.

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CORRELATION OF PROSTAGLANDIN METABOLISING ENZYMES AND PGE_2 SERUM LEVELS WITH VITAMIN D RECEPTOR AND $25(OH)_2D_3$ SERUM LEVELS IN BREAST AND OVARIAN CANCER

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Breast and ovarian cancer are associated with inflammatory processes based on an upregulation of cyclooxygenase-2 (COX-2) expression. The antiproliferative effects of calcitriol ($1,25(OH)_2D_3$) render vitamin D a promising target in cancer therapy. Recent data suggests a correlation between vitamin D and prostaglandin metabolism. The current study aimed to evaluate the expression of prostaglandin-metabolising enzymes COX-2 and 15-hydroxyprostaglandin-dehydrogenase (15-PGDH) compared to the vitamin D receptor (VDR) in benign and malignant breast and ovarian tissues. We determined VDR, COX-2, 15-PGDH and EP_2/EP_4 expression in tissue by real-time PCR and Western blot analysis, as well as $25(OH)_2D_3$ and PGE_2 plasma levels from healthy and cancer patients. We detected an inverse correlation between the COX-2 and VDR expression in cancer patients. Breast cancer patients diagnosed in the wintertime had significantly lower $25(OH)_2D_3$, PGE_2 serum levels of both were higher. These results suggest a link between prostaglandin and vitamin D metabolism.

15

THE COMBINED EFFECT OF $1,25(OH)_2D_3$ WITH CBP OR IONIZING RADIATION ON THE PROLIFERATION OF OVARIAN CANCER SKOV-3 CELLS

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We previously demonstrated that 1,25-dihydroxyvitamin D_3($1,25(OH)_2D_3$) inhibited the growth of human ovarian

cancer cell line SKOV-3. To explore whether $1,25(OH)_2D_3$ can enhance the anti-proliferative effect of carboplatin (CBP) or ionizing radiation (IR), the combination effects on proliferation of SKOV-3 cells were determined. Our results showed that although each of the alone treatment displayed anti-proliferative effect, the growth inhibition of SKOV-3 cells was further enhanced by the combination of $1,25(OH)_2D_3$ and CBP or IR. The greatest effect of inhibition on cell proliferation occurred at 10 nM of $1,25(OH)_2D_3$ combined with 40 mg/l of CBP or 6 Gy IR. Cell cycle analysis indicated that the distribution of G_0/G_1 phase in SKOV-3 cells was significantly increased by $1,25(OH)_2D_3$, whereas, the distribution of G_2/M phase was further increased with its combination with CBP or IR. The contents of reactive oxidative species (ROS) in all combination groups were significantly higher than that of the alone treatment group. This study indicates that $1,25(OH)_2D_3$ enhances the anti-proliferative effect of CBP or IR.

16
RANDOMIZED STUDY OF 4,000 IU, 6,000 IU, 8,000 IU OR 10,000 IU OF CHOLECALCIFEROL IN MEN WITH PROSTATE CANCER: TOXICITY AND 25(OH)D_3 EFFECTS

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Low serum 25(OH) vitamin D_3 [$25D_3$] levels are associated with higher cancer incidence, mortality and overall morbidity. Preclinical studies in prostate and carcinogen-induced lung cancer models indicate that vitamin D deficiency promotes tumor development and growth. At least 60% of normal individuals and patients with cancer have serum $25D_3$ levels below <32 ng/ml. Four different daily doses of cholecalciferol were studied by random assingment in 137 men with prostate cancer with either localized or advanced disease.

Table I.

	Median $25D_3$ (ng/ml)			
	Baseline	1 month	3 months	6 months
4,000 IU	24.7	35.5	49.3	56.4
6,000 IU	27.8	51.9	63.7	68.3
8,000 IU	27.8	41.3	55.5	63.9
10,000 IU	25.4	52.2	72.8	84.1

Doses utilized were: 4,000, 6,000, 8,000 or 10,000 IU. $25D_3$, serum and urine calcium, PTH levels and toxicity were assessed at 1, 3 and 6 months. Among 117 patients analyzed toxicity was negligible. No clinically significant changes in serum or 24 h urine calcium occurred. $25D_3$ levels achieved were proportionate to dose. *Conclusion:* Each dose of VD_3 studied was well tolerated. Exploration of factors associated with response to VD_3 supplementation continue. QD dosing of 4,000 IU or 6,000 IU seem appropriate for study of supplementation in men with prostate cancer.

17
HIGH-DOSE ORAL VITAMIN D_3 ADMINISTRATION TO PROSTATE CANCER PATIENTS INCREASES INTRA-PROSTATE LEVELS OF VITAMIN D METABOLITES

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Objectives: To characterize the effect of high doses of vitamin D3 on its metabolite levels in prostate tissue. *Methods:* Doses of Vitamin D3 (400 IU, 10,000 IU, or 40,000 IU/d; *i.e.* 10 μg, 250 μg or 1000 μg/d) were randomized equally to 48 prostate cancer patients (16 per dose) for 3-6 weaks prior to radical prostatectomy. Prostate tissue and serum samples were obtained at surgery to measure 25-hydroxyvitamin D_3 [25(OH)D_3] and 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. *Results:* Serum 25(OH)D_3 increased significantly in all 3 groups in a dose-response manner ($p<0.01$). Serum $1,25(OH)_2D$ increased significantly with 10,000 and 40,000 IU/d ($p<0.01$) but remained unchanged with 400 IU/d. Prostate 25(OH)D_3 and $1,25(OH)_2D$ were significantly higher with 40,000 IU/d compared to the other doses ($p<0.02$). *Conclusion:* The oral consumption of vitamin D_3 produced the desired higher concentrations of vitamin D metabolites within the prostate tissue.

Serum and prostate levels of vitamin D metabolites at surgery (mean±SD):

Vitamin D_3 dose per day	400 IU/d	10,000 IU/d	40,000 IU/d	p-Value (ANOVA)
Serum 25(OH)D_3 (nmol/l)	69±15	128±30	296±71	<0.001
Serum $1,25(OH)_2D$ (pmol/l)	115±46	136±38	171±33	0.001
Prostate 25(OH)D_3 (nmol/kg)	95±25	118±28	185±76	<0.001
Prostate $1,25(OH)_2D$ (pmol/kg)	29±12	29±10	42±19	0.015

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NEW INSIGHTS INTO THE ROLES OF CYTOCHROME P-450 ENZYMES IN PROSTATE CANCER PREVENTION AND TREATMENT

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The active form of vitamin D, 1 alpha, 25-dihydroxyvitamin D [1 alpha,25$(OH)_2$D], interacts with vitamin D receptor (VDR) and induces anti-proliferative, anti-invasive, pro-apoptotic and pro-differentiation activities in prostate cancer cells. Three cytochrome P-450 (CYP) hydroxylases are responsible for its synthesis and degradation. They include vitamin D-25-hydroxylase [25-OHase] in the liver, and 25(OH)D-1 alpha-hydroxylase [1 alpha-OHase, CYP27B1] and 25(OH)D-24-hydroxylase [24-OHase, CYP24A1] in the kidneys. However, it is now recognized that 1 alpha-OHase and 24-OHase are also expressed in many tissues and cells, including the prostate. Although more than 6 CYP enzymes have been identified with 25-Ohase activity, the two major ones are CYP27A1 and CYP2R1, and both are expressed in the prostate with CYP2R1 as the predominate type. The finding indicates that prostate tissue has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion. Recent evidence indicates that 25-hydroxyvitamin D [25(OH)D] and its analogs can bind to VDR as agonists, without converting to 1 alpha,25$(OH)_2$D or the corresponding 1 alpha-hydroxylated metabolites, to modulate gene expressions, leading to cell growth arrest and other anti-tumor activities. The finding suggests that the circulating levels of 25(OH)D, and the autocrine synthesis of 25(OH)D and thereby the regulation of prostate CYP2R1 expression may play an important role in regulating the growth of prostate cancer. Furthermore, in addition to 1 alpha,25$(OH)_2$D analogs, the presence of 25-OHase, 1 alpha-OHase and 24-OHase in the prostate suggests that the analogs of vitamin D and 25(OH)D, especially those that are resistant to 24-OHase degradation, can be developed and used for the prevention and treatment of prostate cancer.

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EXPRESSION AND FUNCTION OF THE VITAMIN D RECEPTOR IN MALIGNANT GERM CELL TUMOUR OF THE TESTIS

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Testicular germ cell tumours [TGCT] are common in young men. They are clinically and histologically subdivided into seminomas and non-seminomas. 1,25-dihydroxyvitamin D [1,25$[OH]_2D_3$] is the active form of vitamin D and exerts its actions *via* a specific intracellular vitamin D receptor [VDR]. Several investigations showed, in addition to the physiological occurrence of the VDR in various tissues, VDR expression in various human malignancies in the recent years. Furthermore, 1,25$[OH]_2D_3$ plays an important role in the regulation of cell proliferation and differentiation. Existence of the vitamin D receptor [VDR] in normal and malignant tissues has been shown. The anti-proliferative and pro-differentiating effects of 1,25$[OH]_2D_3$ have been described in normal and malignant cell types. We investigated the expression and function of VDR in TGCT. By immunohistochemistry, quantitative RT–PCR and Western blot analyses, we demonstrated for the first time that primary TGCT, as well as TGCT cell lines, express VDR RNA and VDR protein. Furthermore, TGCT cell lines showed a decrease of proliferation after vitamin D stimulation. Furthermore, we investigated the vitamin D regulated genes *VDR, NCOR1, NCOR2, TRIP15, GADD45, MAPKAPK2, CYP24A1* and *CYP27B1* after vitamin D stimulation.

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CALCITRIOL-MEDIATED ANTITUMOR EFFECTS: ROLE IN HUMAN BLADDER CANCER

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1,25 Dihydroxyvitamin D_3 (calcitriol) potentiates the antitumor efficacy of platinum analogs (cisplatin/carboplatin), taxanes (docetaxel/paclitaxel) and nucleosides (gemcitabine) *in vitro* and *in vivo* in a variety of cancers. In muscle-invading bladder cancer, neoadjuvant chemotherapy with gemcitabine and cisplatin has been shown to improve overall survival. *In vitro* and *in vivo*, using human bladder cancer models (T24 and UMUC3), calcitriol enhanced the antitumor activity of gemcitabine/cisplatin as compare to chemotherapy alone as measured by increased apoptosis, a decrease in surviving fraction by clonogenic assay and an increase in T24 tumor regrowth delay. The expression of p73, a p53 homolog, is strongly induced by calcitriol in human bladder cells and p73 sensitizes the tumor to the effects of cisplatin and gemcitabine. We demonstrated that the induction of *p73* by calcitriol contributes to the potentiation of gemcitabine/cisplatin-induced growth inhibition. In a pilot study, sufficient tumor cells can be recovered by lavage from bladder cancer patients undergoing cystectomy to determine p73 status and apoptosis. A phase I study is ongoing to determine the dose, safety and toxicity of calcitriol/cisplatin/gemcitabine in patients with advanced cancer. A neoadjuvant phase II study is planned in patients with

muscle invading bladder cancer with this regime where tumor material can be examined for molecular modulation.

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SERUM LEVELS OF 25(OH)D AND VDR POLYMORPHISMS IN MALIGNANT MELANOMA: RESULTS FROM PILOT STUDIES IN HOMBURG

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Increasing evidence indicates that vitamin D deficiency and distinct functional polymorphisms across the 105 kb vitamin D receptor (*VDR*) gene are associated with various types of cancer. Cancer-associated *VDR* genotypes have been shown to be common in all racial groups, having a minor allele frequency >10% and on average may double the risk of cancer. In a pilot study, we have correlated the serum level of 25-hydroxyvitamin D with tumor thickness at the time of diagnosis and course of disease in patients with melanoma. The study population consisted of 212 patients with histologically proven cutaneous melanomas of different stages: stage I (n=50); stage II (n=20); stage III (n=20); stage IV (n=122). Basal 25-hydroxyvitamin D levels were analyzed (DiaSorin LIAISON 25-OH Vitamin D-Assay) in these patients and compared with a control group (n=80). Additionally, each participant was requested to fill out a questionnaire about the history of sun exposure. Interestingly, basal 25-hydroxyvitamin D levels were lower in melanoma patients as compared to the control group, although this difference was statistically not significant. Moreover, progression of malignant melanoma was associated with statistically significantly reduced 25-hydroxyvitamin D serum levels. In another pilot study, we investigated the presence of distinct functional polymorphisms across the genes for *VDR, CYP24A1, CYP2R1, CYP27A1, CYP27B1* and *DBP* in malignant melanoma as compared to controls. In summary, our findings add to the growing body of evidence that 25-hydroxyvitamin D serum levels, as well as gene polymorphisms of *VDR* and other genes that are relevant to the vitamin D endocrine system, may be of importance for the pathogenesis and progression of malignant melanoma.

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SIGNATURES OF VDR miRNAS AND EPIGENETIC MODULATION OF VITAMIN D SIGNALING IN MELANOMA CELL LINES

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We have previously shown that some melanoma cell lines are resistant to the antiproliferative effects of the biologically active vitamin D metabolite ($1,25(OH)_2D_3$). We now investigated whether combination of $1,25(OH)_2D_3$ with epigenetic modulating drugs may represent a promising tool to overcome the resistance towards the antiproliferative effects of $1,25(OH)_2D_3$ in melanoma cells. We used combination of $1,25(OH)_2D_3$ and the histone deacetylase inhibitor (HDACI) trichostatin A (TSA). Additionally, we studied the anti-proliferative effect of $1,25(OH)_2D_3$ in combination with 5-azacytidine (5-Aza), a DNA methyltransferase inhibitor (DNMTI). Interestingly, additive antiproliferative effects were found after treatment with $1,25(OH)_2D_3$ (10^{-8} M) in combination with TSA (15 ng/ml) in $1,25(OH)_2D_3$-resistant cell lines and after treatment with $1,25(OH)_2D_3$ (10^{-8} M) in combination with 5-Aza (10 μM) in $1,25(OH)_2D_3$-resistant and -responsive cell lines. To gain further insights in the epigenetic modulation of vitamin D signaling in melanoma, we studied the expression of two candidates of VDR microRNAs (miR-125b and miR-27b) and the effect of miR-125b antisense on *VDR* mRNA and protein level. Interestingly, RT-PCR for *VDR* mRNA, miR-125b and miR-27b confirmed inversely correlated expression pattern between *VDR* mRNA and miR-125b in $1,25(OH)_2D_3$-responsive as compared to $1,25(OH)_2D_3$-resistant cell lines. Furthermore, VDR protein levels in melanoma cells were analyzed and showed no difference in $1,25(OH)_2D_3$-responsive as compared to $1,25(OH)_2D_3$-resistant cell lines. Taken together, our findings indicate that the responsiveness of melanoma cells to the anti-proliferative effects of $1,25(OH)_2D_3$ corresponds to the expression level of *VDR* mRNA, which may be regulated by expression of VDR microRNAs (miR-125b and miR-27b). Moreover, our findings indicate that epigenetic modulating drugs modulate vitamin D signaling in melanoma cells and may represent a promising tool to overcome the resistance towards anti-proliferative effects of vitamin D analogs.

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THE DETERMINANTS OF SERUM VITAMIN D LEVELS IN PARTICIPANTS IN A MELANOMA CASE–CONTROL STUDY LIVING IN A TEMPERATE CLIMATE

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Background: Vitamin D is important to cancer prevention/progression. We have reported evidence that

higher vitamin D levels are associated with melanoma thickness at diagnosis and better survival. We have also reported that regular weekend sun exposure is protective for melanoma and we hypothesise that this is associated either with photoadaptation or higher vitamin D levels. These data and new data in which we report the determinants of serum levels in a UK melanoma case–control study are presented. *Methods:* Participants provided data on sun exposure, phenotype, supplemental vitamin D intake and in a proportion a single serum 25-hydroxyvitamin D_3 level. Single nucleotide polymorphisms (SNPs) were typed in genes reported in genome-wide association studies to be associated with serum levels. Estimates of the effect on adjusted serum levels (nmol/l) are given for each genetic/environmental exposure. *Results:* Vitamin D levels were low especially in the sun-sensitive (estimate difference –2.61 nmol/l, p=0.03), those with increased body mass index (BMI) (estimate –0.52 nmol/l for every unit of BMI, p=<0.0001) and for inheritance of the minor allele of rs2282679 (within the gene coding for the vitamin D binding protein) (–5.79 for one copy compared to none, p=<0.0001). The relationship between sun exposure and vitamin D supplementation is described. *Conclusion:* Regular sun exposure provided reasonable levels of vitamin D but required lengthy exposure. The sun-sensitive individuals did not achieve these levels on average without supplementation.

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ANTITUMOR EFFECTS OF CALCITRIOL IN PATCHED1-ASSOCIATED BASAL CELL CARCINOMA INVOLVES INHIBITION OF HEDGEHOG SIGNALING AND INDUCTION OF DIFFERENTIATION

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Activation of the Hedgehog (Hh)-signaling pathway due to deficiency in the Hh receptor Patched1 (Ptch) is frequently found in basal cell carcinoma (BCC). Recent reports provided evidence that Ptch secretes a vitamin D_3-related compound, which functions as an endogenous inhibitor of Hh-signaling. This implicates that Ptch-deficient tumor cells are devoid of this substance, which in turn results in activation of this signaling pathway. Here we show, that the application of the physiologically active form of vitamin D_3, calcitriol, inhibits the proliferation and the growth of BCC of Ptch-mutant mice *in vitro* and *in vivo*. This is accompanied by the inhibition of Hh-signaling, activation of the vitamin D receptor (Vdr) and induction of BCC differentiation. Our data support a model in which Ptch-mediated efflux of vitamin D_3-related compounds controls the activity of two pathways, Hh- and Vdr-signaling, which are relevant to tumorigenesis and tumor treatment. Moreover, our data suggest that calcitriol could be a new therapeutic option in the treatment of BCC, the most common tumor in humans.

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IMMUNOMODULATORY EFFECTS OF 1,25(OH)$_2$D$_3$ IN NORMAL AND TRANSFORMED HUMAN LUNG EPITHELIUM

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25(OH)D_3 is activated to 1,25(OH)$_2$D$_3$ within normal lung epithelium and the deactivating enzyme (CYP24) is over-expressed in many lung carcinomas, suggesting that 1,25(OH)$_2$D$_3$ may play an important role within lung epithelium. The immunomodulatory effects of 1,25(OH)$_2$D$_3$ were studied in two lung cancer cell lines A549 and NCI-H292, one virally transformed cell line 16HBE14o-, and primary small airway epithelial cells SAEC. Basal *CYP24* mRNA expression was highest in A549 cells (A549> NCIH292>>16HBE14o->SAEC). 1,25(OH)$_2$D$_3$ stimulated *CYP24* mRNA expression in all cells except A549. 1,25(OH)$_2$D$_3$ exerted differential immunomodulatory effects on basal and TNFalpha/LPS-induced IL1beta, IL6, IL8 and TNFalpha expression in all cells, except A549, where no effect was detected. 16HBE14o- exhibited similar effects to primary SAEC. These results suggest that 1,25(OH)$_2$D$_3$ may differentially regulate lung inflammation in normal and cancerous lung epithelium. In addition, the choice of cell line is very important for studying the effects of 1,25(OH)$_2$D$_3$ in the lung epithelium.

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VITAMIN D, THE IMMUNE SYSTEM AND CANCER

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Deficiency in vitamin D can be a risk factor for a variety of diseases including chronic inflammatory diseases and cancers. Several types of cancer are infiltrated by pathogenic T-cells, and such cancer can be found in the skin. Tumors are difficult to treat as some of them have a unique way of evading the immune system. However, targeting the T-cells may be a therapeutic approach. The active metabolite of vitamin D, $1,25(OH)_2D_3$, has been shown to induce the generation of regulatory T-cells, which can suppress proliferation and expansion of antigen-specific T-cells. Furthermore, it has been shown that vitamin D may play an important role in directing T-cells to the skin, such as regulatory T-cells. There are several factors in the environment of T-cells that can be used to induce their expression of tissue-specific homing receptors, enabling them to infiltrate tissues where they are needed. Increased understanding of the microenvironment, including vitamin D, and how it affects inflammatory and regulatory T-cells could be a mechanism of increasing immunity in the skin, including antitumor function.

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PROCESS DEVELOPMENTS FOR THE PRACTICAL PRODUCTION OF ELDECALCITOL BY LINEAR SYNTHESIS, CONVERGENT SYNTHESIS, AND BIOMIMETIC SYNTHESIS

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Eldecalcitol [1α,25-dihydroxy-2b-(3-hydroxypropoxy)vitamin D_3], an analog of calcitriol, possesses a hydroxypropoxy substituent at the 2b-position of calcitriol. Eldecalcitol has potent biological effects on bone and is a promising candidate for the treatment of osteoporosis. Recent completion of phase III clinical trials of eldecalcitol produced excellent results and eldecalcitol is now ready for marketing in Japan. Considering the clinical application of eldecalcitol, we have been investigating a practical synthesis of eldecalcitol for industrial scale production. Eldecalcitol was initially synthesized in a liner manner in which the 1,2a-epoxide, prepared from lithocholic acid *via* 25-hydroxycholesterol, served as a key intermediate for the introduction of the hydroxypropoxy substituent. The 27-step linear sequence was, however, suboptimal due to its lengthiness and low overall yield (ca. 0.03%). We developed a convergent approach based on the Trost coupling reaction, in which A-ring fragment (ene-yne part prepared in 10.4% overall yield) and C/D-ring fragment (bromomethylene part obtained in 27.0% overall yield) are coupled to produce triene system of eldecalcitol (15.6%). Although the overall yield of convergent synthesis was better than linear synthesis, significant improvements are still needed, therefore, further biomimetic invesigations on microbiological 25-hydroxylation of steroidal side chain using cholesterol as a starting material are ongoing. Process developments for the practical production of eldecalcitol will be discussed.

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SYNTHESIS OF C15-MODIFIED 16-ENE-ACTIVE VITAMIN D_3 ANALOGS FOR THE RECEPTOR-LIGAND INTERACTION STUDIES

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Crystal structure studies of the complex between the active vitamin D_3 and vitamin D receptor (VDR) ligand binding domain (LBD) indicate that the CD-ring of the ligand molecule is covered by helix 3 (H3) of the LBD, and H3 interacts with H12 in an agonist position through both hydrophobic and polar interactions. The interactions are critical for ligand-dependent VDR transactivation and the expression of biological activities. We hypothesized that the addition of a functional group to C15 at the α or β configuration may alter the interactions in different ways and lead to unique biological profiles. At the C15β configuration, H3 would be pushed upward and would affect the agonistic location of H12, which could lead to a unique biological profile. In the case of C15α modification, new interactions with LBD amino acid residues, which have no interactions with the natural $1\alpha,25(OH)_2D_3$ molecule directly, would be expected. To test this hypothesis, we have synthesized $1\alpha,15\alpha,25(OH)_3D_3$ (**1**), 15α-methoxy-$1\alpha,25(OH)_2D_3$ (**2**), $1\alpha,15\beta,25(OH)_3D_3$, (**3**) and the 16-ene analog of compounds **1** and **3**, because the 16-ene analog of $1\alpha,25(OH)_2D_3$ has a different metabolic pathway associated with a minimal *in vivo* calcemic activity and a potent inhibitory effect on cell growth. The CD-ring part was synthesized from Inhoffen-

Lythgoe diol *via* 15,16-epoxyhydrindan derivative with control of C15 and C20 stereochemistries (steroidal numbering). After coupling with the A-ring part followed by deprotection and HPLC purification, the desired C15-modified 16-ene analogs (**4** and **5**) have been obtained. We are currently studying the crystal structure of the complex between hVDR LBD and the C-15 modified new ligands.

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NOVEL VITAMIN D RECEPTOR LIGANDS HAVING A CARBOXYL GROUP AS A POSSIBLE ANCHOR TO THE CRUCIAL ARGININE RESIDUE IN THE LIGAND-BINDING DOMAIN

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Vitamin D_3 is metabolized into the hormonally active form, 1α,25-dihydroxyvitamin D3 (**1**), *via* 25-hydroxyvitamin D_3 (**2**), which is the most abundant circulating metabolites. The 1α-hydroxyl group, which is recognized by the arginine (R274) and the serine (S237) in the ligand-binding domain (LBD) of vitamin D receptor (VDR), plays a key role in elevating the VDR binding affinity by approximately 1,000-fold for 25-hydroxyvitamin D_3 (**2**) to produce the hormone, 1α,25-dihydroxyvitamin D_3 (**1**). We speculated that this important interaction between the 1α-hydroxyl group in **1** and the arginine (R274) in the LBD of VDR could be reinforced by substituting a well-situated carboxyl group in the A-ring, which could interact better with the guanidinium cation in the arginine residue. Therefore, we designed 25-hydroxyvitamin D_3 analogues having a carboxyl group in the A-ring (**3**) and their methyl esters (**4**). A convergent synthetic method using a palladium catalyst allowed us to reach the novel compounds (**3, 4**). Nine-step conversion of the known epoxide, prepared from 3-buten-1-ol by our method, afforded the requisite A-ring enyne precursor (**5**) for 2α-carboxy-25-hydroxyvitamin D_3 (**3a**) and its methyl ester (**4a**) in excellent yield. Conventional elongation of the substituent in 5 produced the A-ring enyne precursor (**6**) for 2α-carboxymethyl-25-hydroxyvitamin D_3 (**3b**) and its methyl ester (**4b**). The VDR binding affinity of the synthesized analogues demonstrated that the carboxyl group in **4b** would compensate in part the loss of the 1alpha-hydroxyl group, whereas compounds **3a** and **4a** showed a comparable activity to **2**. These results imply that the novel class of the 25-hydroxyvitamin D_3 analogues with a carboxylic acid could have a different activity spectrum to regulating receptors and enzymes on vitamin D system because of the newly-introduced functional group.

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EVALUATION AND CHARACTERIZATION OF 14-EPI-19-NORTACHYSTEROL ANALOGS FROM 14-EPI-19-NORPREVITAMIN D_3

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Active vitamin D_3 exists in the thermal 'pre-vitamin D/vitamin D' equilibrium through [1,7]-sigmatropic rearrangement, and contains 5-10% of its pre-vitamin D form, 1α,25$(OH)_2$preD_3 at 37˚C. To evaluate the biological activities of pre-vitamin D_3, we synthesized stable 14-epi-previtamin D_3 analogs with various C2-functional groups from methyl α-D-glucoside or dimethyl-D-tartrate for the A-ring part, which was coupled with 14-epi-Grundmann's ketone derivatives using the Roche method. Among them, we found that 2α-methyl analogs showed moderate VDR-binding affinity. We then synthesized 2-methyl-19-nor derivatives, which should have no 'pre-vitamin D/vitamin D' equilibrium, from (–)-quinic acid for the A-ring part connected with CD-ring triflate using Sonogashira coupling. During the biological evaluation of these derivatives, however, we found *cis*/*trans* isomerization at the 6,7-double bond to afford the new 14-epi-19-nortachysterol skeleton. Therefore, next we chemically synthesized some 14-epi-19-nortachysterol analogs directly using Stille coupling reaction between vinyl stannane from (–)-quinic acid for the A-ring part and CD-ring triflate, which indicated potent VDR-binding affinity. We also investigated their unique binding configuration with VDR using X-ray crystallographic analysis.

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VITAMIN D AND CANCER: AN OVERVIEW OF EPIDEMIOLOGICAL STUDIES

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In recent years, a rapidly growing number of epidemiological studies have addressed the association between serum vitamin D levels and the incidence of various types of cancer. We conducted and report a systematic review and meta-analyses of the relationship between vitamin D and the occurrence of colorectal adenoma, colorectal cancer, breast cancer, prostate cancer, and ovarian cancer. In meta-analyses, significant inverse associations were found between serum 25(OH)D in

colorectal adenoma, colorectal cancer, and breast cancer, with associations being strongest (summary of covariate adjusted odds ratios, OR, 0.57) for colorectal cancer, especially rectum cancer (OR 0.41). Taken together, these results suggest a protective role of vitamin D on the risk of several types of common cancer. However, the empirical evidence from epidemiological studies, especially longitudinal studies is still very limited for many types of cancer.

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ECOLOGICAL STUDY FINDINGS REGARDING VITAMIN D AND CANCER

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Ecological studies provide the largest and most robust evidence for a beneficial role of solar ultraviolet-B (UVB) irradiance and vitamin D in reducing the risk of cancer incidence and/or death. Such studies use any of several indices for solar UVB doses or irradiance including latitude, summertime UVB, occupation, and death from non-melanoma skin cancer. These studies have been conducted with data from Australia, China, Europe, France, Japan, Spain, and the United States, and using up to 175 countries. Most of these studies include other cancer risk-modifying factors in the analysis, and no factor other than vitamin D production has been proposed to explain the beneficial effects of UVB on cancer risk. The ten types of cancer with the strongest evidence for a beneficial role of solar UVB/vitamin D are bladder, breast, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal cancer, and non-Hodgkin's lymphoma. The five types of cancer with moderate evidence are gallbladder, prostate, renal, vulvar cancer, and Hodgkin's lymphoma. The five types of cancer with limited evidence are brain, cervical, small intestinal, thyroid cancer, and leukemia.

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IS SUNSHINE AND VITAMIN D PROTECTIVE AGAINST MELANOMA ON SHIELDED SITES?

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Cutaneous malignant melanoma (CMM) is related to intermittent sun exposure and sunburn, while the relationship is not clear for regular exposure. Melanomas may arise also on non-UV-exposed areas, such as uvea, vulva, anorectal skin and foot. What is the role of UV for such melanomas? We studied their temporal and latitudinal dependencies among Caucasians in European countries and Australia. The ratios of the incidence rates of these melanomas tend to decrease with increasing rates of CMM. The incidence rates of CMM have increased with time until recently, while those of melanomas on shielded sites seem to have decreased or remained constant and also tend to decrease with decreasing latitude, *i.e.* opposite to what is found for CMM. These observations are discussed in terms of a possible protective effect of UV-induced vitamin D, under the assumption that CMM rates are related to UV exposures, and so are probably vitamin D levels. Possible roles of other, non-vitamin D, UV products in skin, are also discussed.

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INFLUENZA, SOLAR RADIATION AND VITAMIN D

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Seasonal variations of UVB radiation cause seasonal variations of the vitamin D levels. This may influence immune responses and play a role for the seasonality of influenza. Pandemic and non-pandemic influenzas were studied in Sweden, Norway, USA, Singapore and Japan. Weekly/monthly influenza incidence and death rates were evaluated in view of monthly UVB fluency. Non-pandemic influenzas mostly occur in the winter season in temperate regions. UVB calculations show that at high latitudes very little vitamin D is produced in the skin during the winter. In tropical regions, there are two minor peaks in vitamin D photosynthesis, and practically no seasonality of influenza. Pandemics may start with a wave at an arbitrary season, while secondary waves often occur the following winter. Thus, it seems that a low vitamin D level may play a significant role for most influenzas. The data support the hypothesis that a high fluency of UVB radiation (vitamin D level), as in summer, acts in a protective manner with respect to influenza.

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CANCER MORTALITY IN END-STAGE KIDNEY DISEASE PATIENTS IS INDEPENDENT OF VITAMIN D STATUS

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Objective: The aim of this study was to analyse the relationship of 25(OH)D level and mortality in a representative sub-cohort of German patients with end-stage kidney disease (ESKD stage 5 D). *Methods:* From the data- base of the German Renal Registry, 17.291 hemodialysis patients were analysed retrospectively from 1995-2006 according to their vital status. The serum levels of 25(OH)D were divided into quartiles: severe deficiency (<12.5 ng/ml), deficiency (12.5-<20 ng/ml), insufficiency (20-<30 ng/ml), sufficiency (≥30 ng/ml). *Results:* Over the 12-year observation period, 51.3% of the patients were still alive, 19.3% had died due to cardiac complications, 8.1% due to infections, 17.3% by other or unknown reasons; the death rate due to malignancies was only 3.9%. Vitamin D status and mortality shows the lowest relationship for cancer: 25(OH)D levels within the first quartile (<12.5 ng/ml): 55.9% cancer *vs*. 61.4% infectious, *vs*. 59.8% other/unknown, *vs*. 58.7% cardiac; in the forth quartile (>=30 ng/ml): 12.0% cancer *vs*. 9.3% other/unknown, *vs*. 8.7% infectious, *vs*. 8.6% cardiac. *Conclusion:* In patients with ESKD on hemodialysis due to the high prevalence of cardiovascular comorbidities and the suppressed immune system, the mortality rate from organ cancer seems to be independent of the vitamin D status.

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VITAMIN D DEFICIENCY/SECONDARY HYPERPARATHYROIDISM AND THE SEASONAL CHANGES IN GERMANY (51° LATITUDE)

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Data of 3,500 patients from a thyroid outpatient institution were analyzed from 2008 to 2010 using the data (25OH)-vitamin D, PTH (Diasorin-Liaison), bodyweight, age, sex and familial origin (RESidents or IMMigrants) with relation to the seasonal change. -Median 25VD nmol/l: All 34.6/RES 35.8/IM 29.4; VD>75: RES 10% IMM 6%. -Median PTH ng/l: All 62.9/RES 62.7/IM 64.5; PTH<65. RES 55% IMM 52%. After initial plateau (IP) from Jan to Apr (median 24.6) the seasonal upstroke of 25VD starts in May with maximum in Aug (median 46.7), whereas PTH initial plateaus at a median 66.1 with inital downstroke in June and minimum at Oct median 56.4. -Mean VD upstroke from IP to MAX: All 190%; RES 188%; IMM 209%; female 187%; male 183%; age <45 years 235%/>61 years 156%; bodyweight <66 kg and >80 kg 186%; <45 years and <66 kg 247% ; >61 years and >80 kg 168%. Mean PTH downstroke from IP to MIN: All 85%; RES 87%; IMM 84%; female 82%; male 88%; age <45 years 85%/>61 years 84%; bodyweight <66 kg 85%/>80 kg 84%. The data allow a more realistic approach for the evaluation of VD deficiency and secondary hyper-parathyroidism by calculating monthwise an individual correction for seasonal changes in order to estimate the mean metabolically relevant concentration over the year and the respective distance to the border of normal. Beside the seasonal changes, the finding of about 55% patients with PTH over the upper border of normal (65 ng/l) needs discussion about the 'normality' of PTH values.

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VITAMIN D STATUS AND CARDIOVASCULAR DISEASE

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The active form of vitamin D, $1,25(OH)_2D$, has a broad range of actions including various effects on the cardiovascular system. Vitamin D deficiency adversely affects vascular integrity and cardiovascular risk markers such as pro- and anti-inflammatory cytokines, and blood lipids. Vitamin D is a negative endocrine regulator of the renin/angiotensin system, which is responsible for blood pressure control. The scientific evidence for protective vitamin D effects on various cardiovascular risk markers will be presented. Data indicating a biphasic dose-response effect on vascular calcification with deleterious consequences not only of vitamin D deficiency, but also of vitamin D excess will also be presented. Randomized controlled trials with cardiovascular morbidity and mortality as primary endpoints are still lacking. However, prospective cohort studies indicate an increase in multivariate adjusted cardiovascular mortality, especially in those individuals with circulating 25-hydroxyvitamin D concentrations below 25 nmol/l. In Europe and also in the Middle East and South-East Asia, 25-hydroxyvitamin D concentrations below 25 nmol/l are very prevalent, indicating that preventive measures for improving vitamin D status are urgently needed.

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AN OPTIMAL SERUM CALCIDIOL CONCENTRATION FOR CANCER PREVENTION

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It has been demonstrated in several studies that serum calcidiol concentration has an inverse linear relationship with cancer

risk. However, there are also studies showing no such association. Some studies suggest even an opposite finding. The risk of pancreatic cancer seems to increase when serum calcidiol increases. Our results confirm that serum calcidiol is a better predictor of cancer development than calcitriol. In a recent study, we demonstrated that calcidiol is an active hormone in *CYP24*$^{-/-}$ cells. In these cells, calcidiol and calcitriol act synergistically. Therefore, we propose that fluctuations of serum calcidiol concentration define the hormonal activity and cancer development. Using a large serum bank (250,000 sera of Scandinavian men), we found that the smallest risk of prostate cancer was found at a serum calcidiol level of 40-60 nmol/l (16-24 ng/ml). Both lower and higher calcidiol concentrations were associated with a significantly increased risk of prostate cancer. Because some other pathological events (death rate from all causes and from cardiovascular disease, deafness, osteoporosis and systolic blood pressure) show a similar U- or J-shaped dependency on serum calcidiol, it is evident that there is an optimal serum concentration (40-80 nmol/l or 16-32 ng/ml) for the prevention of cancer and other diseases.

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THE D-BATEABLE IOM REPORT: A D-LIGHTFUL PERSPECTIVE

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Over the past decade, several thousand publications have reported that vitamin D deficiency is a worldwide pandemic and that this deficiency was associated with a wide variety of chronic illnesses including heart disease, cancer, autoimmune diseases, infectious diseases, neurocognitive dysfunction, type II diabetes, as well as pre-eclampsia during pregnancy. The Institute of Medicine (IOM) of the United States convened a committee of leading experts in the field of nutrition, bone health and vitamin D and provided their recommendations in November 2010. The committee concluded that the previous recommendation that all children and adults only needed 200 IU of vitamin D a day was woefully inadequate and recommended that children older than one year and all adults up to 70 years require 600 IU of vitamin D daily to maximize bone health. For children 0 to 1 year old, they recommended 400 IU of vitamin D daily and for adults over the age of 70, 800 IU of vitamin D. Although they recognize that essentially every tissue and organ in the body has a vitamin D, receptor and that some of them produce 1,25-dihydroxyvitamin D they were unable to support any of the non-skeletal benefits of vitamin D that have been amply documented in the literature. They also concluded that vitamin D deficiency is less common because they considered a level of 25-hydroxyvitamin D<20 ng/ml as being vitamin D-deficient. There are however several randomized controlled trials demonstrating the beneficial effect of vitamin D in reducing the risk of influenza A infection, improving insulin resistance, reducing risk of cancer and improving vascular function. There is no downside to increasing vitamin D intake for children to 1,000 IU/d and adults to 2,000 IU/d in order to maintain blood levels of 25-hydroxyvitamin D of between 30-100 ng/ml.

Posters

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COMPLEX AND DIFFERENTIAL INTERFERENCE BETWEEN VITAMIN D RECEPTOR ACTION AND HISTONE ACETYLATION

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The dynamic nature of transcriptional regulation by the vitamin D receptor (VDR) involves the sequential recruitment of co-regulators, which in turn associate with histone-modifying enzymes such as histone acetyltransferases or histone deacetylases (HDACs). The monocytic cell line THP-1 is used as a model to investigate transcriptional responses to the treatment with the VDR ligand 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) and the HDAC inhibitor trichostatin A (TsA), as well as their combination. In this study, short-term treatments were performed to focus on primary immediate-early response genes and the effects on mRNA accumulation were measured by quantitative real-time PCR. The primary responses of 35 genes to 1α,25$(OH)_2D_3$ and TsA were characterized by their individual dose and time dependencies. They were subdivided into five classes. The first group was up-regulated by TsA treatment (*e.g. HDAC11*), whereas those of the second group were up-regulated by 1α,25$(OH)_2D_3$ (*e.g.* cathelicidin antimicrobial peptide). Genes of the third class were up-regulated by both treatments (*e.g.* dual specificity phosphatase 10) and the fourth class was characterized by opposing effects of VDR activation and histone hyperacetylation (*e.g.* thrombomodulin). The fifth class was composed of genes showing no response to either treatment (*e.g. VDR*). In conclusion, the described complex and differential interference between the actions of VDR and HDACs in transcription regulation provide a basis for a genome-wide and mechanistic investigation of the observed effects.

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ASSOCIATION OF COMMON SNPS OF GENES REGULATING VITAMIN D HOMEOSTASIS WITH VITAMIN D LEVELS AND LIVER FIBROSIS IN A COHORT OF PATIENTS WITH CHRONIC LIVER DISEASE

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Background: Recently, genome-wide studies identified a number of genetic variants that affect vitamin D levels in healthy populations (rs12785878, near *DHCR7*; rs10741657, near *CYP2R1* and rs7041 vitamin D-binding protein GC; Wang *et al*. Lancet 2010). Since vitamin D deficiency is associated with advanced liver disease, we hypothesized that these variants are associated with vitamin D levels and fibrosis in patients with chronic liver disease. *Patients and Methods:* Overall, 834 patients with chronic liver disease and predominantly Caucasian descent (n=712; 85%) were included. Levels of 25(OH)-vitamin D_3 were determined and liver stiffness was measured using transient elastography (TE). Patients were stratified in different fibrosis stages according to TE results (F0-F1 <7.0 kPa; F2: 7.0-9.0 kPa; F3: 9.0-12.0 kPa; F4 >12.0 kPa). Genotypes were determined using Taqman assays. *Results:* Most patients suffered from chronic viral hepatitis C (58.9%) followed by alcoholic (10.6%) and autoimmune (AIH, PBC, PSC) liver diseases (8.1%). Mean 25(OH)-vitamin D_3 levels were 28.4±15.8 ng/ml. Patients with advanced fibrosis (>12.0 kPa) had significantly lower 25(OH)-vitamin D_3 levels as compared to patients with <12.0 kPa (21.7 *vs*. 29.7 ng/ml; $p<0.001$). 25(OH)-vitamin D_3 levels were inveresely correlated with liver stiffness ($p<0.001$). We detected an association with fibrosis for rs12785878, near *DHCR7*. Interestingly, this SNP was not associated with liver fibrosis in the overall cohort. However, after exclusion of patients with advanced fibrosis (>7.0 kPa), the rare allele was significantly associated with increased liver stiffness (TT & TG *vs*. GG: 5.1±1.0 kPa *vs*. 5.4±1.0 kPa; $p<0.05$). *Conclusion:* A common SNP close to the *DHCR7* gene is associated with 25(OH)-vitamin D_3 serum concentration in patients with chronic liver disease. Interestingly, the association of this SNP with liver fibrosis was restricted to patients with no or mild fibrosis, suggesting that the modulatory role of vitamin D might only be discernible during fibrosis initiation and early stages of fibrosis.

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MODELLING HEPATIC OSTEODYSTROPHY: *ABCB4* KNOCKOUT MICE LACKING THE HEPATOBILIARY PHOSPHOLIPID TRANSPORTER DISPLAY DECREASED BONE MINERAL CONTENT AND CORTICAL DENSITY

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Background: Abcb4 knockout mice (*Abcb4*$^{-/-}$) develop chronic cholangitis and biliary fibrosis due to a biliary phosphatidyl-choline deficiency. Hepatic osteodystrophy is a multifactorial complication of chronic liver diseases, especially in cholestatic patients with abnormalities of calcium homeostasis and vitamin D metabolism. To get further insights into the association between chronic cholestasis and metabolic bone disease, we performed a comprehensive analysis of *Abcb4*$^{-/-}$ mouse bone structure and calcium homeostasis. *Methods:* At 15-20 weeks of age, femurs of *Abcb4*$^{-/-}$ mice and wild-type BALB/cJ mice (n=10-16) were analyzed by X-ray bone densitometry and peripheral quantitative computer tomography (pQCT). Alkaline phosphatase plasma activities, as well as calcium and inorganic phosphate concentrations, were determined by standard assays. Plasma concentrations of 25-OH-vitamin D_3 were measured by chemiluminescence immunoassays. *Results:* Bone analyses demonstrated that femurs of both female and male *Abcb4*$^{-/-}$ mice showed significantly ($p<0.05$) reduced total volumes and mineral contents as compared to wild-type animals; the femoral bone mineral density was not affected. Interestingly, cortical density (reflecting material density of the bone) was significantly ($p<0.05$) decreased in female knockout mice compared to controls. In addition, Abcb4-deficient mice displayed lower plasma calcium concentrations ($p<0.01$), whereas inorganic phosphorus was increased ($p<0.001$), consistent with significantly ($p<0.001$) lower 25-OH-vitamin D_3 levels in *Abcb4*$^{-/-}$ mice. *Conclusion:* Our model provides a basic experimental framework for hepatic osteodystrophy. The

results indicate that the bone-mineralizing defect secondary to liver dysfunction manifests itself in *Abcb4*$^{-/-}$ mice as a total decrease in material density. We speculate that therapeutic interventions correcting low vitamin D levels also affect local and systemic inflammation in *Abcb4*$^{-/-}$ mice; all of these may contribute to hepatic osteodystrophy in patients with chronic cholestasis as well.

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SERUM 25-HYDROXY-VITAMIN D_3 IN CHRONIC LIVER DISEASE: A TRANSIENT ELASTOGRAPHY-BASED STUDY

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Background: Low serum levels of 25-hydroxy-vitamin D_3 have been associated with advanced liver fibrosis and unfavorable outcome of chronic viral hepatitis C (HCV) treatment. Lately, transient elastography (TE) has gained wide acceptance as a non-invasive tool to assess liver fibrosis. In the present study, we investigated serum 25-OH-vitamin D_3 levels in relation to liver stiffness as determined by TE in patients with different types of chronic liver diseases. *Methods:* Overall, the study cohort comprised 787 patients (median age 55, range 17-83 years) with liver diseases of mixed etiology. The HCV group included patients with viral replication (n=491), non-responders to therapy (n=133) and patients after successful treatment (n=55). Liver stiffness was quantified by TE (Fibroscan). Patients were stratified either into a mild fibrosis (TE <6 kPa) or a severe fibrosis (TE >18 kPa) group. For comparison, a subgroup of 206 individuals underwent liver biopsy and fibrosis was staged according to the Desmet and Scheuer score. Serum 25-OH-vitamin D_3 concentrations were determined by chemiluminescence immunoassay. *Results:* The majority of patients (n=490, 62%) presented with 25-OH-vitamin D_3 serum concentrations <30 ng/ml. The levels were significantly (p<0.05) lower in patients with alcoholic or cryptogenic liver diseases than in patients with HCV infection, primary biliary cirrhosis or autoimmune hepatitis. Further analysis revealed a negative correlation between vitamin D_3 levels and both liver stiffness values and histological fibrosis stages (both p<0.001). Patients with fibrosis stages F1 and F2 (n=99) had significantly (p<0.01) higher vitamin D_3 levels (33.2±1.7 ng/ml) than patients with severe fibrosis and cirrhosis (18.5±1.3 ng/ml). Accordingly, TE stratification in patients with mild (n=137) and severe fibrosis (n=284) demonstrated significantly (p<0.001) lower vitamin D_3 levels in the latter group. In our cross-sectional design, we were not able to detect differences in vitamin D_3 levels according to treatment response in patients with HCV. *Conclusion:* 25-OH-Vitamin D_3 serum levels correlate negatively with liver stiffness independently from chronic liver disease etiology, which might reflect impaired hydroxylation capacity or profibrogenic risk conferred by vitamin D deficiency. Low vitamin D_3 levels in patients with cryptogenic liver diseases deserve further studying.

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VITAMIN D AND DEPRESSION IN CHRONIC LIVER DISEASE

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Given the liver's role in vitamin D activation, patients with chronic liver disease (CLD) frequently exhibit vitamin D deficiency [Arteh *et al*. Dig Dis Sci 2010 55:2624]. Vitamin D may be implicated in depression, which is common in patients with CLD. An inverse association of 25-hydroxyvitamin D concentration with depression has been demonstrated [Ganji *et al*. Int Arch Med 2010 3:29]. Vitamin D receptors (VDR) are confirmed in parts of the brain implicated in depression pathophysiology [Eyles *et al*. J Chem Neuroanat 2005 29:21]. Behavioural impairment and increased anxiety are reported in *Vdr* knockout mice compared to controls [Kalueff *et al*. Neurosci Res 2006 54:254]. Human studies have reported *VDR* gene variants to influence depression [Kuningas *et al*. Neurobiol Aging 2009 30:466]. The present quasi-RCT investigates the potential of vitamin D supplementation in ameliorating depression in CLD. Vitamin D supplements were given to patients with serum 25-hydroxyvitamin D deficiency. The control group comprised patients with CLD and no vitamin D deficiency, hence without supplementation. Subjects were followed for 12 months to assess severity scores of depression and 25-hydroxyvitamin D concentrations. Preliminary evidence indicates a potential association between vitamin D deficiency and depression severity.

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EPI-25-OH-VITAMIN D_3 IN INFANTS

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The C3-epimere of 1,25-$(OH)_2$-vitamin-D_3 has been shown to have similar but reduced calcemic effects in bone metabolism when compared to 1,25-$(OH)_2$-vitamin-D_3. To date, significant C3-epimere 25-OH-vitamin-D_3 serum concentrations have been detected in infants less than one year of age only. Thus, monitoring the 25-OH-vitamin-D_3 status of this age group should also consider the level of the epimeric form. Here we describe a new detection method suitable for daily routine analysis by LC-MS/MS that can distinguish between the two 25-OH-vitamin-D_3 forms.

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THE PREVALENCE OF VITAMIN D DEFICIENCY AMONG FEMALE STUDENTS AT QATAR UNIVERSITY

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Objective: The purpose of this analysis was to define the prevalence of vitamin D insufficiency/deficiency in females at Qatar University. *Methods:* Blood samples were obtained from 71 female students at Qatar University, and the vitamin D, calcium, albumin, alkaline phopsphatase and creatinine were measured. A designed questionnaire was used to gather information regarding the time of exposure, the duration, the size of body exposed to the sun, the skin color and dietary intake of vitamin D. The body mass index (BMI) was calculated and the waist circumference was measured. The subjects were aged between 17 and 30 years randomly selected from the population of healthy females at the university. Female students who were taking vitamin D supplement were excluded from the study. *Results:* A high percentage of vitamin D deficiency and/or insufficiency was observed among female students (97.2%): 50.7% had severe vitamin D deficiency, 46.5% had vitamin D insufficiency. All of the subjects showed a serum calcium level below the optimal level. The findings showed a significant relationship between vitamin D deficiency and skin color and hypocalcaemia, the two significant predictors of vitamin D level. All 37 lighter-skinned students (52.1%) had severe deficiency, while 32 students (47.9%) with dark skin had severe deficiency. The results also showed that among females with severe vitamin D deficiency, 51.4% had normal parathyroid hormone, while 25.1% with normal parathyroid hormone level had vitamin D insufficiency. No significant association between vitamin D deficiency and parathyroid hormone levels was detected, (p=0.67). Furthermore, the current study showed no significant correlation between vitamin D deficiency and obesity markers (waist circumference and BMI). *Conclusion:* A high prevalence of physiologically significant hypovitaminosis D accompanied with hypocalcaemia was observed among female students aged between 17 and 30 years at Qatar University, the magnitude of which warrants public health intervention.

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HIGH-DOSE CHOLECALCIFEROL THERAPY IN VITAMIN D-DEFICIENT KIDNEY TRANSPLANT RECIPIENTS AND RISK OF HYPERCALCEMIA: PRELIMINARY REPORT OF A RANDOMIZED CONTROLLED TRIAL (RCT)

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Vitamin D deficiency is common in kidney transplant recipients. Correction of vitamin D deficiency is of particular importance to these patients as vitamin D reduces the risk of various diseases including cancer, cardiovascular and infectious diseases, all of which are frequently encountered after transplantation. To date, there is no general consensus on treatment of vitamin D deficiency after kidney transplantation. We are currently conducting a RCT to evaluate the immunomodulatory effects of high-dose cholecalciferol therapy. A total of 200 kidney transplant recipients with vitamin D deficiency (25-hydroxyvitamin D<50 nmol/l) were randomized to receive placebo or oral cholecalciferol (6,800 IU/day) for one year. In this interim analysis, we evaluated the safety of high-dose cholecalciferol therapy without unblinding the study. Out of 81 patients included, hypercalcemia (total serum calcium>2.65 mmol/l) occurred in 17 participants during the first six months of therapy. From 25-hydroxyvitamin D levels, we deduced that seven of them received placebo. Notably, six of the placebo-treated and three of the verum-treated participants required treatment with cinacalcet for serious secondary hyper-

parathyroidism before transplantation. After halving the dose, calcium levels normalized in almost all verum-treated participants, three required discontinuation. In conclusion, high-dose cholecalciferol therapy after kidney transplantation is well tolerated and hypercalcemia, if present, is reversible after dose reduction.

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NUMEROUS CASES OF SARCOIDOSIS MASQUERADING NEOPLASIA: BY CHANCE OR BY ETIOLOGICAL ASSOCIATION?

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Background: The etiology of sarcoidosis is unknown. An elevated level of calcitriol is a well-known phenomenon in sarcoidosis. *Methods:* The literature was searched for case reports under various conditions. *Results:* The number of case reports in an unlinked appearance was (search key=case report neoplas*) n=334053 and (case report sarcoid*) n=7711 alone. The linked appearance of both diseases sarcoidosis and neoplasia, in a single case was reported in 916 cases (case report neoplas* sarcoid*). A mimicry was reported in 98 cases (neoplas* sarcoid* mimic*), masquerade in 19 (case report neoplas* sarcoid* masqu*) and coincidence in 15 (case report neoplas* sarcoid* coincid*). *Hypothesis:* The process of sarcoidosis following neoplasia could be a programmed cure against malignant cells. This process enhances the pattern of cancer. Monocytes may liberate calcitriol to induce an inhibition of growth. In younger subjects, sarcoidosis mostly overcomes the malignant process. The rare coincidence of sarcoidosis with neoplasia could be a sign of insufficient immune response. *Conclusion:* A spontaneous remission of malignant disease may be managed by the endogenous program of cancer apoptosis (EPOCA) using calcitriol as an antineoplastic agent.

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QUANTITATIVE DETERMINATION OF VITAMIN D METABOLITES IN PLASMA USING A NOVEL MASS SPECTROMETRIC ASSAY

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The plasma concentrations of 25-hydroxy vitamin D (25-OH D) and other metabolites are used as biomarkers for vitamin sufficiency and function. To allow for the simultaneous determination of five vitamin D metabolites, 25-OH D_3, 25-OH D2, 24,25-$(OH)_2$ D_3, 1,25-$(OH)_2$ D_3 and 1,25-$(OH)_2$ D_2, in low volumes of human plasma, an assay using ultra-high performance liquid chromatography–tandem mass spectrometry was established. Plasma samples were spiked with isotope-labeled internal standards and pretreated using protein precipitation, solid-phase extraction and derivatization with 4-phenyl-1,2,4-triazoline-3,5-dione. Recovery rates ranged from 55 to 85%, depending on the vitamin D metabolite; the total sample run time was <5 minutes. Mass spectrometry was conducted using positive ion electrospray ionization on a quadrupole-quadrupole-linear ion trap (QqLIT) instrument after pre-column addition of methylamine to increase ionization efficiency. Intra- and inter-day relative standard deviations were 1.6-4.1% and 3.7-6.8%, respectively. Limits of quantification for these compounds were determined to be between 10-20 pg/ml. The 25-OH D results were compared with values obtained for reference materials (DEQAS). In addition, plasma samples were analyzed with two additional Diasorin antibody assays. All comparisons to conventional methods showed excellent correlations (r^2=0.9738) for DEQAS samples, demonstrating the high degree of comparability of the new UHPLC–MS/MS technique to existing methods.

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EPIGENETIC MODULATION OF VITAMIN D SIGNALING IN AS7B ANGIOSARCOMA CELLS *IN VITRO*

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Laboratory investigations convincingly demonstrate that the vitamin D endocrine system (VDES) regulates in various tissues cellular differentiation, apoptosis, tumor development and progression. Modulation of vitamin D signaling therefore represents a promising new potential target for cancer prevention and treatment. Angiosarcoma is a rare tumor entity that remains difficult to treat. At present, little is known about the expression and function of key components of the VDES in angiosarcoma cells. Using real time PCR (LightCycler) and Western analysis, we have now characterized for the first time the expression of key components of the VDES in AS7B cells (an angiosarcoma cell line that expresses VEGF165). We show that vitamin D receptor (VDR), 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1) and vitamin D-24-hydroxylase (CYP24A1) are strongly expressed in AS7B cells. Moreover, we demonstrate that AS7B cells are resistant against antiproliferative effects of the biologically active vitamin D

metabolite 1,25-dihydroxyvitamin D_2. Additionally, we were able to show that this resistance cannot be overcome by addition of epigenetic modulating drugs such as trichostatin A (inhibitor of histone deacetylases) and 5-azacytidine (inhibitor of DNA methyltransferases). At present, we are investigating which mechanisms may be responsible for the resistance to anti-proliferative effects of biologically active vitamin D metabolites.

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1,25-DIHYDROXYVITAMIN D_3 PROTECTS HUMAN KERATINOCYTES FROM ULTRAVIOLET (UV)- AND IONIZING RADIATION (IR)-INDUCED CELLULAR DAMAGE: *IN VITRO* ANALYSIS OF CELL VIABILITY/PROLIFERATION, DNA DAMAGE AND REPAIR

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We analyzed the capacity of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), which is produced in the skin after exposure to UVB light, to protect human keratinocytes (HaCaT) and cutaneous squamous cell carcinoma cells (SCL-1) from the hazardous effects of UVB- and ionizing radiation *in vitro*. HaCaT and SCL-1 cells were pretreated with $1,25(OH)_2D_3$ over 48 hours and then irradiated once with UVB- or low-dose ionizing radiation. We evaluated the results of several assays (CFUc, WST-1, and CV), comparing cell viability/proliferation of $1,25(OH)_2D_3$-pretreated cells with controls pretreated with the carrier substance (ethanol) alone. Additionally, we analyzed the effects of $1,25(OH)_2D_3$ on induction and removal of UV- and IR-induced DNA damage by detection of CPDs *via* Dot Blot analysis and γH2AX-Foci *via* immunofluorescence in HaCaT-keratinocytes. We showed that $1,25(OH)_2D_3$ at a concentration of 10^{-7} M protects human keratinocytes (HaCaT), as well as squamous cell carcinoma cell lines (SCL-1) from the hazardous effects of UVB-radiation (100 J/cm^2-1000 J/cm^2) *in vitro*. Moreover, it was demonstrated that the number of CPSs induced in HaCaT-keratinocytes after irradiation with UVB (100 J/cm^2-1000 J/cm^2) was reduced after pretreatment with $1,25(OH)_2D_3$. Analysis of the time course revealed that the elimination of UVB-induced DNA damage in HaCaT keratinocytes occurs quicker when cells are pretreated with $1,25(OH)_2D_3$ (as compared to controls). Concerning low-dose IR up to 2 Gy, our findings point to the fact that pretreatment of HaCaT keratinocytes with $1,25(OH)_2D_3$ (10^{-7} M) for 48 hours reduces the formation of γH2AX foci. In summary, our data support the hypothesis that $1,25(OH)_2D_3$ protects cultured human keratinocytes from the hazardous effects of UVB and low-dose IR.

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CROSS-TALK BETWEEN VITAMIN D AND NOTCH SIGNALING PATHWAYS IN MALIGNANT MELANOMA

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NOTCH signaling is of critical importance for the embryonic development and the growth of human melanocytes. NOTCH signaling pathways depend on the presence or absence of several specific receptor proteins and corresponding ligands. We have characterized the immunohistochemical staining pattern of NOTCH receptors 1 and 2 in malignant melanoma, skin and lymph node metastases of malignant melanoma and in benign acquired melanocytic nevi. Additionally, we investigated expression of NOTCH receptor 1 and its corresponding ligand JAGGED 1 in a vitamin D-sensitive human melanoma cell line (MeWo) using real-time PCR and Western blot analysis. We found differential immunohistochemical staining patterns of NOTCH receptors 1 and 2 in tissues analyzed and strong expression of NOTCH 1 and JAGGED 1 at RNA and protein levels in MeWo melanoma cells. Interestingly, treatment of melanoma cells *in vitro* with 1,25-dihydroxyvitamin D_3 (10^{-10} M to 10^{-6} M), the biologically active form of vitamin D, resulted both in a dose-dependent inhibition of cell proliferation and in reduced RNA and protein expression of NOTCH receptor 1 (10^{-6} M). Treatment of MeWo cells *in vitro* with 1,25-dihydroxyvitamin D_3 at a low concentration (10^{-10} M) resulted in an increased expression of JAGGED 1. In summary, our fndings point to cross-talk between vitamin D and NOTCH signaling pathways while regulating the growth of melanoma cells. Moreover, we conclude that both vitamin D analogs and pharmacologic modulation of NOTCH signaling may open new perspectives for the treatment of malignant melanoma.

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BIOSYNTHESIS AND TRANSFORMATION OF STEROLS BY USING STRAINS OF YEAST *SACCHAROMYCES CEREVISIAE*

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The basic ways of biosynthesis and biotransformation of substances from acetyl coenzyme to hormones, vitamin D and their hydroxy derivatives were analyzed. It was shown that the use of genetic engineering makes it possible to

establish the relationship, the sequence of stages of the biosynthesis of cyclic precursors of ergosterol, the mechanisms regulating a processes of biosynthesis, as well as allowing the development of a highly selective system for the allocation of mutations that contribute to the enhanced formation of sterols, which in turn makes it possible to purposefully create sustainable producers.

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SERUM VITAMIN D DEFICIENCY, A NEW EPIDEMIC

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The association of vitamin D deficiency to various systemic, autoimmune and neoplastic conditions is a well documented fact now. We performed a meta-analysis of three studies regarding serum vitamin D levels, and total data of 3,000 patients was analysed. *Objectives:* To review the frequency of vitamin D deficiency in various groups of patients. *Data Source:* We selected three studies all performed by the same authors and published in indexed journals of Pakistan. *Results:* 22% of the patients were male and 78% were female (SD 0.41) with 123 aged below 20 years, 1,785 between 21-40, 714 between 41-60, 358 between 61-80 and 20 above 80 years (SD 0.78). Only 182 patients had a normal vitamin D level above 30 IU (SD 0.23). *Conclusion:* Vitamin D deficiency is highly prevalent in our community and is mostly asymptomatic. Thus, vitamin D supplementation is widely required in Pakistan to reduce its varied and multidimensional effects on health.

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