THE ViDMe STUDY

Vitamin D supplementation in cutaneous malignant melanoma outcome

Sponsor:
University Hospitals Leuven

Coordinating Investigator:
Prof. Marjan Garmyn

Eudract No: 2012-002125-30
Sponsor's Protocol Code Number 2012LRDVDCM

PROTOCOL SYNOPSIS

Title	The ViDMe Project "Vitamin D supplementation in cutaneous malignant melanoma outcome"
EudraCT No.	2012-002125-30
Phase	III
Sponsor	UZ Leuven
Coordinating Investigator	Prof M. Garmyn
Study Objectives	To assess whether vitamin D supplementation, in the follow up period after diagnosis and surgery of the primary tumor, has a protective effect on relapse of cutaneous malignant melanoma and whether this protective effect correlates with vitamin D levels in serum and vitamin D receptor (VDR) immunoreactivity in the primary tumor.
Design	double-blind randomized placebo-controlled study
Treatment Groups	Group 1: optimal standard of care (control group) Group 2: high dose vitamin D3 supplementation added on top of optimal standard of care. Patients will be randomised to treatment or control group in a 1:1 ratio.

Study Flow and Assessments	The study is divided into three parts: • Screening phase: Patients are recruited at the Departments of Dermatology, Oncosurgery or Medical Oncology at the University Hospitals Leuven (Belgium), and other European academic sites.If all eligibility criteria are met, patients are then randomized to the treatment or control group. Randomisation is completed via IVRS. Patients are randomized as they come. • Treatment phase: Vitamin D3 supplementation is given to the patients assigned to the treatment group and placebo to the control group in a double blind manner. Every participant is expected to take in the study drug orally once each month. Study duration is a maximum of 3.5 years or until relapse occurs. • Follow-up phase: Every participant will be followed up every 3 months up to the final study visit. Endpoints will be reported as occurring throughout the follow up. Defined endpoints are adjudicated by an independent Clinical Endpoint Committee (CEC) blinded to the patient treatment allocation. This ensures a consistent and unbiased adjudication of events. Study data are reviewed by a Data and Safety Monitoring Board (DSMB) that reviews key safety data on an ongoing basis.

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Therapy	Ampoules of 100 000 IE cholecalciferol (vitamin D3) are prepared by the pharmacist and will be taken orally at a monthly basis. Duration of the treatment: till the end of the study (max 3.5 years) or until relapse. Placebo capsules, consisting of oil preparation will be prepared in the same way. All patients are treated with optimal standard cutaneous melanoma follow-up.
Population	Adult male or female patients with histologically diagnosed stage IB to III cutaneous melanoma are eligible for the trial.
Inclusion Criteria	1. Older than 18 years and younger than 80 years of age. 2. Histologically proven malignant melanoma, stage IB to III Not participating in other clinical trial. 3. The only treatment for melanoma is surgical treatment. 4. Complete resection of melanoma. 5. Single primary invasive cutaneous melanoma (inclusion within 1 year after diagnosis). 6. Signed ethical committee approved informed consent 7. Serum phosphate and calcium at the entry of the study within normal range of the laboratory reference

Exclusion Criteria	1. Pregnant/lactating women or planning on becoming pregnant during the study 2. Known hypersensitivity to vitamin D or its components. 3. Pre-existing renal stone disease or chronic renal disease with eGRF < 30 mL/min/1.73 m2 or renal dialysis. 4. Liver failure or chronic liver disease with liver enzyme > 2 fold ULN (=upper limit of normal) 5. History of parathyroid disease or granulomatous disease (TBC and sarcoidosis) 6. History of malabsorption syndrome or any medical condition that might interfere with vitamin D absorption. 7. History of other malignancy within the last 5 years except for carcinoma in situ of the cervix or basal cell carcinoma or squamous cell carcinoma of the skin or in situ malignant melanoma. 8. Chronic alcohol abuse. 9. Medical or logistic problems likely to preclude completion of the study.
Study duration	Patients are enrolled during a recruitment phase of three years maximum. Study duration for one patient is maximum 3.5 years or until relapse occurs. The DSMB may terminate the study earlier based on safety concerns.

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Criteria for Evaluation	Primary efficacy endpoint: 1. Relapse free survival Secondary efficacy endpoints: 1. Melanoma subtype, as assessed clinically and histologically 2. Melanoma site, as clinically recorded 3. 25(OH)D3 serum levels at diagnosis and at 6 months intervals 4. Stage of melanoma patient at diagnosis according to the 2009 AJCC Melanoma staging and classification Safety endpoints: • Incidence and severity of adverse events
Sample size	Approximately 500 patients.
Version and date of final protocol	First version, 10-10-2012
Version and date of protocol amendments	Second version, 22-03-2013 Third version, 20-08-2013 Fourth version, 25-11-2015 Fifth version, 27-09-2017 Sixth version, 20-02-2018 Seventh version, 19-03-2019 Eight version, 05-01-2021

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Statistical methods	The analysis of efficacy and safety will be performed for an intention-to-treat (ITT) population, defined as all randomized patients. No interim analysis for efficacy is foreseen. .

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Signatures

This study protocol was subjected to critical review. The information it contains is consistent with the current risk/benefit evaluation of the investigational medicinal product as well as with the ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and the guidelines on Good Clinical Practice.

This protocol is approved by:

Coordinating Investigator:	Date	Prof. Marjan Garmyn
Statistician:	Date	Dr. Kris Bogaerts
Signatory 3 as applicable	Date	Name
Signatory 4 as applicable	Date	Name

Declaration of Investigator

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol. In particular, I agree to adhere to the ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and the Guidelines on Good Clinical Practice.

Responsible Investigator at the Local Study Centre:

Institution	City	Country
Date	Printed Name	Signature

List of Study Core Facilities / Personnel

Sponsor	UZ Leuven Herestraat 40 B-3000 Leuven
Coordinating Investigator	Prof. Marjan Garmyn Head of Dermatology Department UZ Leuven Herestraat 49 B-3000 Leuven
Subinvestigator	Dr Eva Remenyik Dep of Dermatology Medical and Health Science Center University of Debrecen H-4012 Debrecen Nagyerdei krt 98 Hungary
Subinvestigator	Dr Tine Strobbe Dermatology Universitair Ziekenhuis Antwerpen Wilrijkstraat 10 2650 Edegem Belgium
Subinvestigator	Dr Arjen Nikkels Dermatology

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Interactive Voice Response System Centre	Leuven Coordinating Centre U.Z. Gasthuisberg Herestraat 49 bus 7003 B3000 Leuven
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List of Abbreviations

7-DHC	7 Dehydrocholesterol
AE	adverse event
AJCC	American Joint Committee of Cancer
ALM	Acrolentigonous malignant melanoma
BCC	Basal cell carcinoma

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CE	clinical event
CEC	clinical event committee
CMM	Cutaneous malignant melanoma
DSMB	data and safety monitoring board
eCRF	electronic case report form
GCP	good clinical practice
GMP	good manufacturing practice
IMP	investigational medicinal product
IVRS	interactive voice response system
LMM	Lentigo Malignant Melanoma
NMM	nodular malignant melanoma
SAE	serious adverse event
SCC	Squamous cell carcinoma
SSMM	superficial spreading malignant melanoma
SUSAR	suspected unexpected serious adverse reaction
VDR	Vitamin D Receptor

1. INTRODUCTION

1.1. Background

Cutaneous malignant melanoma (CMM) is the most common lethal skin disease, affecting mainly Caucasians worldwide and currently in Flanders the 10th most frequent tumor in males and 5th most frequent tumor in females (Belgian Cancer Registry 2008). Although CMM only accounts for 4 % of all malignant tumors of the skin, it is responsible for 80% of the skin cancer related deaths (Miller, 2006; Jemal, 2008). Incidence rates of this disease have been escalating in the last decades and it is forecasted that the incidence will further exacerbate the coming years (Garbe, 2009). In Belgium, a steady rise in the incidence of cutaneous melanoma has been reported as well (Belgian Cancer Registry, 2008). CMM arises from transformed melanocytes in the skin. These melanocytes are situated in the basal layer of the epidermis. Melanomagenesis implicates a stepwise transformation of melanocytes to melanoma, all or not with intermediate steps comprising ordinary and dysplastic naevi, and subsequent superficial and deep invasion, and metastasis (Miller, 2006). Several risk factors have been linked to the development of malignant melanoma. The strongest risk factors for melanoma are a family history of melanoma (two or more cases of melanoma in first degree relatives), multiple benign (more than 100) or atypical naevi, and a previous melanoma. Immunosuppression, low phototype (characterized by having a fair skin, poor ability to tan or freckled complexion with or without red hair), and exposure to ultraviolet light (especially short bursts of acute exposure in childhood and severe sunburn) are additional risk factors (Bataille, 2008). Each of these risk factors corresponds to a genetic predisposition or an environmental stressor, which contributes to the genesis of melanoma. At least some of these factors are understood to various degrees at the molecular level. For example 25 to 40 percent of the members of melanoma-prone families have mutations in cyclin-dependent kinase inhibitor 2A (CDKN2A). Polymorphisms in the melanocortin 1 receptor (MC1R) have been linked to fair skin and red hair and have been associated with increased risk of melanoma (Miller 2006). Single nucleotide polymorphisms at 9p21 and 22q13 involved in cutaneous nevi development (Falchi, 2009) have also been associated with increased melanoma risk (Bishop 2009).

The most important clinicopathological subtypes of melanoma are superficial spreading malignant melanoma (SSMM), nodular malignant melanoma (NMM), lentigo malignant melanoma (LMM), and acrolentiginous malignant melanoma (ALMM). SSMM is the most frequent subtype within the Caucasian population. Diagnostic signs are progressive asymmetrical enlargement of a pigment lesion which also demonstrates irregularity in colour and irregularity in shape.

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NMM are less frequent, occur mostly in males and are the most aggressive form, since they show a vertical growth phase from the beginning. They present themselves as rapid growing black often ulcerated tumors. LMM arises in a long standing pigmented precursor lesion and is located on chronic photodamaged skin. ALMM appear on palms and soles initially as flat pigmented lesions resembling moles, which start to increase in size and ultimately develop irregular areas of pigmentation. Subungual melanomas are most often located on the thumb nail of the great toe and present themselves as a narrow pigmented band, which slowly widens and ultimately produces a subungual mass. Different risk factors are associated with different subtypes of CMM and different locations of malignant melanoma: while chronic photodamage preferentially associates with LMM and malignant melanoma on head and neck region, a high count of naevi preferentially associates with malignant melanoma on the trunk, indicating that different pathways may lead to the development of malignant melanoma, i.e. a pathway through chronic sun exposure, and a melanomagenetic pathway (Whiteman, 2003). Different subtypes of malignant melanomas show also a significant genetic heterogeneity: CMM arising in sites intermittently exposed to the sun harbor BRAF and/or N-ras mutations more frequently; while malignant melanomas on chronically sun exposed skin (LMM) and ALM show more frequent c-Kit mutations (Curtin 2006). While cutaneous malignant melanoma, when diagnosed and treated in an early stage (pure radial or early invasive growth phase) has a very good prognosis and can still be treated in a curative way via surgery, metastatic melanoma decreases the chances of survival markedly, because of the inherent resistance of the melanoma cells to classical cytotoxic chemotherapy. The most important predictor of progression of malignant melanoma and survival is Breslow thickness of the primary tumor at diagnosis. Breslow thickness is a histological characteristic of the tumor and refers to the thickness of the tumor, measured as the distance between the granular layer of the epidermis and the deepest tumor cell in millimetres. According to the recent final version of the 2009 American Joint Committee of Cancer (AJCC) melanoma staging and classification, primary tumor ulceration and primary tumor mitotic rate are considered as independent prognostic factors for survival and have been included in tumor staging (Balch 2009). For instance, patients with a melanoma equal or less than 1 mm thickness, without ulceration or mitosis and without nodal or distant metastasis (T1aN0M0, stage IA) have a very good prognosis, reflected by a five and ten year survival of respectively 97 and 93%, whereas patients with an ulcerated melanoma of over 4 mm thickness, but without nodal or distant metastasis at diagnosis (T4bN0M0) have a 5 and 10 year survival of 53% and 39% respectively. Once distant metastasis occurs, the prognosis is bad, i.e. a patient with distant metastatic melanoma to the viscera (stage IV, M1c) has a 1 year survival of 33% (Balch 2009). Other known predictive prognostic factors are presence and distribution of tumor infiltrating lymphocytes, tumor site, sex, and social deprivation. (Balch, 2009; Newton Bishop 2009).

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Vitamin D status: current knowledge

There are 3 sources of vitamin D: endogenous production in skin upon absorption of ultraviolet (UV) B (290-315 nm) radiation, vitamin D in foods and finally dietary supplements. Since a standard western diet contains only very few food components, which contain vitamin D (fatty fish and liver extracts) our most important source of vitamin D is endogenous conversion of 7-dehydrocholesterol (7-DHC) to previtamin D3 by UVB, which isomerizes to vitamin D3 by thermal energy. However, this vitamin D synthesis is only reliably available year-round at latitudes between 40 ° north and 40 ° south, indicating that in our regions the required levels of UVB are low or non-existent for a significant proportion of the year. Data indicate that indeed insufficient vitamin D levels are widespread both in the American and European population (Holick 2006; Lee 2009; Adams 2010,) especially in wintertime (Rhodes 2010).

Vitamin D (from the food, via supplements or produced by UVB in the skin) is biologically inert and the biological effect of vitamin D results only as a consequence of its sequential metabolisation in the liver into 25-hydroxy-vitamin D3 (25(OH)D3) and then in the kidney into the steroid hormone, 1alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3). The classical signaling pathway of 1,25(OH)2D3 employs the vitamin D receptor (VDR), which is a transcription factor for 1,25(OH)2D3 target genes, including the cyclin dependent kinase inhibitors, p21 and p27.

Vitamin D receptor (VDR) is believed to be located in the nucleus prior to activation by 25(OH)2D3, which dissociates from the serum vitamin D-binding protein, enters the cell by diffusion and binds the VDR. Ligand binding produces conformational changes, resulting in dimerization, necessary for high affinity interaction with target gene promoter at the vitamin D response element (Osborne, 2002).

The gene encoding VDR maps to the chromosomal region 12q13 and contains numerous common variants, that are hypothesized to influence the function and or expression of VDR (Egan 2009).

It is generally agreed that the serum concentration of 25(OH)D3 in normal subjects is the best indicator for vitamin D status and currently a serum concentration below 10 ng/ml (<25/ nmol/L) is considered as vitamin D deficient, and a serum concentration below 20 ng/ml (< 50 nmol/L) as vitamin D insufficient. The optimal level for health is believed to be reached at 30 ng/ml (75 nmol/L), while some individuals may be at risk for toxicity (hypercalcaemia, vomiting thirst, polyuria, ectopic calcifications) when their 25(OH)D3 levels are in the range of 100-150 ng/ml (or 250-300 nmol/L). Current recommendation for additional vitamin D intake via supplements, in addition to skin synthesis and nutritional intake is 1000 IU/d. A good safety profile for this amount has been shown in more than 50 000 subjects over a several year treatment period (Norman and Bouillon, 2010), while doses for chemoprevention studies (colon, prostate and breast cancer) range from 2000 to 4000 IU /day (ClinicalTrials.gov). A recent IWT/TBM project investigating the effect of vitamin

D supplementation on Chronic Obstructive Pulmonary Disease (COPD) on 90 patients in a placebo controlled setting, has shown that a single monthly dose of 100 000 IU, given during a year is safe, is able to increase the 25 (OH)D3 levels in serum, from insufficient levels (<20 ng/ml) to 50 ng/ml(Lehouck, 2012). , and has no immunosuppressive effects (personal communication, Prof Wim Janssens and Prof Chantal Mathieu, UZ KULeuven).

Another important finding is that vitamin D levels are influenced by the genetic variability of every individual. Data from a very recent genome wide association study (Wang 2010) show that the following genes contribute to the variability of serum concentrations of 25(OH)D3: 7-DHC reductase (responsible for the availability of 7-DHC in the skin), the liver 25-hydroxylase CYP2R1 (involved in the conversion of vitamin D into 25-hydroxyvitamin D3) and CYP24A1 (key degradation enzyme). Additionally polymorphisms in GC, the gene encoding vitamin D-binding protein, had the greatest effect on serum 25-hydroxyvitamin D3 concentration.

Vitamin D and melanoma: current knowledge.
Laboratory data, animal studies, epidemiological observations and clinical studies indicate that Vitamin D may have a preventive effect on melanoma development and may affect tumor progression or melanoma outcome

The VDR has been identified in cultured melanoma cells, in melanoma xenografts and in primary melanoma tissue (reviewed by Osborne 2002 and Egan 2009). Vitamin D metabolites have been shown to inhibit proliferation and induce differentiation in melanoma cell lines. In vivo 1,25 (OH)2 D3 has been shown to suppress growth in human melanoma derived xenografts in immunosuppressed mice. Pretreatment of mouse melanoma cells with 1,25(OH)2D3 resulted in inhibition of migration through extracellular matrix and adherence of the cells to reconstituted basement membrane (Matrigel) type IV collagen and inhibition of the formation of lung metastases, indicating an inhibitory effect on tumor invasion and metastasis.

Skin is an important target site for the actions of vitamin D. Photochemical reactions that produce pre Vitamin D3 (cholecalciferol) only take place in the skin. Keratinocytes and other cells in the skin are capable of synthesizing hormonally active vitamin D metabolite, 1,25(OH)2D3 and there is evidence that 1,25(OH)2D3 can protect skin cells from UV damage (De Haes 2005; Gupta 2007).

Epidemiological evidence for a possible protective influence of sunlight/vitamin D on melanoma risk is suggested by studies showing lower than expected incidence in persons with outdoor occupations, which, according to the authors, could reflect at least in part a level of protection afforded by their greater reserves of vitamin D. The majority of melanomas occur at body sites only receiving intermittent sun exposure, consistent with possibility of inadequate production of

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vitamin D as possible cofactor in sites with limited exposure to sun, like trunk and extremities. FokI and BsmI polymorphisms in the VDR, which may influence expression or activity of this protein are associated with an altered melanoma risk (Gandini, 2009). However large epidemiological studies have failed to find an association between dietary vitamin D intake and melanoma incidence (Weinstock 1992; Asgari 2009) and between serum vitamin D levels and melanoma risk (Randerson-Moor 2009).

Added value compared to current knowledge/state of the art initiatives

Vitamin D levels may have a protective effect on malignant melanoma outcome, as suggested by a reported association of higher 25(OH)D3 serum levels at diagnosis with lower Breslow thickness at diagnosis. Increased 25(OH)D3 serum levels at diagnosis were also independently protective of relapse and death (Newton Bishop, 2009). In the present proposal, we expand these investigations on the potential protective effect of vitamin D on melanoma outcome in a more comprehensive way, because of the following reasons:

1. We will test the protective effect of vitamin D supplementation to melanoma patients (in a placebo controlled and double blind way) against relapse (primary endpoint).

2. We will also immunohistochemically assess the expression of the Vitamin D Receptor (VDR) expression in the primary tumor, and investigate whether VDR immunoreactivity in the primary tumor correlates with relapse (primary endpoint) and melanoma stage at diagnosis. (secondary endpoint)

3. We will monitor increases in vitamin D levels after supplementation (secondary endpoint). This will allow us to assess whether every patient is characterized by the same increase in vitamin D, or whether serum levels following intake depends on genetic variability. Furthermore, this will allow us to monitor compliance to the supplementation.

4. We will correlate vitamin D levels at diagnosis with melanoma site (secondary endpoint) and subtype (secondary endpoint).

1.2. Rationale of the study

The **rationale** for this project is the need to **improve the outcome of malignant melanoma of the skin**, the most common lethal skin disease, with a large impact in terms of years of life lost. While early stage malignant melanoma (pure radial, early invasive growth phase) can be cured, late stage (metastatic) melanoma decreases the chances of survival markedly because of its resistance to chemotherapy (Balch 2009). Predictors of melanoma progression and survival include histological characteristics of the primary tumor at diagnosis: Breslow thickness, presence of ulceration and number of

mitoses. Improvement of melanoma outcome can be achieved by diagnosing the tumor in an earlier stage (thinner Breslow, absence of mitosis and absence of ulceration) and by decreasing the chance of relapse after surgery of the tumour.

We hypothesize that high dose vitamin D supplementation after surgery of the primary tumor can decrease the chance of relapse, based on previous in vitro and vivo data, which support an anticancer effect of vitamin D.

The active metabolite of vitamin D, 1,25(OH)2D3, demonstrates relevant anti-cancer effects on melanoma cells, not only antiproliferative, prodifferentiating and proapoptotic effects, but also inhibitory effects on tumor invasion and metastasis (Osborne, 2002 and Egan, 2009). These pleiotropic anticancer effects are mediated by the vitamin D receptor (VDR).

Patients in stage IV (metastatic malignant) melanoma show reduced 25(OH)D3 serum levels and a recent study revealed an association of higher 25(OH)D3 serum levels with lower Breslow thickness of the tumor at diagnosis. (2009, Nürnberg, 2009, Newton Bishop 2009)

Serum concentration of 25(OH)D3 is the best indicator of the vitamin D status, which is determined by UVB induced production of vitamin D in the skin, dietary intake and vitamin D supplementation (Sage, 2010). In addition vitamin D status may also be influenced by genetic variants of certain proteins involved in the vitamin D pathway (Wang 2010).

The proposed project will assess whether high dose **vitamin D supplementation after surgery of the primary tumor affects malignant melanoma outcome**. More specifically the following questions will be addressed:

1. Is vitamin D supplementation after diagnosis and surgery of the primary tumor protective against a relapse of malignant melanoma?
2. Does this protective effect on relapse correlate with VDR immunoreactivity in the primary tumor?

1.3. Risk-benefit assessment

In every clinical trial, anticipated benefits need to be weighed against the study associated risks. It is generally agreed that the serum concentration of 25(OH)D3 in normal subjects is the best indicator for vitamin D status and currently a serum concentration below 10 ng/ml (<25/ nmol/L) is considered as vitamin D deficient, and a serum concentration below 20 ng/ml (< 50 nmol/L) as vitamin D insufficient. The optimal level for health is believed to be reached at 30 ng/ml (75 nmol/L), while some individuals may be at risk for toxicity (hypercalcaemia, vomiting, thirst, polyuria, ectopic calcifications) when their 25(OH)D3 levels are in the range of 100-150 ng/ml (or 250-300 nmol/L). Current recommendation for additional vitamin D intake via supplements, in addition to skin synthesis and nutritional intake, is

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1000 IU/ d. A good safety profile for this amount has been shown in more than 50 000 subjects over a several year treatment period (Norman and Bouillon, 2010), while doses for chemoprevention studies (colon, prostate and breast cancer) range from 2000 to 4000 IU /day (ClinicalTrials.gov). A recent publication (Lehouck et al, 2012) investigating the effect of vitamin D supplementation on Chronic Obstructive Pulmonary Disease (COPD) on 90 patients in a placebo controlled setting, has shown that a single monthly dose of 100 000 IU, given during a year is safe, is able to increase the 25 (OH)D3 levels in serum, from insufficient levels (<20 ng/ml) to 50 ng/ml, and has no immunosuppressive effects.

The anticipated benefits high dose vitamin D supplementation for study participants are expected better long-term outcomes such as reduced occurrence of relapse and longer relapse free survival period. Given the increased mortality of melanoma patients that progress to a higher stage (Balch et al, 2009) and the patients' burden that is associated with this course (including intensive medical treatment, financial impact and/or inability to work), the ViDMe study offers a valuable adjuvant, low invasive treatment opportunity for participating patients. In view of these facts, the risk benefit ratio seems well balanced in the ViDMe trial so that the benefits outweigh the risks. To account for the described risks, the ViDMe study will assess complications/adverse events associated with vitamin D supplementation. A DSMB Committee will analyze key safety aspects in regular intervals during the ongoing trial to ensure patient safety.

2. STUDY OBJECTIVES

2.1. Primary objective

The primary endpoint for this study is

- Relapse free survival.

2.2. Secondary objective

Secondary efficacy endpoints are:

- Melanoma subtype, as assessed clinically and histologically
- Melanoma site, as clinically recorded
- 25(OH)D3 serum levels at diagnosis and at 6 months intervals
- Stage of melanoma patient at diagnosis according to the 2009 AJCC Melanoma staging and classification

Safety endpoints:

- Incidence and severity of adverse events

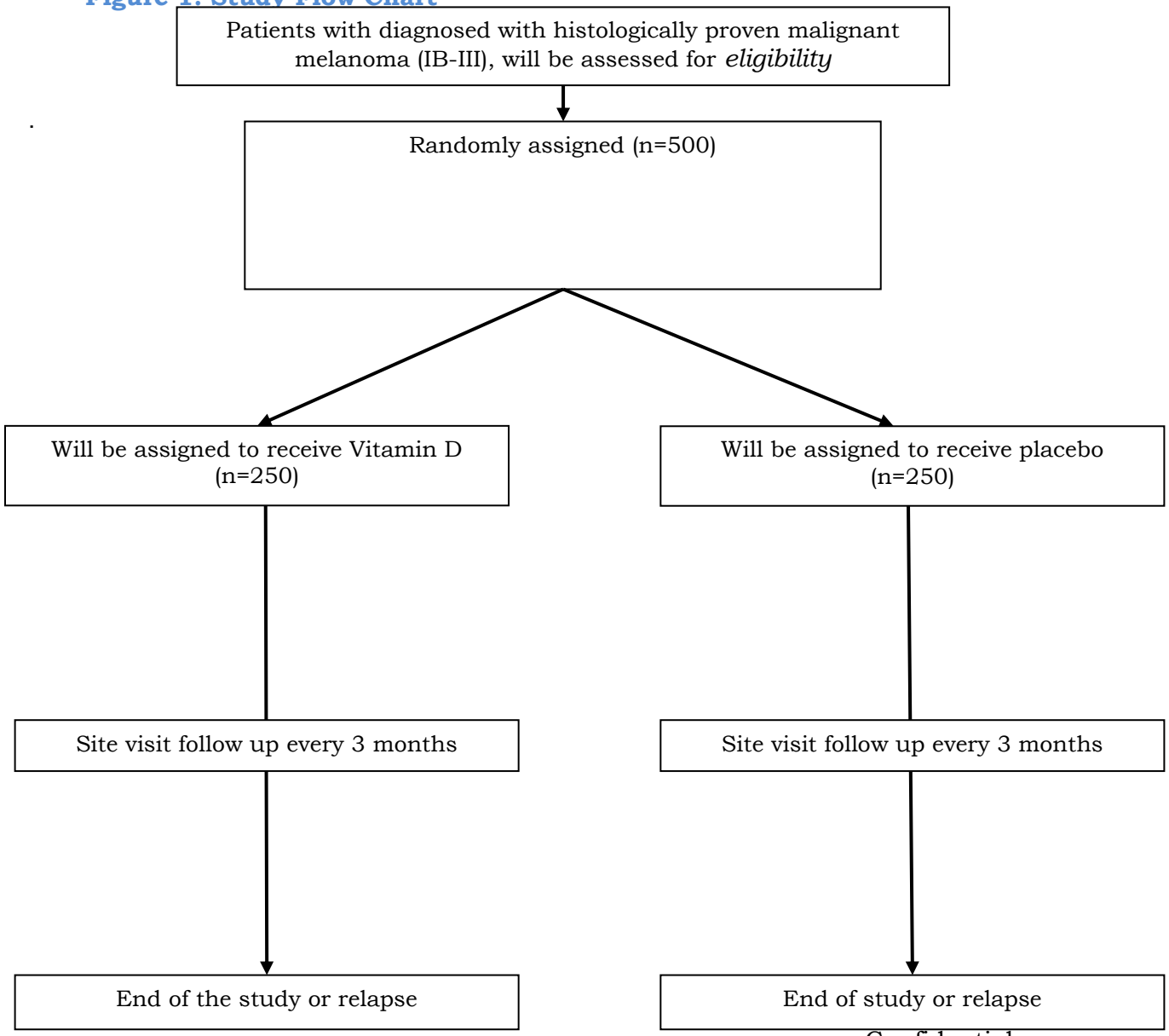
3. OVERALL DESIGN AND PLAN OF THE STUDY

3.1. Overview of study flow

To investigate whether vitamin D supplementation after diagnosis affects malignant melanoma outcome (primary endpoint relapse free survival), stage IB-III patients will be enrolled from fall 2012 to fall 2023. Stage IA patients will not be included in the study because they only have a very small chance to relapse. This double-blind randomized study will comprise 500 patients (stage IB-III). This patient cohort will be randomized in a 1:1 ratio to vitamin D supplementation or placebo. We will also assess the expression of the Vitamin D Receptor (VDR) expression in the primary tumor. A blood sample will be taken for DNA analysis (genetic variability in the vitamin D pathway). We will check 25(OH)D3 serum levels both at diagnosis, and every 6 months until the end of the study. This patient cohort will exist of 3 subgroups (patients diagnosed within 6 or 12 months before start of the study and newly diagnosed patients). Inclusion of previously diagnosed patients will allow us to assess whether delayed supplementation has a similar effect on the relapse free survival rate as immediate supplementation after surgery of the tumor.

In this randomized vitamin D supplemented placebo-controlled trial (both arms), we will assess whether vitamin D supplementation in the follow up period after diagnosis has a protective effect on relapse (primary endpoint) of the malignant melanoma and whether this protective effect correlates with the vitamin D receptor immunoreactivity in the primary tumor.

Figure 1: Study Flow Chart



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3.2. Justification of study design

A previous study has demonstrated the association of higher 25-hydroxyvitamin D3 serum levels with lower Breslow thickness of the tumor at diagnosis (Newton-Bishop, 2009), suggesting a protective effect of higher vitamin D levels on disease progression of cutaneous malignant melanoma.

To further proof the protective effect of Vitamin D supplementation on melanoma disease progression, this study will give high dose vitamin D supplementation to the melanoma patients after surgery of the primary tumor (which is the current best standard of care), in a placebo controlled and double blind way. The effect of high dose vitamin D supplementation in double blind placebo controlled way will be primarily tested on relapse free survival.

Patients will be randomly assigned in a 1:1 ratio to receive a monthly oral dose of 100 000 IU of vitamin D (1ml of D-Cure) or placebo (1 ml of olive oil). A block randomization method stratified on time of diagnosis (newly diagnosed, 0-6 months or 6-12 months before start of the study) will be used to overcome seasonal influences on baseline characteristics.

We will also assess the expression of the Vitamin D Receptor (VDR) expression in the primary tumor. A blood sample will be taken for DNA analysis (genetic variability in the vitamin D pathway). We will check 25(OH)D3 serum levels both at diagnosis, and every 6 months until the end of the study. If the level of 25(OH)D3 serum is above 80 ng/ml, study drug will be interrupted and during drug free interval the 25(OH)D3 serum levels will checked every 3 months. When serum 25 (OH)D3 falls below 50 ng/ml, study drug is restarted

The patient cohort will exist of 3 subgroups (patients diagnosed within 6 or 12 months before start of the study and newly diagnosed patients). Inclusion of previously diagnosed patients will allow us to assess whether delayed supplementation has a similar effect on the relapse free survival rate as immediate supplementation after surgery of the tumor.

3.3. Study population

3.3.1. Number of patients

A total of approximately 500 male and female patients will be enrolled in the ViDMe study in this multiple centre study.

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3.3.2. Inclusion criteria

1. Older than 18 years and younger than 80 years of age.
2. Histologically proven malignant melanoma, stage IB to III Not participating in other clinical trial.
3. The only treatment for melanoma is surgical treatment.
4. Complete resection of melanoma.
5. Single primary invasive cutaneous melanoma (inclusion within 1 year after diagnosis).
6. Signed ethical committee approved informed consent
7. Serum phosphate and calcium at the entry of the study within normal range of the laboratory reference

3.3.3. Exclusion criteria

1. Pregnant/lactating women or planning on becoming pregnant during the study
2. Known hypersensitivity to vitamin D or its components.
3. Pre-existing renal stone disease or chronic renal disease
4. With eGRF < 30 mL/min/1.73 m2 or renal dialysis.
5. Liver failure or chronic liver disease with liver enzyme > 2 fold ULN (=upper limit of normal)
6. History of parathyroid disease or granulomatous disease (TBC and sarcoidosis)
7. History of malabsorption syndrome or any medical condition that might interfere with vitamin D absorption.
8. History of other malignancy within the last 5 years except for carcinoma in situ of the cervix or basal cell carcinoma or squamous cell carcinoma of the skin or in situ malignant melanoma.
9. Chronic alcohol abuse.
10. Medical or logistic problems likely to preclude completion of the study.

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4. STUDY CONDUCT

4.1. Schedule of procedures

The schedule of study assessment is listed in Table 1.

Table 1: Schedule of assessments

	Screening	Randomization = day 0	Every 3 months FU	Every 6 months FU	End of Study (fall 2023) or Progression/Relapse
Informed Consent	X				
Demographics	X				
Medical History	X				
Melanoma Risk Factors	X				
Sun exposure Behaviour	X				X
Physical Examination	X		x		X
Vitamin D levels		X		X	X
DNA analysis		x			
Safety Laboratory#	X		X	x	X
Concomitant Medications	X	X	X		X
Adverse Event / Endpoint Collection		X	X		X
24 hour urine calcium					X

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Safety laboratory

Screening and end of study:
Hemoglobin, haematocrit, WBC count with differential, RBC count and platelet count
Renal function (creatinine)
Liver function (AF, ALT, gamma-GT, LDH)
Serum calcium and serum phosphate, and albumin

Every 3 months:
serum calcium, serum phosphate,

Every 6 months
WBC count plus differential

4.2. Patient screening and randomization

Patients are recruited upon presentation and recruitment is based on the stage of melanoma.

Stage IB-III patients will be enrolled from fall 2012 to fall 2023.

After staging and surgical treatment, and after obtaining written informed consent, patient will undergo screening at the investigational site to ensure that the patient meets all other in- and exclusion criteria. Written informed consent must be obtained from patients prior to any study specific procedure (for details see section 8.1). After consenting patients undergo the procedures listed for the screening visit. If the patient meets all in- and exclusion criteria, the patient will then be randomized using a specifically designed Interactive Voice Response system (IVRS) on an equal basis (1:1) to either vitamin D or placebo stratified on time of diagnosis (newly diagnosed, 0-6 months or 6-12 months before start of the study). The IVR system will supply a patient number that will be used as the key identifier for the entire study period. Patients in both arms will receive similar looking capsules, containing either 100 000 IE cholecalciferol or placebo oil. The randomization schedule will be kept under closure at the data management centre. All other study staff members, dermatologists, trail nurses, and patients will be blinded to the treatment allocation. The DSMB members may be unblinded to assess the safety of the patients. The study complies with the Helsinki declaration of medical research and needs approval by the hospital medical ethics committee. Written informed consent will be obtained prior to enrolment.

In these stage IB-III melanoma patients (500 patients), we will take a blood sample for serum analysis (25(OH)D3 levels) at the moment of diagnosis and, every 6 months until relapse or until the end of the study.

Every participant will also be asked to complete a questionnaire regarding melanoma risk factors, including amount and type of sun exposure and to undergo skin examination for the assessment of phototype, naevus phenotype and actinic damage. We will measure patient's height and weight, which will be used to estimate body mass index (BMI).

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Our power calculations are based on a patient enrolment rate of 500 melanoma patients until the end of the study. In order to meet those targets, a multicentre study is necessary, via participation of other academic centers.

4.3. Description of study assessments

4.3.1. Medical history / demographics

A complete medical history is obtained from each patient at the screening visit. Demographics, including gender, age and country of birth of all four grandparents, and the smoking status are recorded.

4.3.2. Staging of melanoma

Patients are ranked according to the following staging system:

Pathological staging of the primary tumor: is performed at the department of Pathology of the participating centers and is based on the Breslow thickness of the tumor, mitotic rate and presence or absence of ulceration. Breslow thickness of the tumor is measured by a calibrated eye piece graticule, and reflects the distance from the granular layer of the epidermis to the deepest tumor cell in millimetres. Mitotic rate is determined by the hot spot approach (as recommended by the melanoma Staging Committee) and expressed as the number of mitoses per square millimetre (i.e. 11 high power fields) of primary tumor. Presence or absence of ulceration is determined on Hematoxilin and Eosin sections.

Staging for regional metastatic melanoma: is performed by routine physical examination, including palpation of the lymph nodes and ultrasound examination of the lymph node regions. Ultrasound of the drainage region with FNAC for cytological evaluation will be performed in patients with suspicious nodes by ultrasound. In case of lymph node metastasis PET-CT and brain MRI is performed to exclude general metastatic loci, and patients undergo a complete lymphadenectomy.

Patients with negative nodes (clinically and by ultrasound) but with primary tumor pathology at intermediate or high risk for tumor metastasis (Breslow > 1 mm or ≤ 1 mm but with ulceration and/ or mitosis ≥1/mm^2), are candidate for lymphatic mapping with sentinel lymph node biopsy. When the sentinel node is positive these patients will have a PET-CT and brain MRI to exclude general metastatic loci, and undergo a complete lymphadenectomy.

Nodal micrometastasis are detected by routine H&E staining, supplemented by immunohistochemistry with a cocktail of antibodies directed against Melan-A, tyrosinase and gp100 (HMB45). Processing of the sentinel node and reporting of the results will be done according to EORTC guidelines. Stage IB-III patients are eligible for the vitamin D supplementation study if the only treatment is surgery. (Mohr et al, 2009).

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4.3.3. Follow-up for relapse

Patients are taught the process of self-examination; Follow-up intervals are three-monthly. Since up to 70% of the first recurrence is found in the loco regional area, follow up visits will consist of routine physical examination, including full skin assessment and palpation of regional lymph nodes. Ultrasound of the lymph nodes will be performed in all patients at an interval of 6 months, or in case of palpable lymph nodes. Ultrasound of draining lymph nodes by experienced radiologists has higher sensitivity to detect relapse compared with clinical examination. (PET)CT to exclude distant metastasis and MRI to exclude brain metastasis will be performed in patients with new symptoms and physical findings. Routinely PET(CT) and MRI are not used in the follow up period, since there is lack of prospective studies proving the efficacy of these expensive imaging techniques in routine follow up. Since both arms in the study are followed identically, the primary endpoint of the study will not be hampered by this less invasive and aggressive follow-up approach. (Mohr et al, 2009) (Garbe et al, 2012).

4.3.4. Clinical laboratory

Blood sampling and sample analysis is performed at the clinical centre. The local investigator is responsible for proper judgment of abnormal blood test results, and is responsible to appropriate patient care following clinically relevant pathological results. Please refer to section 6.4.4 to determine whether an abnormal laboratory value may constitute an adverse event.

The following analysis is to be obtained:

Screening and end of study:
- Hematology: hemoglobin, hematocrit, WBC count with differential, RBC count and platelet count
- Biochemistry and enzymes: serum creatinine, serum calcium, serum phosphate, AF, ALT, gamma GT, LDH

Randomisation (Day 0):
- Baseline serum vitamin D level.
- Bloodsample for DNA analysis

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Every 3 months:
- serum calcium
- serum phosphorus
- 25(OH)D3 serum levels, in case of drug interruption when serum 25(OH)D3 levels are > 80 ng/ml

Every 6 months:
- 25(OH)D3 serum levels
- WBC plus differential

End of study: 24 hrs urine calcium

SNP analysis will be performed by the laboratory of Professor Lambrechts, VIB
Peripheral blood from study participants will be sampled in K2EDTA tubes.
The samples are coded and only then transferred to VIB. So VIB only has coded information from the patients. Subsequently, tubes will be transferred to the Vesalius Research Center (Leuven) and genomic DNA will be extracted by a standard salting out method and stored at -80 °C until further analysis. Genetic variants affecting VD levels will be genotyped in a blinded manner using iPLEX technology on a MALDI-TOF based MassARRAY Compact Analyser (Sequenom Inc., CA, USA). The Sequenom's iPLEX technology efficiently multiplexes up to 32 different SNP's in one PCR reaction, thereby providing rapid and accurate SNP genotypes at an affordable price. Only samples for which ≥ 80% SNPs have succeeded will be considered for further analysis. SNPs with a call rate < 98% will be re-designed and genotyped again to reach success rates close to 100%. At least 5% of the samples will be genotyped in duplicate, to prove accuracy of the genotyping protocol. We expect that approximately 2 Sequenom assays will have to be applied on the DNA of participating individuals to cover all 19 SNPs. Multiple Single Nucleotide Polymorphisms (SNPs) have been reported to affect VD signaling.
Following SNPs of the VDR will be determined: rs2228570 (Fok1), rs731236 (Taq1), rs1544410 (Bsm1), rs7975232 (Apa1), rs757343 (Tru91). Following 14 SNP's of the VD pathway genes will be determined: rs12785878 (DHCR7 gene), rs10741657 (CYP2R1 gene), rs59443548, rs1803361 rs2229381, rs11559242, rs41272685, rs41272687 (6 SNP's of CYP27A1 gene), rs10877012 (CYP27B1), rs2296241, rs2248359, rs6013897 (CYP24A1), rs4588, rs7041 (2 SNPs for DPB gene). The SNP analysis will be in collaboration with the laboratory of Prof. Lambrechts.

4.3.5. Concomitant medications

All patients participating in the trial should receive treatment according to evidence-based guidelines. Concomitant medications are recorded throughout the study.

4.3.6. Safety

- serum calcium, phosphorus, to detect for vitamin D intoxication (every 3 months)

- measurement of total WBC and differential (every 6 months) and a record of infection rate (every 3 months) to check for immunosuppressive effects

Adverse clinical events

All serious events will be reported to the Leuven coordinating Centre. See also section 6.4.

4.3.7. Data Safety Monitoring Board (DSMB)

All safety parameters and adverse events will be reviewed by a DSMB. The DSMB is unblinded and composed of medical experts not involved in the study: a dermatologist, oncologist and statistician. They will assess the safety of patients based on the safety parameters and adverse events. In particular, at regular intervals an unblinded interim analysis will be performed to assess the difference between the intervention arms and to exclude an increase in relapse rate in de vitamin D supplemented arm. No early stopping for efficacy will be allowed. Details of the DSMB responsibilities will be documented in a charter.

4.3.8. Study duration for study patients

Each enrolled patient will remain in the study throughout the entire study duration, with a maximum study follow-up of 3.5 years for each patient. A study patient's participation may be terminated early because of relapse/progression which is the main study end point or due to a reasonable cause, such as the investigator's medical decision. At any time, the patient has the right to withdraw consent without a negative impact on her/his medical treatment.

4.3.9. Duration of the whole study

Our power calculations are based on a patient enrolment rate of 500 melanoma patients during a recruitment phase of three years maximum. Concerning the enrolment period: the first patient will be recruited as a fall 2012 and the last patient in fall 2023. In orde to meet those targets, a multiple center study is necessary, via participation of other academic centers.

The DSMB may terminate the study earlier based on safety concerns at any time. The sponsor may also terminate the study early for reasonable cause. Competent authorities/ethics committees retain the right for premature termination of the study according to applicable regulations. The study may also be terminated early if the work performed is not compliant with Good Clinical Practice.

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1. D-Cure® (cholecalciferol)

5.1.1. General information

- Name investigational product: D-Cure® (amp. cholecalciferol 100.000 U.I./ml)

  Content of 1 ml amp.: Cholecalciferol 100.000 U.I./ml -DL α-Tocopherol. acetas –

  Sorbitol. oleic. polyoxyaethylenat. - Aetherol. aurantii corticis dulcis - olie van olijfolie ad 1 ml

- Administration route: orally
- General properties: clear, (light) yellow oil

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* D-Cure® amp. 100.000 U.I/ml

Licence number: BE436073

Licence holder: Laboratoires SMB NV, Herdersliedstraat 26-28, 1080 Brussel

Manufactured by SMB Technology S.A., 39 rue du Parc Industriel, Marche-en-Famenne

- Quality control tests for + specifications:

Since D-Cure® is a licenced drug, all quality control tests are performed by Laboratoires SMB.

For more information about D-Cure®: see the scientific package leaflet in attachment.

* D-Cure® ampoules 100.000 U.I/ml are delivered in a blinded manner by Laboratoires SMB NV.

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5.1.2. Labelling

- Local Pharmacy labels the D-Cure® ampoules
  - Example of label

Protocol: ViDMestudy Investigator: of local academic center
Vitamin D 100.000 IU vs placebo
Pro ampoules 1ml
Lot: ….. Expiration date: …….
Store at roomtemperature
Exclusively for clinical research

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5.1.3 Storage

D-Cure® (oral ampoules) will be stored at room temperature, below 25 °C. Temperature (minimum and maximum temperature) is recorded electronically every working day.

5.1.4 Transfer

After release by a responsible person, the IMP's can be transferred to the investigator, using a signed transfer document. IMP will not be transferred to another site.

5.1.5. Use, accountability and destruction

Pharmacists of the University Hospitals Leuven, who are independent from the clinical study team, will randomly assign participants by using a computer-generated randomization list and prepare the study medication. A diary will be given to the patient for dates when to take the medication (numbered syringes) on a monthly base, at a dose of 100 000 IU (1 ml of D-cure® suspension in) or 1 ml of placebo (olive oil). Vitamin D and placebo are prepared by Laboratoires SMB NV in oral ampoules , identical in taste and appearance. Oral ampoules are numbered according the randomization schedule. At each visit 3 numbered ampoules are given to the patient, Patient will take empty ampoules back to the control visit (every 3 months). Number of ampoules and date the Vitamin D/placebo is taken are registered. Subsequently empty ampoules are destroyed

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5.2. Olive oil

5.2.1. General information

Name investigational product: Olive oil

Olive oil will be used as matching placebo for vitamin D (cholecalciferol)

Composition: Tocopherol Acetate, sweet orange peel oil, Polyglyceryl oleate, olive oil refined ad 1ml

- Administration route: orally
- General properties: clear, (light) yellow oil

Olive oil

- Quality control tests for + specifications:

Since Olive oil is manufactured by Laboratoires SMB and all quality control tests are performed by Laboratoires SMB.

Placebo ampoules are delivered in a blinded manner by Laboratoires SMB NV.

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5.2.2. Labelling

Local pharmacy labels the placebo ampoules.

Example of label

Protocol: ViDMestudy Investigator: of local academic center
Vitamin D 100.000 IU vs placebo
Pro ampoule 1ml
Lot: ….. Expiration date: …….
Store at roomtemperature
Exclusively for clinical research

5.2.3. Storage

The -Olive oil (oral ampoules) will be stored at room temperature, below 25°C. Temperature (minimum and maximum temperature) is recorded electronically every working day.

5.2.4. Transfer

After release by a responsible person, the IMP's can be transferred to the principal investigator of each site, using a signed transfer document. IMP will not be transferred to another site.

5.2.5 Use, accountability and destruction

See active product

6. ADVERSE EVENTS AND CLINICAL EVENTS

6.1. Definitions

6.1.1. Adverse event

An adverse event (AE) is defined as any untoward medical occurrence in a patient administered an investigational medicinal product and which does not necessarily have a causal relationship with this treatment.

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6.1.2. Serious and non-serious adverse events

All AEs fall into either the category “non-serious” or “serious”. A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event

A non-serious adverse event is any untoward medical occurrence that does not fulfil any of the criteria listed above.

Adverse events with respect to abnormal assay results and precautionary safety procedures to be taken:

- Clinical significant hypercalcemia: defined as 2 independent measurements (2 different laboratories), above the reference value; action taken: end of study

- HypervitaminoseD: defined as 25(OH)D3 > 80 ng/ml; action taken: discontinue study drug and restart monthly treatment with study drug when 25(OH)D3 falls below 50 ng/ml on 3 monthly monotoring .

- Hypovitaminose D:

  * Serum 25(OH)D3 < 10 ng/ml at baseline; action taken: supplementation of extra vitamin (D-cure): 25000IU / month,

  * Serum 25(OH)D3 < 10 ng/ml at 6 months or later; action taken: end-of-study

  * Serum 25(OH)D3 > 10 ng/ml and < 20 ng/ml at baseline: supplementation of extra vitaminD (D-cure): 25000IU/month

  * Serum 25(OH)D3 > 10 ng/ml and < 20 ng/ml at 6 months or later: supplementation of extra vitaminD (D-cure): 25000IU/month

- Hypophosphatemiae: < 1.5 mg/dl: further investigation

- Hyperphosphatemia: > 6mg/dl: further investigation

Clarification of the difference in meaning between “severe” and “serious”

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The term “severe” is often used to describe the intensity (severity) of a specific event (see next section below); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

6.1.3. Severity assessment

The investigator will use the following terms to assess the severity of the adverse event:

Mild:	Causing no limitation of usual activities; the patient may experience slight discomfort
Moderate:	Causing some limitation of usual activities; the patient may experience annoying discomfort
Severe:	Causing inability to carry out usual activities; the patient may experience intolerable discomfort or pain

6.1.4. Causality assessment

The investigator will use the following causality terms to assess the relationship of the adverse event or clinical efficacy event to the use of the investigational medicinal product:

Reasonable causal relationship with the investigational medicinal product:	There is evidence or argument to suggest a causal relationship with the investigational medicinal product administered
No reasonable causal relationship with the investigational medicinal product:	There is no evidence or argument to suggest a causal relationship with the investigational medicinal product administered

Similarly, the investigator will assess the relationship of the AE/CE to the therapy associated interventions.

6.2. Clinical handling

During and after a patient's participation in the trial, the investigator needs to ensure that adequate medical care is provided to a patient for any AE, including clinically significant laboratory values, or CE. The investigator should inform a patient when medical care is needed for concurrent illness of which the investigator becomes aware.

All SAEs that occur in the course of a clinical study regardless of the causal relationship must be monitored and followed up until the outcome is known. There

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must be documented reasonable attempts to get this information. It is the responsibility of the investigator to ensure that any necessary additional therapeutic measures and follow-up procedures are performed. The clinical course of the AE/CE will be followed up according to accepted standards of medical practice, even after the end of the period of observation until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up.

6.3. Collection period

In this study, the collection period for serious adverse events starts at randomisation and ends when the patient completes the study or at premature termination.

6.4. Documentation and reporting

6.4.1. Documentation of SAEs

The investigator must document all SAEs that occur during the observation period in the SAE module provided in the eCRF. Every attempt should be made to describe all SAEs in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms should not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, signs and symptoms must be recorded individually.

6.4.2. Documentation and reporting of SAEs

If the AE is serious, the investigator must complete an “eSAE Report Form” at the time the SAE is detected. This form must be provided immediately, i.e. ***within one working day*** upon becoming aware of the SAE to the SAE processing centre.

The SAE initial report should be as complete as possible, including details of the current illness and the serious AE, the reason why the event was considered serious, date of onset and stop date (if applicable), diagnostic procedures and treatment of the event, relevant medical history and concomitant medication and action taken with investigational medicinal product (if applicable).

Information not available at the time of the initial report (e.g., an end date for the SAE or laboratory values received after the report) must be documented and reported as a follow up. Should the SAE result in death, a full pathologist’s report should be supplied, if possible.

The SAE processing centre will identify missing information for each reported SAE. Requests for follow up will be sent for further processing to the investigator. Follow up information are required at regular intervals from the investigators until all queries are resolved or no further information can be reasonably expected. All responses to queries

and supply of additional information by the investigator should follow the same reporting route as the initial report and must be sent without delay.

6.4.3. Reporting of serious adverse events to competent authorities and concerned ethics committees

A SUSAR (suspected unexpected serious adverse reaction) is an AE that is:

- serious and
- unexpected (meaning that nature or severity of the AE is not consistent with the IMP reference safety information, which is the Investigator's Brochure) and
- is judged by either the investigator or the sponsor as having a reasonable suspected causal relationship with the investigational medicinal product.

SUSARs are subject to expedited reporting. The sponsor reports all SUSARs to the Competent Authority and the concerned Ethic Committee according to applicable regulations and timelines.

The sponsor also prepares and submits annual safety reports to Competent Authority and Ethics Committee.

6.4.4. Documentation of abnormal laboratory values

Abnormal laboratory parameters per se will not be recorded as adverse events. They will only be reported as AEs if the deviation from the normal range is assessed as "clinically significant" by the investigator and/or when they result in precautionary safety measures. All other abnormal laboratory values will be documented and will only be considered within the context of an event which constitutes an AE.

The following laboratory values will be reported as adverse events:

Hypercalcemia: defined as 2 independent measurements (2 different laboratories) above the reference value.

Hypervitaminose D: defined as serum 25(OH)D3 level > 80 ng/ml

Hypovitaminose D:

Deficient: serum 25(OH)D3 < 10 ng/ml

Insufficient: serum 25(OH)D3 >10 ng/ml and < 20 ng/ml

- Hypophosphatemiae: < 1.5mg/dl
- Hyperphosphatemia: > 6mg/dl

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6.4.5. Documentation and reporting of IMP exposure during pregnancy or lactation

In principle, pregnancy and the lactation period are exclusion criteria. In the event of a pregnancy occurring during the course of this study, the study should be ended and the patient should be closely followed-up during the entire course of the pregnancy and postpartum period. When a pregnancy is detected without an adverse outcome, the investigator should complete a report and send this to the SAE processing centre without delay. It should be clearly stated that *no AE* was observed. In this case, there is no need to complete the AE module in the eCRF. Parental and neonatal outcomes must be recorded even if they are completely normal and without AEs. Off-spring should be followed up for at least 8 weeks after delivery. All serious or non-serious adverse events associated with the pregnancy will be handled as described in the previous sections

7. STATISTICAL METHODS

7.1. Sample Size

Approximately 500 patients for the treatment group and for the control group are required. Assuming similar times to relapse as retrospectively observed at the University hospital Leuven, 3 year of recruitment and a total study duration of 3.5 years, 500 patients will have 90% power to detect a hazard ratio of 0.40 in favour of the vitamin D supplemented arm by means of Cox proportional hazards model stratified for time since diagnosis (3 strata: 0, 6 or 12 months ago). The hazard ratio of 0.40 is in correspondence with the intermediate effect size that was reported by Newton-Bishop et al (2009) and a potential increase in VD serum levels of 70 nmol/L which is 80% of the observed effect in a study with CPOD patients (Lehouck et al, 2012). It is anticipated that no patient will be lost for follow-up.

7.2. Statistical Methods

The primary analysis is an intent-to treat analysis of all randomised patients. Patients will be analysed according to the treatment group to which they were randomised, irrespective of which study drug was given or if any study drug was received. Additional populations like a per protocol population may be defined in the statistical analysis plan

The difference in relapse-free survival will be compared between the two treatment groups by means of a Cox proportional hazards model stratified for time since diagnosis (3 strata: 0, 6 or 12 months ago). It will be verified whether the treatment effect is stratum dependent. If so, the strata will be analysed separately. Furthermore, using a Cox proportional hazards model it will be investigated whether the potential treatment effect is different for VDR immunoreactivity and genetic variability in the Vitamin D pathway. A correction for other baseline covariates like gender, age and

baseline Vitamin D level will be performed as sensitivity analyses. No interim analysis for efficacy is foreseen. Potential subgroups analysis like for instance calcium serum levels will be defined in the statistical analysis plan

The evolution in 25(OH)D3 levels in function of genetic variability in the Vitamin D pathway will be analysed by a linear mixed model. Sensitivity analyses will include patient's compliance, seasonal effects and patient's baseline characteristics like age and gender.

In order to examine the effect of the actual 25(OH)D3 levels rather than just supplementation, the evolution of 25(OH)D3 levels and relapse-free survival will be jointly modelled.

The potential correlation between VDR immunoreactivity and stage at diagnosis will be assessed by means of a Kruskal Wallice test.

The potential correlation between vitamin D levels at diagnosis with melanoma site and melanoma subtype will be assessed by means of an analysis of variance. This analysis can only be performed for newly diagnosed patients because vitamin D levels at diagnosis will not be available for patients who had their diagnosis 6 or 12 months ago.

Full details of the statistical analyses will be described in a statistical analysis plan which will be finalized before database lock.

8. ETHICAL, ADMINISTRATIVE AND LEGAL ASPECTS

8.1. Ethical conduct of the study and informed consent

The study is conducted according to the protocol and in strict compliance with ICH GCP, the Declaration of Helsinki and all applicable regulatory requirements.

Before the start of the study, the study protocol, the investigator brochure and other applicable documents are submitted to an independent Ethics Committee (EC) and responsible national and local authority, as required by the participating country regulation. The study can start only after a favourable EC opinion and a regulatory authorisation has been obtained. The sponsor informs the investigator in writing that all ethical and legal requirements have been met before the first patient is enrolled in the study. After the protocol has been accepted, substantial amendments to this protocol require the corresponding assessment processes by competent authorities and/or ECs as described above. After the end of the study, a final study report is prepared and distributed to regulatory authorities and ECs as required by applicable regulations.

Before a patient can participate in the study, patient's informed consent needs to be obtained according to GCP and the legal requirements of the country concerned. Patient information sheet and consent form must have been reviewed and approved by

the responsible EC. The investigator or an authorised designate explains the nature, purpose, scope and course of the study, including information on the investigational medicinal product, potential benefits and risks to the patient. In addition to oral information, the patient receives a written patient information sheet containing all relevant information. Sufficient time will be allowed to discuss any questions raised. Only after this process is completed, consent for participation may be given. Consent must be obtained prior to any study specific procedure and with sufficient time before a study related intervention as per local requirements. The consent form must be personally signed and dated by the individual giving consent and by the investigator or designee who lead the informed consent process with the patient. The consent form must be retained by the investigator as part of the study records. In addition, the patient receives a copy of the patient information sheet and a copy of his/her signed and dated consent form. Confirmation that consent was obtained is also documented in the medical records and on the eCRF. Should a protocol amendment be made, the patient information sheet may need to be revised to reflect the change(s) of the protocol. After the EC has approved the revised information sheet and consent form, it is the responsibility of the investigator to inform all active patients affected by the change, and to receive their written consent for continuation in the study.

All patients must be identifiable throughout the study at the study site. The investigator will maintain a personal list of patient numbers and patient names for data reconciliation.

8.2. Study-specific committees

8.2.1. Study Executive Committee

An Executive Committee is appointed. This smaller body provides a more rapid management body for day to day or operational level decision and consultation. The functions of the Executive Committee are to prepare, update, and maintain the trial protocol and corresponding forms, review performance reports of the clinical centres, resolve operational problems and to supervise data preparation, data analysis and publication of study reports.

8.2.2. Data & Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) is formed to monitor patient safety as the study progresses. The DSMB has been selected by and communicates directly to the study's Executive Committee. The DSMB is authorised to make recommendations to the Executive Committee and sponsor regarding safety issues. If a serious concern with the safety of the patients in the trial would arise, the DSMB may recommend early termination of the study. Working procedures governing the convening, execution and the responsibilities of the DSMB are specified separately in a prospectively written charter. The DSMB should initially review safety issues every

three months. The review includes, but may not be limited to, unexpected serious adverse events, mortality and complications of high dose vitamin D supplementation

8.2.3. Clinical endpoint committee

Endpoints will be reported as occurring throughout the follow up. Defined endpoints are adjudicated by an independent Clinical Endpoint Committee (CEC) blinded to the patient treatment allocation, ensuring a consistent and unbiased adjudication of events

8.3. Case report forms

All case report forms will be electronic using Open Clinica, a web-based tool, provided by LCC. The site staff will enter and edit the data via a secure network, with secure access. An electronic audit trail will be maintained. The investigator will confirm the accuracy of the data by using an electronic signature.

8.4. Quality control

8.4.1. Study monitoring and inspections

All efforts will be done to reassure maximal quality control. Study will be performed according GMP. Direct access to source data is available. Access is carried out with strict adherence to all confidentiality regulations.

8.5. Confidentiality

All local legal requirements regarding data protection must be adhered. All study documents and study findings are regarded as confidential. The confidentiality of patients participating in the study must be maintained. Throughout documentation and evaluation, the patients are identified on eCRFs and other documents that are transferred outside the study centre by their patient number. Documents identifying the patient (e.g. the signed informed consent, health records) must be maintained confidential by the investigator.

8.6. Use of records and archiving

8.6.1. Publication and use of study findings

The Parties agree that publications or presentations of any of the results from the Study shall be in accordance with accepted scientific practice, academic standards and customs.
The Parties agree that at first they will strive to make a joint publication coordinated by Sponsor. After such joint publication the following shall be agreed:
As a general principle, the Parties agree that prior to submission of a publication or any other dissemination of the results, including oral presentation, the Sponsor shall

have the right to prior review and comments on the content of the material to be published or presented. Sponsor may request, within thirty (30) days following the receipt of the publication or any other dissemination of the results, that Sponsor's confidential information would be deleted before proceeding with the publication or any dissemination of the results.
Authorship and other related publications questions shall be addressed in accordance with the principles of the 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals' and in accordance with the requirements of the respective medical journal

8.6.2. Archiving of study records

Essential documents should be retained according to the legal requirements

8.7. Insurance/Indemnity

Sponsor shall be liable, even without fault, for any damages incurred by a Study Patient and linked directly or indirectly to the participation to the Study.

Sponsor shall enter into an insurance agreement in order to cover the liability for any damages incurred by a Study Patient included in the Belgian participating site.

If an insurance coverage is required by local laws of non-Belgian Participating Sites, these Participating Sites shall have and maintain in full force and effect during the term of this Agreement (and following termination of the trial to cover any claims arising from the trial) adequate insurance coverage for possible damages linked directly or indirectly to the patients' participation to the trial at Participating Sites.

8.8. Financial Aspects

There is partial funding of the study by the Belgian agency for science and technology (Agentschap voor Innovatie door Wetenschap en Technologie – IWT), Het Anti-Kankerfonds and Kom op Tegen Kanker.

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