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The Potential of Vitamin D Supplementation to Enhance Prognosis of Colorectal Cancer Patients: Role of Vitamin D on Inflammatory Modulation.

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List of abbreviations

Abbreviation	Full form
BL	Baseline
BMI	Body Mass Index
CDCP1	CUB Domain-Containing Protein 1
CIN	Cervical Intraepithelial Neoplasia
CRC	Colorectal Cancer
CRP	C-reactive Protein
CVD	Cardiovascular Disease
CXCL	C-X-C Motif Chemokine
CSS	Colorectal Cancer Specific Survival
DFS	Disease Free Survival
FU1	Follow-Up 1 (end of loading dose)
FU2	Follow-Up 2 (end of maintenance dose and end of trial).
HRQoL	Health Related Quality of Life
ICD-10	International Classification of Diseases-10th Revision
IFN-γ	Interferon-gamma
IL	Interleukin
IQR	Interquartile Range
ITT	Intention-To-Treat
JAK/STAT3	Janus kinase/Signal Transducer and Activator of Transcription 3
KM	Kaplan-Meier
MMP-1	Matrix Metalloproteinase-1
MET-h/wk	Metabolic Equivalent Task Hours per Week
NF-kB	Nuclear factor-kappa light chain B
OS	Overall Survival
PP	Per-Protocol
PCa	Prostate cancer
PFS	Progression Free Survival
RCT	Randomised Controlled Trial
RFS	Recurrence Free Survival
SMD	Standardised Mean Difference
SNP	Single Nucleotide Polymorphism

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Abbreviation	Full form
TNF-α	Tumor necrosis factor-alpha
TNM	Tumor Node Metastasis
VDR	Vitamin D Receptor
VIDS	Vitamin D_3 Supplementation
25(OH)D	25-hydroxyvitamin D
95% CI	95% Confidence Interval

1 Introduction

1.1 Epidemiology of Colorectal Cancer

Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality worldwide, with over 1.9 million new cases and more than 900,000 deaths annually (Bray et al. 2024). This burden is expected to rise in the coming decades due to aging populations and lifestyle changes. Surgery is the primary treatment for CRC, with clinical outcomes heavily influenced by the stage at diagnosis. Despite extensive collaborative efforts and investments in primary prevention, early detection, and treatment, the 5-year relative survival rates for CRC remain suboptimal, ranging from over 90% for stage I to less than 20% for stage IV cancers (American Cancer Society 2023). Moreover, there is significant unexplained variability in disease progression among patients diagnosed at the same stage (Siegel et al. 2023; van den Berg et al. 2021). The TNM (tumor-node-metastasis) staging system is useful for predicting patient outcomes in various cancers, including CRC, but its focus on tumor-centric factors limits its applicability in personalized patient management as it neglects host-related factors that influence cancer progression. Consequently, several host-related factors beyond staging may be crucial for prognosis and, if modifiable, could offer opportunities for personalized tertiary prevention. In this context, post-surgical vitamin D status and systemic inflammatory response are emerging as key modifiable prognostic factors, potentially providing additional insights into cancer prognosis beyond the stage at diagnosis.

1.2 Post-Operative Vitamin D Status and Colorectal Cancer Prognosis

Large scale epidemiological studies indicate a high prevalence of vitamin D insufficiency or deficiency in post-surgical patients with a prior CRC diagnosis (Aguirre et al. 2016; Calmarza et al. 2018; Li et al. 2021; Maalmi et al. 2017). Additionally, lower serum levels of 25-hydroxy-vitamin D (25(OH)D), a recognized indicator of vitamin D status, are linked to increased mortality. Among CRC patients, those with vitamin D deficiency (25(OH)D <30 nmol/L) and insufficiency (25(OH)D 30 - 50 nmol/L) have a significantly poorer prognosis compared to those with sufficient vitamin D levels (25(OH)D >50 nmol/L) (Maalmi et al. 2018; Maalmi et al. 2017; Wu et al. 2020; Zgaga et al. 2014).

The physiological effects of vitamin D are mediated through vitamin D receptors (VDRs), which are widely expressed in various human tissues, including bones, the stomach, and kidneys (Chen et al. 2022b). Nevertheless, it is unclear if and to what extent the prognostic value of post-operative vitamin D status may be influenced by genetically determined VDR

function. Earlier research found no associations between VDR polymorphisms rs731236 (*Taq1*), rs2228570 (*Fok1*), rs11568820 (*Cdx2*), and rs1989969 (*VDR-5132*) and CRC survival (Perna et al. 2013). However, new evidence suggests the role of *Cdx2*, a functional polymorphism located in the promotor region of the VDR gene, in modifying the relationship between vitamin D status and CRC survival. In two extensive cohorts of CRC patients from the UK, a strong inverse relationship between 25(OH)D levels and CRC-specific survival was observed in patients with the GG genotype of *Cdx2*, but not in individuals with the AA/AG genotypes (Vaughan-Shaw et al. 2020b). These findings suggest that the prognostic significance of vitamin D status may be VDR genotype-dependent, emphasizing the need for validation in independent cohorts to identify patients who could benefit most from vitamin D supplementation for more effective, personalized interventions.

1.3 Post-Operative Systemic Inflammation and Colorectal Cancer Prognosis

The prognosis of CRC is significantly influenced by the host's inflammatory response at both the tumor micro-environment and systemic levels (Dolan et al. 2017; Rossi et al. 2017). Systemic inflammation is a prominent feature of cancer, closely associated with both tumorigenesis and tumor progression (Balkwill and Mantovani 2001; Pęczek et al. 2023; Wen et al. 2022). Pre-operative biomarkers related to inflammation, such as neutrophils, monocytes, and lymphocytes from white blood cell counts, are valuable predictors of clinical outcomes in CRC, including survival and disease recurrence following surgery (Yamamoto et al. 2021). Additionally, pre-operative inflammatory cytokines like C-reactive protein (CRP), interleukin-6 (IL-6), and the glycoprotein chitinase-3-like protein 1 (YKL-40) have prognostic significance in CRC (Dolin et al. 2023). However, since surgery and its immediate post-operative complications can significantly trigger systemic inflammation (Watt et al. 2017), the prognostic value of post-operative systemic inflammation might be more pertinent for patient monitoring in CRC management.

Recent years have witnessed growing evidence on the value of post-treatment inflammatory blood biomarkers to predict CRC outcomes (Chan et al. 2018; Gwenzi et al. 2024; Gwenzi et al. 2023b; Li et al. 2018a; Matsuoka et al. 2020; Thiagarajan et al. 2021; Yasui et al. 2021). Although anti-inflammatory drugs can reduce inflammation, nutrients with low dietary inflammation scores have shown benefits in modulating inflammation in non-critically ill patients (Kaluza et al. 2019; Ugai et al. 2022; Zitvogel et al. 2017). In this regard, the potential anti-inflammatory effects of micronutrients such as vitamin D could be of interest. The role of

vitamin D in inflammation modulation is well-documented in mechanistic and pre-clinical studies (Na et al. 2022; Pereira et al. 2024), and observational studies have reported inverse associations between serum 25(OH)D and pro-inflammatory biomarkers in CRC patients (Sha et al. 2023; Väyrynen et al. 2016). However, clinical evidence on the effects of vitamin D supplementation (VIDS) is limited and shows mixed results. Thus, given the high prevalence of vitamin D inadequacy and elevated post-operative systemic inflammation in CRC patients undergoing surgery, further clinical studies are needed to explore the potential benefits of VIDS in correcting low 25(OH)D levels and mitigating tumor-promoting inflammation. Future research should also assess the impact of post-operative inflammatory modulation by VIDS on clinical outcomes.

1.4 Vitamin D Supplementation and Systemic Inflammation in Patients with Cancer

Low serum levels of 25(OH)D have been linked to poor survival outcomes in patients with various types of cancer, including CRC (Maalmi et al. 2018), breast (Thanasitthichai et al. 2019), prostate (McGrowder et al. 2022), lung (Weinstein et al. 2022), pancreatic (Rasmussen et al. 2021), and liver cancers (Fang et al. 2020). This has led to the suggestion that VIDS might improve the prognosis of cancer patients, though evidence from randomized controlled trials (RCTs) is still limited (Chen et al. 2022b; Kanellopoulou et al. 2021). Nevertheless, several meta-analyses of RCTs have consistently shown a significant 13% reduction in cancer mortality with VIDS in older adults (Haykal et al. 2019; Keum et al. 2019; Zhang and Niu 2019). Additionally, VIDS has demonstrated benefits in reducing recurrence and improving metabolic profiles in patients with adenomas (Vahedpoor et al. 2018).

Although the precise mechanisms through which vitamin D may affect cancer outcomes remain unclear, recent studies suggest it might involve the modulation of inflammatory processes (Chen et al. 2022b; Dolin et al. 2023; Zhan et al. 2024). Inflammatory markers are associated with tumor growth, higher tumor grade, and increased mortality in cancer patients (Marques et al. 2021; Seidu et al. 2020). Therefore, it is plausible that VIDS could serve as a supportive therapy to enhance cancer outcomes by modulating inflammation. Despite the conflicting evidence from RCTs, which often involve smaller patient cohorts, VIDS has shown significant effects in reducing serum IL-6 and tumor necrosis factor-alpha (TNF-α) in patients with breast cancer (El-Bassiouny et al. 2022; Mohseni et al. 2017; Naderi et al. 2022). A comprehensive systematic review and evaluation of clinical trial data is needed to determine the effects of VIDS on serum inflammatory biomarkers in patients with cancer.

1.5 Vitamin D Supplementation and Post-Operative Systemic Inflammation in Colorectal Cancer Patients

Although routine clinical assessment of vitamin D deficiency is not yet standard practice in managing CRC patients, there is increasing advocacy for screening and normalizing 25(OH)D levels through supplementation to potentially improve prognosis (Grant 2020). Despite the limited evidence from RCTs, a recent meta-analysis revealed a significant 35% reduction in all-cause mortality among CRC patients receiving VIDS (Vaughan-Shaw et al. 2020a). Furthermore, VIDS has been linked to potential benefits such as enhancing chemotherapy efficacy, reducing chemotherapy-induced side effects (Peng et al. 2020), and improving health-related quality of life (HRQoL) in CRC patients (Martínez-Alonso et al. 2016). These results are particularly noteworthy as they come from studies where VIDS was provided regardless of the patients' initial vitamin D status and other factors that might influence the effectiveness of supplementation.

Calcitriol, the most active form of vitamin D, functions through VDRs present in various tissues (Chen et al. 2022b). Clinical evidence on the effects of VIDS on systemic inflammatory response among CRC patients is limited. Although the effects were not statistically significant, a previous RCT showed reduction in serum levels of TNF-α, IL-6, and CRP by weekly 50,000IU of VIDS among stage II and III CRC patients (Haidari et al. 2020). However, the true effects of VIDS might have been underestimated due to methodological issues, such as administering uniform VIDS doses without considering crucial factors like baseline vitamin D status, body mass index (BMI), and dosage regimen (bolus vs. daily) (Brenner 2023). The benefits of VIDS could be maximized through personalized interventions tailored to the individual needs of CRC patients. Supplementation appears most beneficial for those with vitamin D deficiency (Brenner et al. 2017), suggesting that targeted VIDS aiming to achieve and maintain adequate 25(OH)D levels may be most effective (Ross et al. 2011).

1.6 Aims of the dissertation

The relationship between post-operative vitamin D status, systemic inflammation, and CRC prognosis requires further study. This dissertation investigates the potential of VIDS to improve CRC patient outcomes by modulating systemic inflammation. Key objectives include:

1. Assessing whether the prognostic role of post-operative vitamin D status on long-term CRC survival outcomes is influenced by the VDR *Cdx2* genotype in a large cohort.

2. Conducting a systematic review and meta-analysis of RCTs to evaluate the impact of VIDS on systemic inflammatory biomarkers in cancer or pre-cancerous patients.

3. Evaluating the effects of personalized VIDS on post-operative systemic inflammatory biomarkers in CRC patients with low vitamin D status through a randomized placebo-controlled trial.

The findings from this research could significantly impact CRC management. Given the high prevalence of vitamin D inadequacy among operable CRC patients and its link to poor clinical outcomes, routine screening and correction of vitamin D levels in clinical settings may be beneficial. Beyond its known benefits for bone and muscle health, VIDS may serve as a supportive anti-inflammatory therapy post-surgery. VIDS is also potentially cost-effective due to its safety, affordability, and availability. By elucidating the connections between vitamin D, systemic inflammation, and CRC prognosis, this dissertation could inform the development of new therapeutic and tertiary prevention strategies to enhance patient outcomes.

2 Materials and Methods

2.1 Vitamin D Status, *Cdx2* Genotype, and Colorectal Cancer Survival: Population-Based Patient Cohort.

The physiological effects of vitamin D are mediated through VDRs, which are widely expressed in various human tissues. However, as already highlighted before, it is unclear to what extent the prognostic value of post-operative vitamin D status is influenced by genetically determined VDR *Cdx2* function. Therefore, this study aimed to thoroughly examine the individual and combined associations of serum 25(OH)D levels and VDR *Cdx2* polymorphisms with various survival outcomes in a large cohort of CRC patients to identify those who may benefit most from VIDS.

2.1.1 Study details

In this project, I utilized data and serum samples from the DACHS study ["***Da****rmkrebs: **Ch**ancen der Verhütung durch **S**creening" (Colorectal Cancer: Chances of Prevention by Screening*")], a population-based case-control study with long-term follow-up of patients who had a first diagnosis of CRC, recruited in south-west Germany between 2003 and 2021. The DACHS study adheres to the Declaration of Helsinki guidelines and was approved by the state medical boards of Baden-Württemberg and Rhineland-Palatinate, as well as the University of Heidelberg ethics committees (ethical code: 310/2001, approved on December 06, 2001). All participants provided written informed consent.

Details of the DACHS study have been documented previously (Brenner et al. 2014; Brenner et al. 2011; Carr et al. 2016; Maalmi et al. 2017; Walter et al. 2016). Briefly, eligible patients were identified from 22 participating clinics based on a first diagnosis of CRC (ICD-10 codes C18–C20). Patients were informed about the study either shortly before or after surgery by clinicians or via mail after discharge. Trained interviewers conducted personal interviews using standardized questionnaires to gather sociodemographic, lifestyle, and medical information. Medical data on tumor stage, location, and therapy were obtained from hospital records. Blood samples were collected post-interview, and serum aliquots were stored at -80°C. Participants were followed up on therapy and health outcomes at 3, 5, and 10 years after CRC diagnosis. Vital status data were sourced from population registries, while cause of death information was obtained from health authorities. Recurrence and treatment details were collected using standardized follow-up questionnaires.

For the current study, 2819 patients with incident CRC, along with available serum 25(OH)D measurements and *Cdx2* genetic polymorphism data, were included. These patients were recruited from 2003 to 2010 and followed up for a median period of approximately 10 years (**Figure 1**).

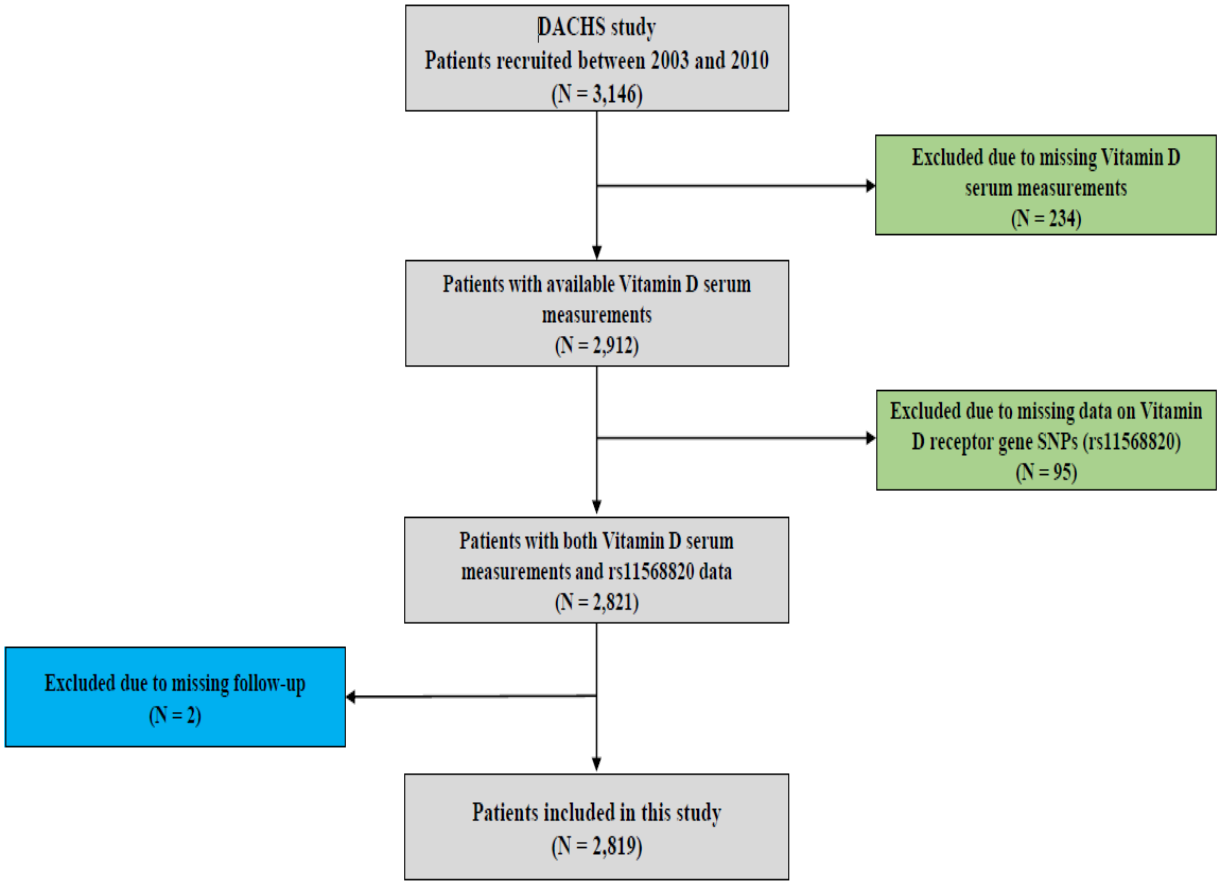


Figure 1. Patient selection flow chart: DACHS study.

2.1.2 Serum vitamin D measurements

Serum 25(OH)D levels were measured at the German Cancer Research Center using High Performance Liquid Chromatography-Electro Spray Ionization-Mass Spectrometry. This method was standardized with the Standard Reference Material 972a provided by the National Institute of Standards and Technology (Phinney 2008). Vitamin D status was classified based on serum 25(OH)D concentrations according to the United States-American Institute of Medicine guidelines: deficient (< 30 nmol/L), insufficient (30 to < 50 nmol/L), and sufficient (≥ 50 nmol/L) (Ross et al. 2011).

2.1.3 Genotyping for *Cdx2*

The process of identifying VDR gene single-nucleotide polymorphisms (SNPs) for this study has been detailed in previous publications (Chen et al. 2022a; Guo et al. 2023; Perna et al.

2013). Briefly, DNA was extracted from blood samples or, when blood samples were unavailable, from buccal swab samples using standard techniques (Gupta et al. 2020). Genotyping was performed using Illumina array technologies (San Diego, California, USA). PLINK (version 1.9) was utilized to extract *Cdx2* SNP genotypes AA, AG, and GG. For the analyses, *Cdx2* genotypes were categorized into a binary variable: the rarer variants AA and AG were grouped together, and GG was considered as the other category.

2.1.4 Outcomes

Survival outcomes were defined as follows: overall survival (OS) was measured as death from any cause, CRC-specific survival (CSS) as death specifically from CRC, recurrence-free survival (RFS) as recurrence of or death from CRC, and disease-free survival (DFS) as recurrence of CRC or death from any cause. Follow-up times for these survival endpoints were calculated in days from the date of blood sample collection to the date of the event occurrence. Patients were censored at the last known date they were alive or free of recurrence if they did not experience a specific endpoint.

2.1.5 Statistical analyses

I used descriptive statistics to analyze the characteristics of the study population. For survival analysis, Cox regression models were employed to calculate hazard ratios (HRs) for the individual and combined associations of predictors [serum 25(OH)D levels and *Cdx2* genetic variants] with survival outcomes (OS, CSS, RFS, and DFS). For combined associations, analyses were stratified by *Cdx2* as a binary variable. Two adjustment models were used to evaluate the predictor-outcome associations. Model 1 adjusted for sex (male/female), age (30–59/60–69/70–79/>80 years), and season of blood collection (winter, spring, summer, autumn). Model 2 included additional adjustments for tumor detection mode (screening/other), cancer site (colon/rectum) and stage (I-IV) at diagnosis, chemotherapy use (yes/no), surgery (yes/no), history of cardiovascular disease (CVD) (yes/no), diabetes (yes/no), hypertension (yes/no), lifetime smoking exposure (never/<10/10-19/20-29/≥30 pack-years), BMI (normal/overweight/obese), physical activity (quartiles of average lifetime Metabolic Equivalent of Task hours per week), and time between diagnosis and blood collection (<1 month/≥1 month). Additionally, I assessed interactions between 25(OH)D as a continuous variable and *Cdx2* as a categorical variable concerning survival by including their product terms in model 2.

For Cox regression model diagnostics, interactions between time and covariates were evaluated. Interactions between predictors and covariates were examined by adding product terms to the regression models and analyzing the corresponding Wald test statistics. Survival outcomes based on serum 25(OH)D status and *Cdx2* genotype were also assessed and presented as Kaplan-Meier (KM) survival curves. All statistical analyses were conducted using R statistical software (version 4.2), with two-sided significance levels set at p-values < 0.05.

2.2 Effects of vitamin D supplementation on inflammatory response in patients with cancer and precancerous lesions: Systematic review and meta-analysis of randomized trials.

The understanding of how vitamin D may modulate the inflammatory response primarily comes from pre-clinical studies, with clinical evidence being limited and sometimes contradictory. Thus, the objective of this study was to systematically search for, review, appraise, and conduct a meta-analysis of the existing evidence from published RCTs on the effects of VIDS on serum inflammatory biomarkers in patients with cancer or pre-cancerous lesions.

2.2.1 Study details

The protocol for this systematic review was registered in the International Prospective Register of Systematic Reviews prior to data extraction (PROSPERO, registration no. CRD42022295694). The systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al. 2021).

2.2.2 Search strategy and data extraction

The focus of this review was on original RCTs involving patients with cancer or precancerous lesions where the intervention included VIDS, with or without additional interventions. I excluded observational studies, unpublished studies, abstracts, reviews, dissertations, theses, editorials, study protocols, clinical guidelines, commentaries, and letters. Studies were included in the meta-analyses if they reported follow-up means and corresponding standard deviations of inflammatory serum biomarkers for both the intervention and control groups.

Systematic searches were conducted using Medline (PubMed interface), the Cochrane Central Register of Controlled Trials (CENTRAL), and ISI Web of Science databases from inception

until November 2022. Studies were screened for inclusion in the review. The study identification and selection process is illustrated in **Figure 2**, and the search strings are detailed in **Table 1**. Medical subject headings (MeSH), free-text words, synonyms, and related terms for concepts such as "vitamin D supplementation," "cancer," "adenoma," "inflammatory biomarker," and "randomized controlled trial" were used in database searches. There were no time restrictions on the searches, but non-English publications were excluded.

EndNote software version 9 was used for reference management. Data extracted from included studies using a standardized form included: first author, publication year, country, number of participants, cancer site and stage, sex, VIDS dosage, mean baseline serum concentration of 25(OH)D, compliance rate, outcome biomarker under investigation, mean/standard deviation of serum biomarker levels at follow-up for intervention and control groups, and maximum follow-up time. For studies that did not report any of the predefined data domains, I contacted the corresponding authors to request the missing details.

2.2.3 Assessment of study quality

I employed the Cochrane risk-of-bias (CRoB 2) tool (Higgins et al. 2011) to evaluate the quality of the included randomized trials. This assessment covered various domains such as completeness of outcome data, blinding, sequence generation, allocation concealment, and selective outcome reporting. Additionally, I assessed the risk of bias and categorized it as low, high, or uncertain based on the extracted data.

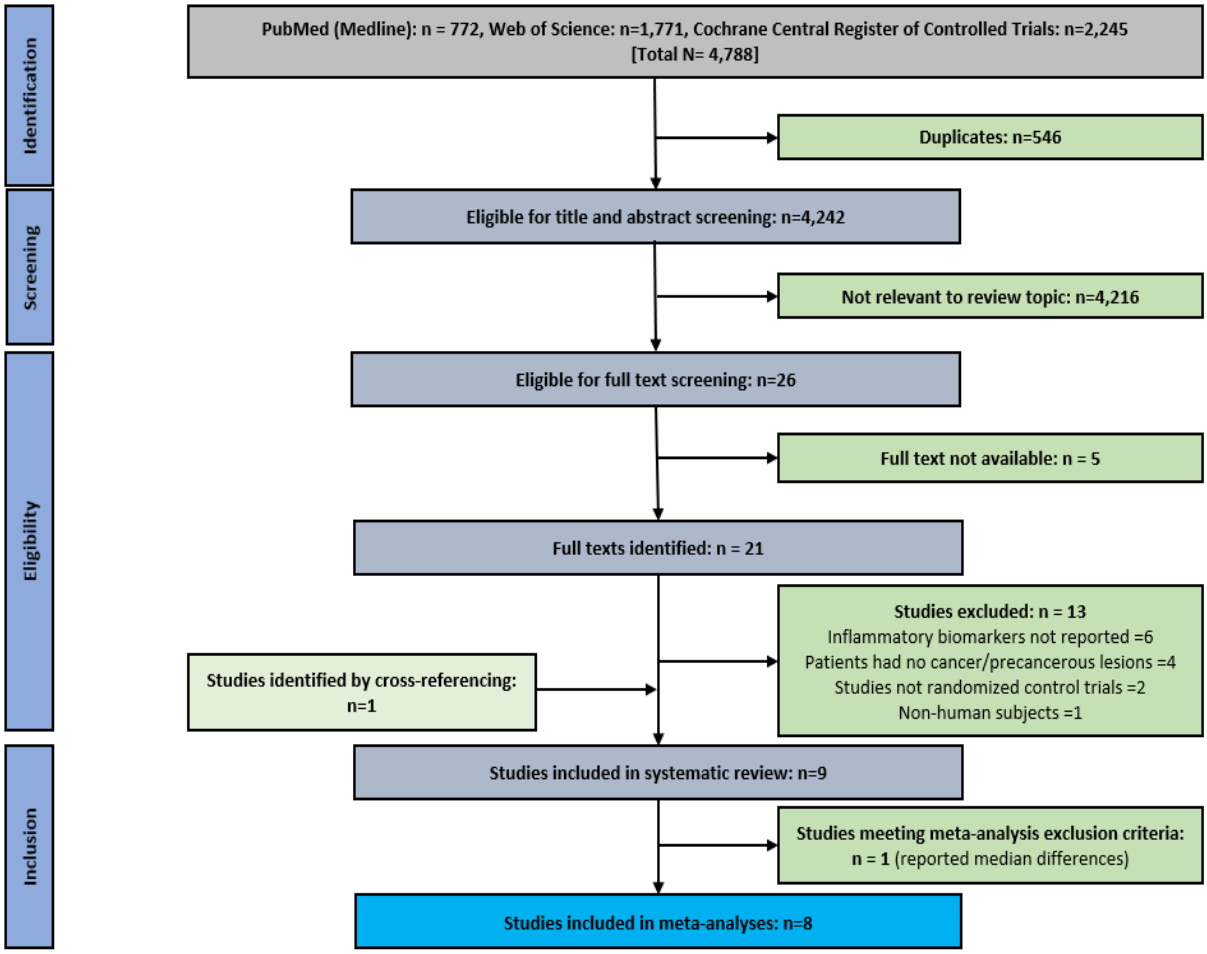


Figure 2. PRISMA Flow Diagram.

2.2.4 Statistical analyses

Serum inflammatory biomarker levels were reported in various units across different RCTs. To standardize the comparison, I used standardized mean differences (SMDs) between intervention and control groups for biomarker levels at follow-up in the meta-analyses. Effect sizes were categorized as large (SMD > 0.7), moderate (SMD 0.4 - 0.7), or small (SMD < 0.4) (Higgins et al. 2011). I summarized the SMDs with their 95% CIs and displayed the results in forest plots. Meta-analyses were not performed if fewer than two studies were available for a specific biomarker.

Table 1. PubMed, Web of Science and Cochrane CENTRAL database search strings (from inception until 30.11.2023).

Database		Search string	Hits
PubMed	1.	"neoplasms"[MeSH Terms] OR "adenoma"[MeSH Terms] OR "carcinoma"[MeSH Terms] OR "cancer"[Title/Abstract] OR "neoplas*"[Text Word] OR "malignanc*"[Text Word] OR "tumor*"[Text Word] OR "tumour*"[Text Word]	4,991,732
	2.	"cholecalciferol"[MeSH Terms] OR "calcitriol"[MeSH Terms] OR "calcifediol"[MeSH Terms] OR "alfacalcidol"[Text Word] OR "vitamin d"[Text Word] OR "vitamin d3"[Text Word] OR "supplement*"[Title/Abstract]	479,887
	3.	"biomarkers"[MeSH Terms] OR "inflammation"[MeSH Terms]	1,231,431
	4.	"placebos"[MeSH Terms] OR "placebo"[Text Word] OR "control"[Text Word] OR "randomized"[Title/Abstract] OR "randomized controlled trial"[Publication Type]	4,963,508
	5.	clinicalstudy [Filter] OR randomizedcontrolledtrial [Filter]	1,105,947
	6.	**#1 (population) AND #2 (intervention) AND #3 (outcome) AND #4 (study design) AND #5 (study design 2)**	**772**
Web of Science	1.	AB=(neoplasms) OR ALL=(adenoma) OR ALL=(tumor) OR ALL=(carcinomas) OR TI=(cancer) OR ALL=(malignan*)	3,737,814
	2.	TI= (vitamin d) OR TI=(supplement*) OR AB=(vitamin) OR ALL=(cholecalciferol) OR ALL=(calcitriol) OR ALL=(calcifediol) OR ALL=(alfacalcifediol) OR ALL=(vitamin d3)	303,768
	3.	AB=(biomarkers) OR AB=(inflammat*)	1,105,330
	4.	ALL=(randomized controlled trial) OR ALL=(placebo) OR ALL=(random*) OR ALL=(control)	7,591,194
	5.	**#1 (population) AND #2 (intervention) AND #3 (outcome) AND #4 (study design)**	**1,771**
Cochrane (CENTRAL)	1.	(neoplasms):ab OR (cancer):ti,ab,kw OR (adenoma) OR (tumor) OR (carcinomas OR malignan*)	240,592
	2.	(vitamin d OR supplement*):ti,ab,kw OR (vitamin):ab OR (cholecalciferol OR calcitriol OR calcifediol OR alfacalcifediol OR vitamin d3)	93,957
	3.	(biomarkers OR inflammat*):ab	103,892
	4.	(randomized controlled trial OR placebo OR random* OR control)	1,944,901
	5.	**#1 (population) AND #2 (intervention) AND #3 (outcome) AND #4 (study design)**	**2,245**

To explore the sources of heterogeneity and variation in intervention effects, I conducted subgroup analyses based on intervention duration, baseline 25(OH)D status, VIDS dosage regimen, cancer/precancerous condition, and study country of origin. Heterogeneity was visualized using forest plots and statistically assessed using Cochran's Q test and the I^2 index, where < 25% indicated low heterogeneity, 25% – 50% moderate heterogeneity, and > 50% high heterogeneity. When possible, sensitivity analyses were conducted to address high heterogeneity. Publication bias analyses were not performed for meta-analyses with fewer than 10 studies. All statistical analyses were conducted using random effects models with the Review Manager (RevMan) software, version 5.4. A two-sided p-value of 0.05 was set as the level of significance for all tests.

2.3 Anti-inflammatory effects of personalized vitamin D supplementation among colorectal cancer patients: randomized trial.

Earlier RCTs involving patients with cancer have demonstrated mixed effects of VIDS on selected pro-inflammatory biomarkers including TNF-α, IL-6, and CRP (Gwenzi et al. 2023b). However, the potential benefits of VIDS might be enhanced through personalized interventions tailored to the specific needs of patients. Therefore, in this study, I aimed to evaluate the impact of personalized oral VIDS on blood-based inflammatory biomarkers in CRC patients with low vitamin D status through a randomized, double-blind, placebo-controlled clinical trial.

2.3.1 Study details

My study utilized data from the ongoing VICTORIA trial, officially titled "*Personalized vitamin D supplementation for reducing or preventing fatigue and enhancing quality of life of patients with colorectal tumor-randomized intervention trial*" (EudraCT-No: 2019-000502-30; DRKS00019907). The trial design has been previously detailed in the protocol (Schöttker et al. 2020). In summary, this is a multicenter, parallel-group, randomized, double-blind, placebo-controlled clinical trial. CRC patients aged 18 and older are recruited from five German rehabilitation clinics. To be included in the study, patients must have been diagnosed with CRC and treated within the last 12 months (including surgical tumor removal, chemotherapy, or radiotherapy) and must have completed at least three weeks of in-patient rehabilitation in a participating clinic. Key exclusion criteria are serum 25(OH)D levels ≥ 60 nmol/L, high-dose VIDS (≥ 2000 IU daily or equivalent), high-dose calcium supplementation (> 1000 mg daily), hypercalcemia, hypercalciuria, and severe renal impairment (eGFR < 30 ml/min/1.73m^2).

The study received ethical approval from the Ethics Committee of the State Chamber of Medicine in Rheinland-Pfalz, the local Ethics Committee of the Chamber of Medicine Westfalen-Lippe, and the Federal Institute for Drugs and Medical Devices (BfArM). All participants provided written informed consent before enrollment in the VICTORIA trial. The primary outcome of the VICTORIA trial is "cancer-related fatigue," with secondary outcomes to be addressed after the completion of recruitment and follow-up in 2025. The current post-hoc analysis on the impact of VIDS on inflammatory biomarkers is based on data from 126 patients recruited between September 23, 2020, and July 19, 2023 (see **Figure 3**).

2.3.2 Intervention and control arms

Participants were randomly allocated in a 1:1 ratio to either the VIDS group or the placebo group. The randomization list was generated by computer and managed by the pharmacy at Heidelberg University Hospital. Both patients and study staff were blinded to the group assignments (double-blind trial). To maintain blinding, the placebo capsules were identical in appearance, weight, quantity, and packaging to the verum capsules. For the first 11 days, a personalized loading dose was administered based on each participant's 25(OH)D level and BMI at screening. This dose was calculated using the equation provided by Jansen et al., aiming for optimal 25(OH)D levels of 75–100 nmol/L (Jansen and Svendsen 2014):
Loading dose = 165 x BMI [kg/m^2] x (70-baseline 25(OH)D level [nmol/L]) [1].

To avoid administering excessively high doses of VIDS, the loading dose was spread over 11 days in units of 20,000 or 40,000 IU per day or placebo, rather than a single large bolus. After the loading dose, a maintenance dose of 2000 IU per day was given until the trial concluded after 12 weeks (84 days). In the control group, patients received a placebo following the same schedule as the intervention group.

2.3.3 Laboratory methods

Blood samples were collected at three distinct time points: baseline (BL), visit 1 on trial days 12–21 (i.e., the end of the loading dose and the end of the rehabilitation clinic stay, designated as FU1), and visit 2 at trial weeks 13–16 (i.e., the end of the maintenance dose and the end of the trial, designated as FU2).

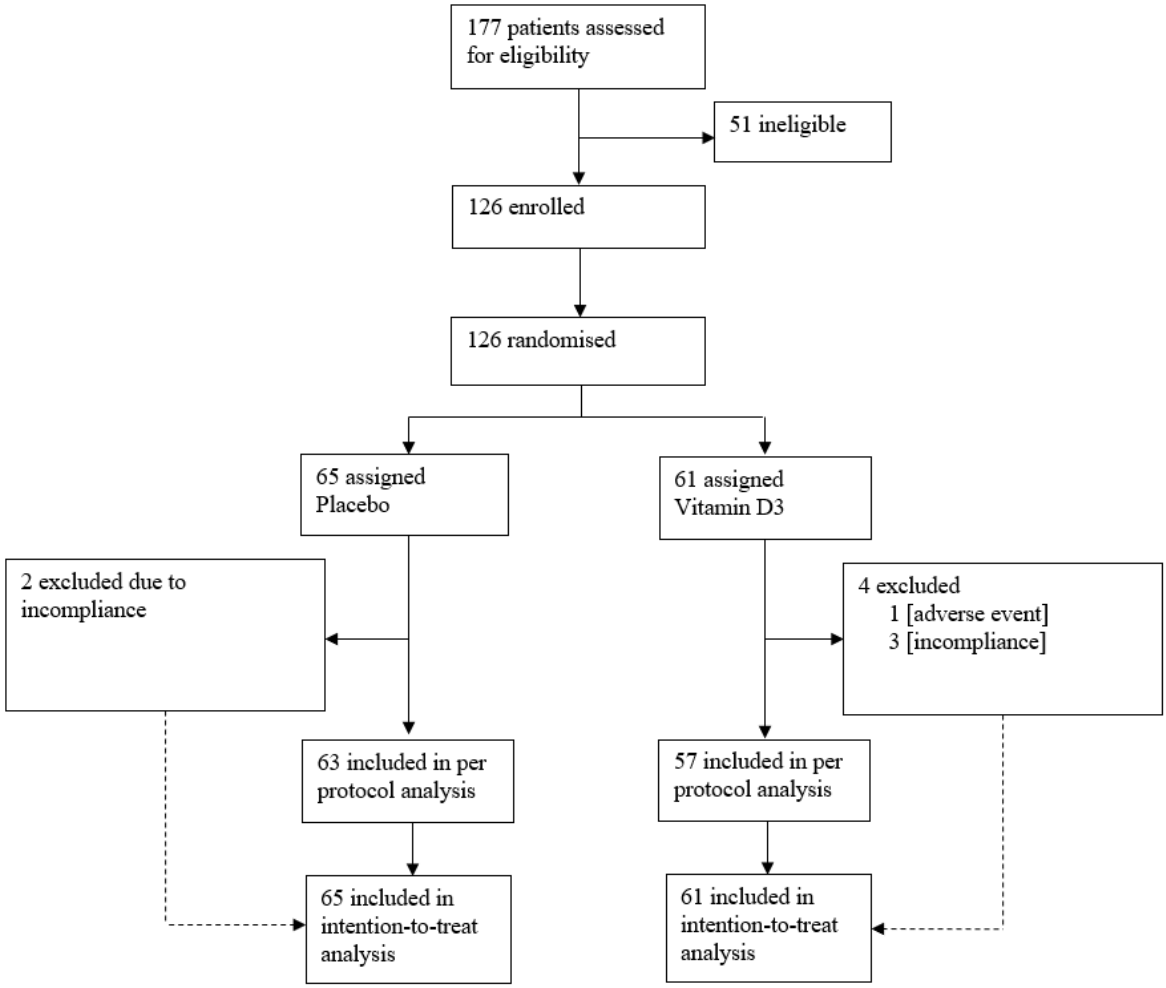


Figure 3. Patient selection flow diagram: VICTORIA trial.

25(OH)D Measurements

The serum 25(OH)D measurements were conducted in German certified laboratories using the LIAISON® 25 OH VITAMIN D TOTAL chemiluminescent immunoassay of DiaSorin, Saluggia, Italy. Based on the specifications of the manufacturer, the detection range is 10.0 – 375.0 nmol/L, while the intra- and inter-assay coefficients of variation (CV) are 5.4% and 10.6%, respectively.

Inflammatory Biomarker Measurements

Inflammatory biomarkers were assessed by the Olink Target 96 Inflammation panel which allows the measurement of 92 blood-based biomarkers per sample (see the list of all biomarkers of this panel in **Appendix Table 1**). Measurements were performed on serum samples collected at BL and at FU2. Blood samples were sent to the study centre and stored at −80°C until biomarker measurements took place. For the biomarker measurements, 10–25 µl of serum was

extracted from aliquots that had been thawed twice and sent with dry ice for analysis in the laboratory of Olink Proteomics, Uppsala Science Park, SE-75183 Uppsala, Sweden. The Olink panels are based on a proximity extension assay technology (PEA) (Assarsson et al. 2014; Lundberg et al. 2011). The average intra-assay CV among all 92 measured biomarkers was <10% at both BL and FU2. The inter-assay CV was between 10% and 12% at BL and FU2, respectively. Furthermore, the quality of each serum sample was assessed by Olink technology and the biomarker levels were reported as Normalized Protein eXpression (NPX) values, a relative quantification based on the Log2 scale. I excluded biomarkers with ≥ 25% of the values below the lower limit of detection (LOD) from the analyses (see **Table 2**). For the remaining biomarkers with less than 25% of values below the LOD, I replaced biomarker values below the LOD by LOD/√2. I conducted the normalization of raw data with the R (R Core Team, 2020, version 3.6.3) package "OlinkAnalyze", developed and maintained by the Olink Proteomics Data Science Team.

2.3.4 Outcomes

This was a post-hoc analysis because the inflammatory biomarkers of the OLINK inflammation panel are not mentioned as primary or secondary outcomes in the study protocol of the VICTORIA study. This post-hoc analysis included all study participants recruited between 23 September 2020 and 19 July 2023, completed the study until 22 November 2023 at the latest, and were unblinded on 22 November 2023 or earlier. Following a pre-defined statistical analysis plan, my analyses were be based on two approaches: (1) Confirmatory analysis to assess the effects of VIDS on the following biomarkers, which were selected based on evidence from a recent review summarizing the diagnostic and prognostic value of these biomarkers in CRC patients (Maryam et al. 2023): IL-6, interferon-gamma (INF-γ) and matrix metalloproteinase-1 (MMP-1) and (2) Exploratory analysis to assess the effects of VIDS on all the other remaining biomarkers of the Olink Target 96 Inflammation panel. Safety outcomes have been previously reported in the interim analysis (Kuznia et al. 2022). In all analyses, the outcome variable was the change in the biomarker level (measured by relative quantification based on the Log2 scale) between BL and FU2.

Table 2. Biomarkers excluded due to high proportion (≥25%) of values below the Limit of Detection.

Abbreviation	Biomarker name	Proportion < LOD
ARTN	Artemin	0.7057903
Beta-NGF	Beta-nerve growth factor	0.92957746
FGF-23	Fibroblast growth factor 23	0.28794992
GDNF	Glial cell line-derived neurotrophic factor	0.3458529
IL-1 alpha	Interleukin-1 alpha	0.83255086
IL-17A	Interleukin-17A	0.38810642
IL-20	Interleukin-20	0.43192488
IL-20RA	Interleukin-20 receptor subunit alpha	0.43661972
IL-22 RA1	Interleukin-22 receptor subunit alpha-1	0.5743349
IL-24	Interleukin-24	0.88419405
IL-2RB	Interleukin-2 receptor subunit beta	0.41314554
IL13	Interleukin-13	0.70892019
IL2	Interleukin-2	0.87167449
IL33	Interleukin-33	0.82629108
IL4	Interleukin-4	0.39280125
IL5	Interleukin-5	0.50547731
LIF	Leukemia inhibitory factor	0.43035994
NRTN	Neurturin	0.41471049
NT-3	Neurotrophin-3	0.30359937
TSLP	Thymic stromal lymphopoietin	0.45539906

Abbreviations: LOD, lower limit of detection

2.3.5 Statistical analyses

Patient characteristics at BL were assessed for serum 25(OH)D, IL-6, INF-γ and MMP-1 concentrations, as well as for age, sex, cancer stage at diagnosis, time since diagnosis, time since surgery, previous chemotherapy, previous radiotherapy, comorbidities (diabetes, history of myocardial infarction or stroke or congestive heart failure), BMI, smoking status, alcohol consumption, physical activity, and frailty.

Main outcome results were based on the intention-to-treat (ITT) analysis which included all randomized patients who were recruited until 19 July 2023 (n = 126, of whom 65 were in the placebo group and 61 in the VIDS group). In the per-protocol (PP) analysis, I excluded a total of six study participants (see **Figure 3**) who either failed to comply with the trial medication (< 80% compliance, n = 5) or were discontinued from treatment (one patient was discontinued from treatment in the intervention arm due to hypercalcemia). The percentage difference in the

original biomarker serum concentrations between the placebo and intervention groups at the end of the trial was reported as calculated from the formula:

Percentage Actual Difference = $(2^{\log 2 \text{ difference}} - 1) \times 100\%$ [2].

Assuming a normal distribution for the change of IL-6, INF-γ, MMP-1 and the other OLINK inflammation panel biomarker serum concentrations (measured by relative quantification based on the Log2 scale) from BL to FU2, I performed multivariable linear regression models to estimate the effects of VIDS on biomarkers of inflammation based on β-coefficients with their respective 95% CIs and p-values. In addition, the estimated change in the original biomarker serum concentrations due to VIDS was calculated from the β-coefficients using the formula:

Percentage change = $(2^{\beta} - 1) \times 100\%$ [3].

The linear regression models included the treatment group (placebo or VIDS) and the following BL variables: concentration of the respective inflammatory biomarker (continuous), age (continuous), sex, serum 25(OH)D (continuous), BMI (continuous), cancer stage (I, II, III, or IV), time since surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), previous chemotherapy and previous radiotherapy. Within-study-arm means of the changes of the serum inflammatory biomarker levels from BL to FU2 were presented with their respective 95% CIs. For the three biomarkers in the confirmatory analysis part, I applied the two-sided significance level of 0.05 using Bonferroni correction for multiple testing, i.e. p-values < 0.0166 were considered statistically significant. In the exploratory analyses with the remaining biomarkers, the ITT approach was applied to obtain β-coefficients and their respective p-values with the aim of generating hypotheses. All statistical tests were performed using R-statistical software (version 4.3) and two-sided test significance levels were set at p-values < 0.05.

I performed multiple imputation of missing values (covariates only) using the MICE package in R statistical software. Five imputation datasets with 30 iterations were applied using the following imputation model including all assessed variables that theoretically predict inflammatory response: treatment arm (dichotomous: placebo or VIDS), baseline age (continuous), sex (dichotomous), school education (≤ 9, 10-11, ≥ 12 years), serum 25(OH)D level (continuous), cancer stage (I, II, III, or IV), time since diagnosis (continuous), time since CRC surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), time since last chemotherapy (No chemotherapy, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), time since last radiotherapy (No radiotherapy, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), planned chemotherapy or radiotherapy in next 3 months (No, yes chemotherapy, yes radiotherapy, yes both), stoma at baseline

(dichotomous), subjective pain burden (continuous scale from 0-5), subjective exhaustion burden (continuous scale from 0-5), diabetes at baseline (dichotomous), cardiovascular disease at baseline (dichotomous, defined by coronary heart disease, history of myocardial infarction or revascularization of coronary arteries), heart failure at baseline (dichotomous), history of stroke (dichotomous), chronic obstructive pulmonary disease at baseline (dichotomous), asthma at baseline (dichotomous), renal failure at baseline (dichotomous), arthropathy at baseline (dichotomous, defined as arthritis, arthrosis or other rheumatic joint disease), diarrhoea in last week (none, a little, moderate, a lot), baseline BMI (continuous), baseline smoking status (never, former, current), baseline alcohol consumption (none, low, moderate, high), baseline physical activity meeting WHO recommendation (dichotomous) (Erben et al. 2019; Topolski et al. 2006), baseline red meat consumption (never, up to 3 times a month, 1-3 days a week, 4-6 days per week, daily), frailty (non-frail, pre-frail, frail), baseline global quality of life (continuous scale from 1-7), baseline and 12-week OLINK inflammation panel biomarker levels of all biomarkers meeting inclusion criteria (continuous), and baseline and 12-week follow-up "Quality Control Warning" about blood sample from OLINK inflammation panel measurement's output.

2.3.6 Additional analyses

The mean 25(OH)D levels, the change in 25(OH)D levels, and the proportion of subjects exhibiting inadequate 25(OH)D levels (i.e., levels <50 nmol/L) in the intervention and placebo groups at BL, FU1, and FU2 were presented with their respective 95% CIs.

3 Results

3.1 Vitamin D Status, *Cdx2* Genotype, and Colorectal Cancer Survival: Population-Based Patient Cohort.

3.1.1 Description of the study population

A total of 2819 patients were included in the analyses (see **Table 3**). Approximately 60% of the patients were male, with a median age at diagnosis of 69 years (interquartile range: 62–76 years). Over half of the patients were diagnosed at stages I or II, while about 14% were diagnosed at stage IV CRC. A majority, 59%, had serum 25(OH)D levels in the deficient range. About 65% of the patients had the GG genotype for *Cdx2*. Serum 25(OH)D levels did not significantly differ by *Cdx2* genotype, with roughly 60% of patients being vitamin D deficient across all three genotypes (GG, AG, and AA), and around 15% having sufficient vitamin D levels (chi-square p-value = 0.64) (see **Table 4**). The interquartile range for BMI was 23.6–29.0 kg/m², with a median BMI of 26.1 kg/m². Approximately half of the patients were recruited within 30 days of their primary CRC diagnosis. After a median follow-up of 9.4 years, 1521 deaths were recorded, 798 of which were due to CRC.

3.1.2 Vitamin D status and survival

The associations between serum 25(OH)D levels and survival outcomes are presented in **Table 5**. After adjusting for sex, age, and season of blood draw, patients with vitamin D insufficiency and sufficiency demonstrated significantly better survival outcomes compared to those with vitamin D deficiency. Although the associations between vitamin D status and survival outcomes were somewhat reduced after adjusting for all covariates, they remained statistically significant. The fully adjusted HR (95% CI) for sufficient versus deficient vitamin D status were 0.71 (0.59–0.84) for OS, 0.76 (0.60–0.95) for CSS, 0.79 (0.64–0.98) for RFS, and 0.69 (0.58–0.82) for DFS. No significant interactions were found between vitamin D status and categorical covariates, therefore subgroup analyses for these variables were not performed.

Table 3. Main characteristics of colorectal cancer patients in the DACHS cohort.

Characteristic		**n**	**%**
Sex	Female	1136	40.3
	Male	1683	59.7
Age at diagnosis	Median (IQR)	69 (62 - 76)	
	30-59	553	19.6
	60-69	914	32.4
	70-79	923	32.7
	80+	429	15.2
TNM Cancer Stage	I	650	23.1
	II	879	31.3
	III	889	31.7
	IV	391	13.9
Cancer Site	Colon	1687	59.8
	Rectum	1132	40.2
Serum 25(OH)D	< 30 nmol/L	1675	59.4
	30-49 nmol/L	695	24.7
	≥50 nmol/L	449	15.9
Cdx2 genotype	AA	122	4.3
	AG	872	30.9
	GG	1825	64.7
BMI [kg/m^2]	Median (IQR)	26.1 (23.6 – 29.0)	
	<25	1077	38.2
	25-<30	1208	42.9
	≥30	534	18.9
Screen-detected tumor		658	23.4
Surgical treatment		2744	97.3
Chemotherapy		1287	45.9
History of CVD		709	25.8
History of diabetes		521	18.5
History of hypertension		1445	51.3

continued on next page

Characteristic		n	%
Smoking, Lifetime pack-years	Never	1280	45.7
	< 10	501	17.9
	10 - 19	365	13.0
	20 - 29	278	9.9
	≥ 30	379	13.5
Alcohol intake[1]	None	818	29.4
	Low	1253	45.1
	High	710	25.5
Physical activity[2]	Low	923	33.4
	Moderate	920	33.3
	High	917	33.2
School education	<9 years	1914	68.1
	9-10 years	470	16.7
	≥10 years	428	15.2
Late entry[3]	≤1 month	1400	51.9
	>1 month	1300	48.1
Season of blood draw	Spring	780	27.7
	Summer	756	26.8
	Autumn	671	23.8
	Winter	612	21.7

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CVD, cardiovascular disease; IQR, interquartile range; MET-h, Metabolic equivalent task hours; TNM, tumor node metastasis.

[1]Commonly used sex-specific definitions (women: cut-off=16 g ethanol/day; men: cut-off=24 g ethanol/day); [2]Definitions according to MET-hours/week in the last 12 months categorized in tertiles (low <80; moderate 81-<146.5; high >146.5); Season of blood draw (spring: ''March, April, May'', summer: ''June, July, August'', autumn: ''September, October, November'', winter: ''December, January, February''). [3]Late entry was defined as time between CRC diagnosis and blood collection.

Table 4. Distribution of serum 25(OH)D level by *Cdx2* genotype in the DACHS cohort.

Vitamin D status	***Cdx2* genotype**		
	GG (%)	**AG (%)**	**AA (%)**
Deficient	1,100 (60)	502 (58)	73 (60)
Insufficient	443 (24)	220 (25)	32 (26)
Sufficient	282 (15)	150 (17)	17 (14)
Total	1,825 (100)	872 (100)	122 (100)

Chi-square = 2.53; degrees of freedom = 4; p-value = 0.64; Frequencies are presented as n (%)

3.1.3 VDR *Cdx2* locus genotypes and survival

Hazard ratios for the associations between *Cdx2* genotypes and survival outcomes are shown in **Table 5**. After adjusting for sex, age, and season of blood draw, no significant associations were found between VDR genotypes and any of the survival outcomes. Similar findings were observed after adjusting for all relevant covariates. The fully adjusted HR (95% CI) for the AA/AG genotype compared to the GG genotype were 0.99 (0.88–1.11) for OS, 0.93 (0.80–1.09) for CSS, 0.97 (0.84–1.11) for RFS, and 0.98 (0.88–1.10) for DFS.

3.1.4 Joint associations of vitamin D status and VDR *Cdx2* locus genotypes with survival

The survival curves for the combined associations of vitamin D status and *Cdx2* genotypes are illustrated in **Figure 4**. For patients with the GG genotype, survival outcomes were consistently higher for those with sufficient or insufficient vitamin D levels compared to those with vitamin D deficiency across all measures. In contrast, no clear associations were observed between vitamin D status and survival outcomes for patients with the AA or AG genotypes. These patterns were also confirmed in the multivariable analyses presented in **Table 6**. For patients with the GG genotype, the adjusted HR (95% CI) for those with sufficient vitamin D (25(OH)D > 50 nmol/L) or insufficient vitamin D (25(OH)D between 30 and 50 nmol/L) compared to those with deficient vitamin D (25(OH)D < 30 nmol/L) were as follows: 0.63 (0.50–0.78) and 0.69 (0.56–0.84) for OS, 0.68 (0.50–0.90) and 0.71 (0.55–0.92) for CSS, 0.66 (0.51–0.86) and 0.73 (0.58–0.91) for RFS, and 0.62 (0.50–0.77) and 0.68 (0.56–0.83) for DFS.

Table 5. Individual associations of serum 25(OH)D concentration and *Cdx2* genotype with the different survival outcomes in the DACHS cohort

Survival Endpoint	Predictor		N/events	Hazard Ratio (95% CI)	
				Model 1*	Model 2**
Overall	Serum 25(OH)D (nmol/L)	< 30	1673/1012	1.00 (ref)	1.00 (ref)
		30-49	695/317	**0.67 (0.59 – 0.75)**	**0.73 (0.63 – 0.85)**
		≥50	449/190	**0.62 (0.53 – 0.72)**	**0.71 (0.59 – 0.84)**
	Cdx2 genotype	GG	1824/999	1.00 (ref)	1.00 (ref)
		AA or AG	993/520	0.95 (0.86 – 1.06)	0.99 (0.88 – 1.11)
CRC specific	Serum 25(OH)D (nmol/L)	< 30	1657/542	1.00 (ref)	1.00 (ref)
		30-49	686/154	**0.61 (0.51 – 0.73)**	**0.72 (0.59 – 0.89)**
		≥50	442/102	**0.61 (0.49 – 0.75)**	**0.76 (0.60 – 0.95)**
	Cdx2 genotype	GG	1806/534	1.00 (ref)	1.00 (ref)
		AA or AG	979/264	0.90 (0.78 – 1.05)	0.93 (0.80 – 1.09)
Recurrence-free	Serum 25(OH)D (nmol/L)	< 30	1662/612	1.00 (ref)	1.00 (ref)
		30-49	690/187	**0.65 (0.55 – 0.76)**	**0.76 (0.64 – 0.90)**
		≥50	444/119	**0.62 (0.51 – 0.76)**	**0.79 (0.64 – 0.98)**
	Cdx2 genotype	GG	1811/603	1.00 (ref)	1.00 (ref)
		AA or AG	985/314	0.95 (0.83 – 1.09)	0.97 (0.84 – 1.11)
Disease-free	Serum 25(OH)D (nmol/L)	< 30	1661/1034	1.00 (ref)	1.00 (ref)
		30-49	690/331	**0.67 (0.59 – 0.76)**	**0.73 (0.62 – 0.85)**
		≥50	444/194	**0.60 (0.51 – 0.70)**	**0.69 (0.58 – 0.82**)
	Cdx2 genotype	GG	1810/1026	1.00 (ref)	1.00 (ref)
		AA or AG	985/533	0.95 (0.86 – 1.06)	0.98 (0.88 – 1.10)

***** Adjusted for sex, age and season

******Additionally, adjusted for cancer stage at diagnosis, tumor location, tumor detection mode, chemotherapy, history of cardiovascular diseases, history of diabetes, history of hypertension, smoking, body mass index, physical activity, and late entry in months.

Trend analyses for vitamin D status were significant for all outcomes among patients with the GG genotype (p-trend < 0.01). Conversely, no consistent patterns or significant trends were observed among those with the AA/AG genotypes, except for DFS (p-trend = 0.04). However, tests for interaction between vitamin D status and genotype regarding survival outcomes did not reach statistical significance.

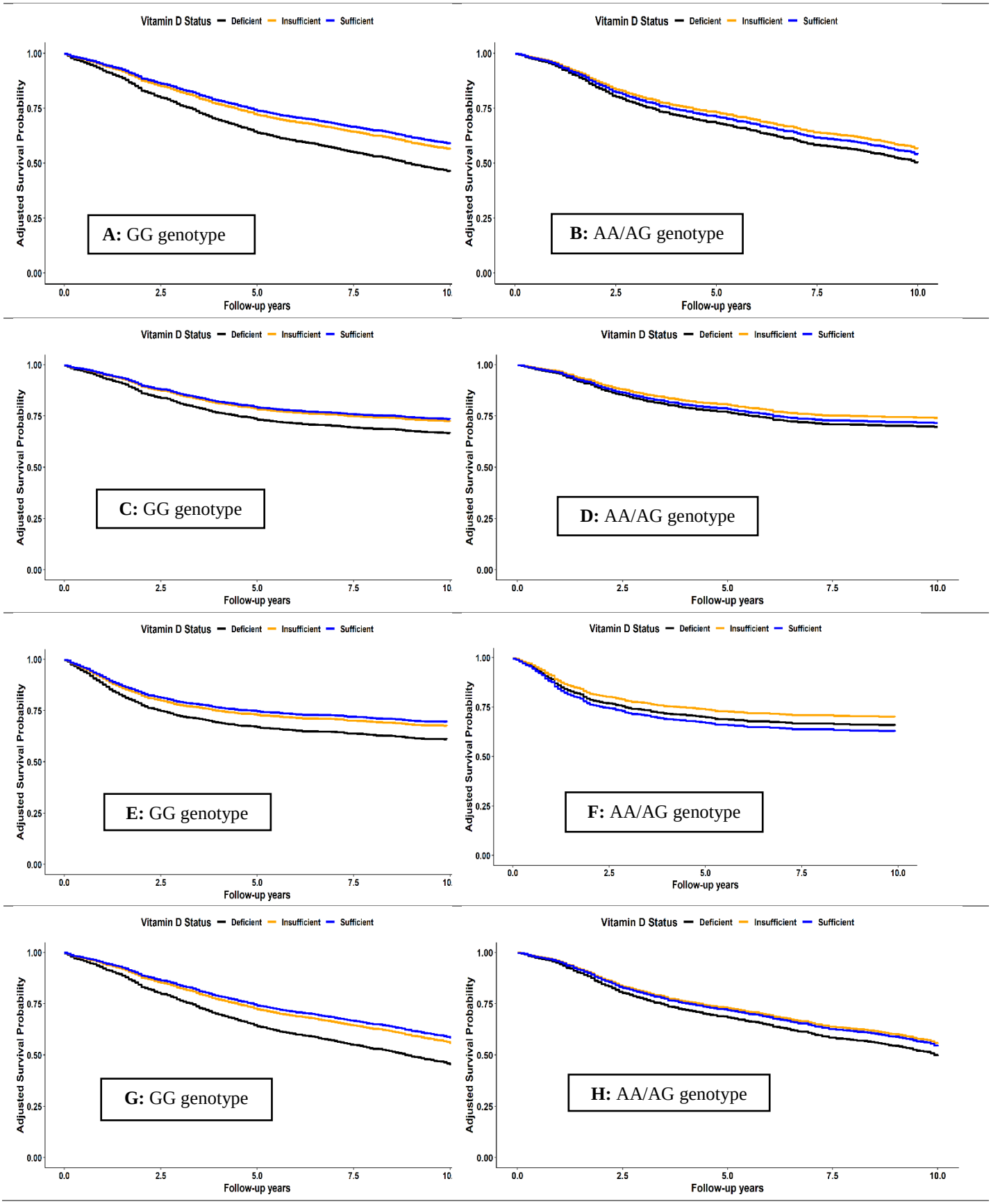


Figure 4. Survival curves for joint associations of vitamin D status and *Cdx2* genotype with overall survival (A and B), CRC-specific survival (C and D), recurrence-free survival (E and F) and disease-free survival (G and H).

Table 6. Joint associations of serum 25(OH)D concentration and *Cdx2* genotype with the different survival outcomes in the DACHS cohort.

Survival endpoint	*Cdx2* genotype	25(OH)D	N/events	Adjusted Hazard Ratio (95% CI) *	P_{trend}
Overall	GG	< 30 nmol/L	1099/683	1.00 (ref)	**< 0.001**
		30-49 nmol/L	443/200	**0.69 (0.56 – 0.84)**	
		≥50 nmol/L	282/116	**0.63 (0.50 – 0.78)**	
	AA or AG	< 30 nmol/L	574/329	1.00 (ref)	0.08
		30-49 nmol/L	252/117	**0.77 (0.61 – 0.98)**	
		≥50 nmol/L	167/74	0.85 (0.64 – 1.13)	
	$P_{interaction}$	0.33			
CRC specific	GG	< 30 nmol/L	1091/370	1.00 (ref)	**0.002**
		30-49 nmol/L	439/102	**0.71 (0.55 – 0.92)**	
		≥50 nmol/L	276/62	**0.68 (0.50 – 0.90)**	
	AA or AG	< 30 nmol/L	566/172	1.00 (ref)	0.24
		30-49 nmol/L	247/53	0.74 (0.52 – 1.05)	
		≥50 nmol/L	166/40	0.87 (0.59 – 1.29)	
	$P_{interaction}$	0.88			
Recurrence-free	GG	< 30 nmol/L	1090/411	1.00 (ref)	**< 0.001**
		30-49 nmol/L	440/120	**0.73 (0.58 – 0.91)**	
		≥50 nmol/L	281/71	**0.66 (0.51 – 0.86)**	
	AA or AG	< 30 nmol/L	572/200	1.00 (ref)	0.99
		30-49 nmol/L	250/67	0.79 (0.58 – 1.07)	
		≥50 nmol/L	163/47	1.13 (0.79 – 1.61)	
	$P_{interaction}$	0.50			
Disease-free	GG	< 30 nmol/L	1089/696	1.00 (ref)	**< 0.001**
		30-49 nmol/L	440/209	**0.68 (0.56 – 0.83)**	
		≥50 nmol/L	281/121	**0.62 (0.50 – 0.77)**	
	AA or AG	< 30 nmol/L	572/338	1.00 (ref)	**0.04**
		30-49 nmol/L	250/122	**0.78 (0.62 – 0.99)**	
		≥50 nmol/L	163/73	0.81 (0.61 – 1.08)	
	$P_{interaction}$	0.40			

*****Additionally, adjusted for cancer stage at diagnosis, tumor location, tumor detection mode, chemotherapy, history of cardiovascular diseases, history of diabetes, history of hypertension, smoking, body mass index, physical activity, and late entry in months.

$P_{interaction}$ was obtained by fitting a non-stratified full model with the interaction term for vitamin D serum level as a continuous variable and *Cdx2* as a binary variable.

3.2 Effects of vitamin D supplementation on inflammatory response in patients with cancer and precancerous lesions: Systematic review and meta-analysis of randomized trials.

3.2.1 Search strategy and study selection

The study selection process is illustrated in **Figure 2**. Out of 4788 individual studies, 26 were selected for full-text screening. Additionally, one more study was included via cross-referencing (Li et al. 2018b). In total, nine studies (El-Bassiouny et al. 2022; Haidari et al. 2020; Hopkins et al. 2011; Li et al. 2018b; Mohseni et al. 2019; Naderi et al. 2022; Shahvegharasl et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) were included in this systematic review, and eight of these studies were incorporated into the meta-analyses, involving a total of 592 patients. Other studies were excluded based on predefined criteria detailed in **Table 7.**

3.2.2 Description of studies included in the meta-analyses

General information about the included studies is summarized in **Table 8**. The eight studies incorporated into the meta-analyses had sample sizes ranging from 30 to 100 participants. Six of these studies were conducted in Iran. Four studies (El-Bassiouny et al. 2022; Mohseni et al. 2019; Naderi et al. 2022; Shahvegharasl et al. 2020) focused on the effects of VIDS on inflammatory markers in breast cancer patients, while two studies (Vahedpoor et al. 2017; Vahedpoor et al. 2018) examined patients with cervical intraepithelial neoplasia (CIN). The remaining studies investigated the impact of VIDS on inflammatory biomarkers in CRC patients (Haidari et al. 2020) and patients with colorectal cancer adenoma (Hopkins et al. 2011). Five trials administered VIDS as weekly (Haidari et al. 2020; Mohseni et al. 2019; Shahvegharasl et al. 2020) or bi-weekly (Vahedpoor et al. 2017; Vahedpoor et al. 2018) oral bolus doses of 50,000 international units (IU), while the other three trials (El-Bassiouny et al. 2022; Hopkins et al. 2011; Naderi et al. 2022) provided daily doses ranging from 20 IU to 4,000 IU.

Table 7. Excluded studies and reasons for exclusion in the systematic review and meta-analysis.

Reason	Study reference
Inflammatory biomarkers not reported (n=6)	1. Ahearn TU, Shaukat A, Flanders WD, Rutherford RE, Bostick RM. **A randomized clinical trial of the effects of supplemental calcium and vitamin D3 on the APC/β-catenin pathway in the normal mucosa of colorectal adenoma patients**. Cancer Prev Res (Phila). 2012 Oct;5(10):1247-56. doi: 10.1158/1940-6207.CAPR-12-0292. Epub 2012 Sep 10. PMID: 22964475; PMCID: PMC3466388. 2. Apoe O, Jung SH, Liu H, Seisler DK, Charlamb J, Zekan P, Wang LX, Unzeitig GW, Garber J, Marshall J, Wood M. **Effect of Vitamin D Supplementation on Breast Cancer Biomarkers: CALGB 70806 (Alliance) Study Design and Baseline Data**. Am J Hematol Oncol. 2016 Jul;12(7):4-9. PMID: 29081880; PMCID: PMC5656380. 3. Arnaout A, Robertson S, Pond GR, Vieth R, Jeong A, Hilton J, Ramsey T, Clemons M. **Randomized window of opportunity trial evaluating high-dose vitamin D in breast cancer patients.** Breast Cancer Res Treat. 2019 Nov;178(2):347-356. doi: 10.1007/s10549-019-05392-9. Epub 2019 Aug 9. PMID: 31399931. 4. Gao Y, Um CY, Fedirko V, Rutherford RE, Seabrook ME, Barry EL, Baron JA, Bostick RM. **Effects of supplemental vitamin D and calcium on markers of proliferation, differentiation, and apoptosis in the normal colorectal mucosa of colorectal adenoma patients**. PLoS One. 2018 Dec 17;13(12):e0208762. doi: 10.1371/journal.pone.0208762. PMID: 30557404; PMCID: PMC6296527. 5. Peppone LJ, Ling M, Huston AJ, Reid ME, Janelsins MC, Puzas JE, Kamen C, Del Giglio A, Asare M, Peoples AR, Mustian KM. **The effects of high-dose calcitriol and individualized exercise on bone metabolism in breast cancer survivors on hormonal therapy: a phase II feasibility trial**. Support Care Cancer. 2018 Aug;26(8):2675-2683. doi: 10.1007/s00520-018-4094-4. Epub 2018 Feb 22. PMID: 29470705; PMCID: PMC6019129.

Reason	Study reference
	6. Urashima M, Okuyama M, Akutsu T, Ohdaira H, Kaji M, Suzuki Y. **Effect of Vitamin D Supplementation on Survival of Digestive Tract Cancer Patients with Low Bioavailable 25-Hydroxyvitamin D levels: A Post Hoc Analysis of the AMATERASU Randomized Clinical Trial.** Cancers (Basel). 2020 Feb 4;12(2):347. doi: 10.3390/cancers12020347. PMID: 32033150; PMCID: PMC7072519.
Participants had no cancer/precancerous lesions (n=4)	1. Avcioglu G, Özbek Ipteç B, Akcan G, Görgün B, Fidan K, Carhan A, Yilmaz G, Kozaci LD. **Effects of 1,25-Dihydroxy vitamin D_3 on TNF-α induced inflammation in human chondrocytes and SW1353 cells: a possible role for toll-like receptors**. Mol Cell Biochem. 2020 Jan;464(1-2):131-142. doi: 10.1007/s11010-019-03655-z. Epub 2019 Nov 16. PMID: 31734843. 2. Chandler PD, Scott JB, Drake BF, Ng K, Manson JE, Rifai N, Chan AT, Bennett GG, Hollis BW, Giovannucci EL, Emmons KM, Fuchs CS. **Impact of vitamin D supplementation on inflammatory markers in African Americans: results of a four-arm, randomized, placebo-controlled trial**. Cancer Prev Res (Phila). 2014 Feb;7(2):218-25. doi: 10.1158/1940-6207.CAPR-13-0338-T. Epub 2013 Dec 10. PMID: 24327720; PMCID: PMC4038929. 3. Crew KD, Anderson GL, Hershman DL, Terry MB, Tehranifar P, Lew DL, Yee M, Brown EA, Kairouz SS, Kuwajerwala N, Bevers T, Doster JE, Zarwan C, Kruper L, Minasian LM, Ford L, Arun B, Neuhouser M, Goodman GE, Brown PH. **Randomized Double-Blind Placebo-Controlled Biomarker Modulation Study of Vitamin D Supplementation in Premenopausal Women at High Risk for Breast Cancer (SWOG S0812).** Cancer Prev Res (Phila). 2019 Jul;12(7):481-490. doi: 10.1158/1940-6207.CAPR-18-0444. Epub 2019 May 28. PMID: 31138522; PMCID: PMC6609474. 4. Duggan C, de Dieu Tapsoba J, Mason C, Imayama I, Korde L, Wang CY, McTiernan A**. Effect of Vitamin D3 Supplementation in Combination with Weight Loss on Inflammatory Biomarkers in Postmenopausal**

Reason	Study reference
	Women: A Randomized Controlled Trial. Cancer Prev Res (Phila). 2015 Jul;8(7):628-35. doi: 10.1158/1940-6207.CAPR-14-0449. Epub 2015 Apr 23. PMID: 25908506; PMCID: PMC4491001.
Studies were not RCTs but protocol proposals (n=2)	1. Augustin LS, Libra M, Crispo A, Grimaldi M, De Laurentiis M, Rinaldo M, D'Aiuto M, Catalano F, Banna G, Ferrau' F, Rossello R, Serraino D, Bidoli E, Massarut S, Thomas G, Gatti D, Cavalcanti E, Pinto M, Riccardi G, Vidgen E, Kendall CW, Jenkins DJ, Ciliberto G, Montella M. **Low glycemic index diet, exercise and vitamin D to reduce breast cancer recurrence (DEDiCa): design of a clinical trial**. BMC Cancer. 2017 Jan 23;17(1):69. doi: 10.1186/s12885-017-3064-4. PMID: 28114909; PMCID: PMC5259892. 2. Haidari F, Abiri B, Iravani M, Razavi SM, Sarbakhsh P, Ahmadi-Angali K, Vafa M. **Effects of vitamin D and omega-3 fatty acids co-supplementation on inflammatory biomarkers, tumor marker CEA, and nutritional status in patients with colorectal cancer: a study protocol for a double blind randomized controlled trial.** Trials. 2019 Dec 9;20(1):682. doi: 10.1186/s13063-019-3719-3. PMID: 31815661; PMCID: PMC6900845.
Intervention was on non-human subjects (n=1)	1. Al-Rasheed NM, Al-Rasheed NM, Bassiouni YA, Hasan IH, Al-Amin MA, Al-Ajmi HN, Mohamad RA. **Vitamin D attenuates pro-inflammatory TNF-α cytokine expression by inhibiting NF-κB/p65 signaling in hypertrophied rat hearts**. J Physiol Biochem. 2015 Jun;71(2):289-99. doi: 10.1007/s13105-015-0412-1. Epub 2015 May 1. PMID: 25929726.

Intervention follow-up periods varied from 8 to 24 weeks. Compliance rates were reported to exceed 80% in five studies (Haidari et al. 2020; Hopkins et al. 2011; Mohseni et al. 2019; Vahedpoor et al. 2017; Vahedpoor et al. 2018), while the remaining three studies (El-Bassiouny et al. 2022; Naderi et al. 2022; Shahvegharasl et al. 2020) did not report compliance rates. Baseline mean serum 25(OH)D levels were documented in seven studies. Four studies (El-Bassiouny et al. 2022; Hopkins et al. 2011; Mohseni et al. 2019; Naderi et al. 2022) reported sufficient mean 25(OH)D levels [i.e., 25(OH)D > 20 ng/mL] in the intervention group, while three studies (Haidari et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) reported deficient mean 25(OH)D levels [i.e., 25(OH)D < 12 ng/mL].

Five studies (Haidari et al. 2020; Hopkins et al. 2011; Shahvegharasl et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) examined the effects of VIDS on serum CRP concentrations. Serum levels of TNF-α were reported in four studies (Haidari et al. 2020; Hopkins et al. 2011; Mohseni et al. 2019; Naderi et al. 2022), and IL-6 levels were reported in four studies (El-Bassiouny et al. 2022; Haidari et al. 2020; Hopkins et al. 2011; Naderi et al. 2022). Two studies (Hopkins et al. 2011; Naderi et al. 2022) reported serum levels of interleukin-10 (IL-10). Four biomarkers were not included in the meta-analyses due to an insufficient number of studies (see **Table 9**). Additionally, two studies (Haidari et al. 2020; Hopkins et al. 2011) explored the effects of VIDS combined with calcium/omega-3 fatty acid co-supplements (see **Table 10** for study details). All studies used the enzyme-linked immunosorbent assay (ELISA) technique to measure serum inflammatory biomarkers, except one (Li et al. 2018b) which did not specify the assay technique used.

Table 8. General information of studies included in the meta-analyses.

First author, year, reference	Country	Mean Age (SD)	Cancer site&stage	F (%)	Baseline 25(OH)D ng/mL (intervention/ placebo)	Intervention (Vitamin D3 Dosage)	Number of participants (intervention/placebo)	Biomarker investigated	Biomarker Serum Level at Follow-Up: Mean (SD)		Follow-up
									Intervention	Placebo	
Hopkins et al, 2011	USA	60.2 (8.1)	Colorectal Adenoma	30	21.0 /20.4	400 IU twice daily	22/21	CRP (µg/ml)	0.99 (1.97)	1.88 (4.16)	24 weeks
						400 IU twice daily	22/21	TNF-α (pg/ml)	2.73 (2.52)	4.57 (2.05)	
						400 IU twice daily	22/21	IL-6 (pg/ml)	0.67 (3.76)	1.41 (2.67)	
						400 IU twice daily	22/21	IL-10 (pg/ml)	0.43 (1.38)	0.53 (1.96)	
Vahedpoor, et al 2017	Iran	36.9 (7.4)	CIN, I	100	10.8/11.2	50 000 IU every 2 weeks	29/29	CRP (µg/ml)	1.96 (3.72)	1.64 (4.29)	24 weeks
Vahedpoor et al, 2018	Iran	41.9 (7.2)	CIN, II-III	100	11.5/12.4	50 000 IU every 2 weeks	29/29	CRP (µg/ml)	3.80 (1.57)	4.84 (3.01)	24 weeks
Shahvegharasl et al, 2019	Iran	41.1 (5.6)	BC, I-III	100	NR	50 000 IU every week	22/22	CRP (µg/ml)	4.19 (3.89)	3.30 (3.25)	8 weeks
Mohseni et al, 2019	Iran	47.7 (8.0)	BC	100	28.0/15.3	50 000 IU every week	26/26	TNF-α (pg/ml)	14.5 (1.60)	25.6 (3.20)	8 weeks
Haidari et al, 2020	Iran	57.1 (11.4)	CRC, II/III	23.8	11.6/11.2	50 000 IU every week	21/20	CRP (µg/ml)	1.44 (0.8)	1.49 (0.98)	8 weeks
				23.8	11.6/11.2	50 000 IU every week	21/20	TNF-α (pg/ml)	4.93 (2.34)	6.76 (2.88)	
				23.8	11.6/11.2	50 000 IU every week	21/20	IL-6 (pg/ml)	33.54 (28.8)	41.64 (51.26)	
El-Bassiouny et al, 2022	Egypt	49.6 (5.8)	BC, II	100	21.4/20.7	20 IU daily	50/50	IL-6 (pg/ml)	39.68 (10.47)	64.79 (14.8)	12 weeks
[1]Naderi et al, 2022	Iran	48.0 (8.0)	BC, 0-II	100	41.2/43.4	4000 IU daily	10/10	TNF-α (pg/ml)	17.96 (4.37)	22.24 (3.91)	12 weeks
								IL-6 (pg/ml)	0.3 (0.19)	0.48 (0.19)	
								IL-10 (pg/ml)	83.04 (67.31)	75.85 (43.55)	

Notes: F female; USA United States of America; BC Breast Cancer; CIN Cervical Intraepithelial Neoplasia; NR Not Reported; IU International Units; i.v intravenous; SD Standard Deviation; CRP C-reactive protein; TNF-α tumor necrosis factor alpha; IL interleukin; µg microgram; ng nanogram; pg picogram; ml millilitre.
[1]Study compared vitamin D supplementation group and those on yoga intervention.
Only two studies reported mean time of blood sample collection after surgery: Li et al (day 1 – 6 after surgery for the follow-up) and Naderi et al (>3 years post-operatively for both baseline and follow-up)

Table 9. Additional information on biomarkers not included in meta-analyses.

First author, year	Country	Mean Age (SD)	Cancer site&stage	F (%)	Mean baseline 25(OH)D ng/mL (intervention /placebo)	Intervention (Vitamin D3 Dosage)	Participants (intervention /placebo)	Biomarker investigated	Biomarker Serum Level at Follow-Up (ng/mL): Mean (SD)		Follow -up
									Intervention	Placebo	
Shahvegharasl, 2019	Iran	41.1 (5.6)	BC, I-III	100	NR	50 000 IU every week	22/22	Ang-2	1.61 (0.88)	2.07 (1.93)	8 weeks
								VEGF-A	0.29 (0.18)	0.21 (0.13)	
								Hif-1	1.30 (0.70)	1.30 (0.30)	
Mohseni, 2019	Iran	47.7 (8.0)	BC	100	28.0/15.3	50 000 IU every week	26/26	TGF-β	0.29 (0.04)	0.13 (0.01)	8 weeks
Li, 2018	China	55.4 (10.2)	Gastric Cancer	35.7	NR	220IU i.v daily	14/16	IL-8 (pg/ml)	6.74*	10.32*	1 week

Notes: F female; BC Breast Cancer; 25(OH)D 25-hydroxyvitamin D; NR Not Reported; IU International Units; SD Standard Deviation; Ang-1 Angiopoietin 1; VEGF-A vascular endothelial growth factor-A; Hif-1 hypoxia inducible factor 1; TGF-β transforming growth factor beta; ng/mL nanograms/millilitre.
*****Median values for IL-8 serum levels were reported.

Table 10. General information of studies on the effects of vitamin D and co-supplements on inflammatory biomarkers.

First author, year	Country	Mean Age (SD)	Cancer site&stage	F (%)	Baseline mean 25(OH)D ng/mL (intervention/ placebo)	Intervention (Vitamin D3 Dosage)	Number of participants (intervention/placebo)	Biomarker investigated	Biomarker Serum Level at Follow-Up: Mean (SD)		Follow-up
									Intervention	Placebo	
Hopkins, 2011	USA	60.2 (8.1)	Colorectal Adenoma	30	21.0 /20.4	400 IU twice daily + Ca 2g/day	21/21	CRP (µg/ml)	2.21 (3.06)	1.93 (2.94)	24 weeks
						400 IU twice daily + Ca 2g/day	21/23	TNF-α (pg/ml)	4.00 (1.62)	3.62 (1.75)	
						400 IU twice daily + Ca 2g/day	21/23	IL-6 (pg/ml)	1.62 (3.25)	1.39 (4.49)	
Haidari, 2020	Iran	57.1 (11.4)	CRC, II/III	50	9.7/11.2	50 000 IU every week + ω3FA twice/day	20/20	CRP (µg/ml)	0.55 (0.48)	1.49 (0.98)	8 weeks
				50	9.7/11.2	50 000 IU every week + ω3FA twice/day	20/20	TNF-α (pg/ml)	4.86 (2.12)	6.76 (2.88)	
				50	9.7/11.2	50 000 IU every week + ω3FA twice/day	20/20	IL-6 (pg/ml)	34.56 (40.7)	41.64 (51.26)	

Notes: F female; USA United States of America; IU International Units; Ca calcium; ω3FA omega 3 fatty acids; SD Standard Deviation; CRP C-reactive protein; TNF-α tumor necrosis factor alpha; IL interleukin; µg microgram; ng nanogram; pg picogram; ml millilitre.

3.2.3 Risk of bias assessment

The results of the risk of bias assessment are detailed in **Table 11**. Five studies (Hopkins et al. 2011; Li et al. 2018b; Shahvegharasl et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) were deemed to have good overall quality. Two studies (Haidari et al. 2020; Mohseni et al. 2019) were assessed as having fair quality, while the remaining two (El-Bassiouny et al. 2022; Naderi et al. 2022) were rated as having poor quality. Additionally, three studies (El-Bassiouny et al. 2022; Haidari et al. 2020; Naderi et al. 2022) exhibited a high attrition rate (>15%). Regarding blinding methods, one study used triple blinding, six employed double blinding, and one used single blinding (results not shown).

Table 11. Risk of Bias Evaluation with the Cochrane Risk of Bias 2 Tool.

First author, year	RS	AC	SR	OB	BPP	BOA	IOD	OSQ
Hopkins, 2011	U	L	L	L	L	L	L	Good
Vahedpoor, 2017	L	L	L	L	L	L	L	Good
Li, 2018	L	L	L	L	L	L	L	Good
Vahedpoor, 2018	L	L	L	L	L	L	L	Good
Shahvegharasl, 2019	L	L	L	L	L	L	L	Good
Mohseni, 2019	L	L	U	L	L	L	L	Fair
Haidari, 2020	L	L	L	L	L	L	H	Fair
El-Bassiouny, 2022	U	U	U	L	U	L	H	Poor
Naderi, 2022	L	U	L	L	H	L	H	Poor

Notes: Study quality assessment domains are graded as either low (L), high (H) or unknown (U) risk of bias. Overall quality of study is graded **Good quality:** if all criteria met (i.e. low for each domain), **Fair quality:** if one criterion not met (i.e. high risk of bias for one domain) or two criteria unclear, and the assessment that this was **unlikely** to have biased the outcome, and there is no known important limitation that could invalidate the results, **Poor quality:** One criterion not met (i.e. high risk of bias for one domain) or two criteria unclear, and the assessment that this was **likely** to have biased the outcome, and there are important limitations that could invalidate the results **OR** Two or more criteria listed as high or unclear risk of bias.

Abbreviations
RS: Bias arising from the random sequence generation (selection bias)
AC: Bias arising from allocation concealment (selection bias)
SR: Bias due to selective reporting
OB: Bias from other sources
BPP: Bias arising from blinding of participants and personnel (performance bias)
BOA: Bias arising from outcome assessment (Detection bias)
IOD: Bias arising from incomplete outcome data (attrition bias)
OSQ: Overall study quality (Good, Fair or Poor)

3.2.4 Effect of vitamin D supplementation on C-reactive protein

The meta-analysis of CRP serum levels included five studies (Haidari et al. 2020; Hopkins et al. 2011; Shahvegharasl et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) with a total of 244 patients with cancer or precancerous lesions. VIDS did not significantly impact CRP serum levels after 8 to 24 weeks of supplementation (SMD, 95% CI: -0.09, -0.35 to 0.16) (see **Figure 2, panel A**). The quality of four studies (Hopkins et al. 2011; Shahvegharasl et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) was rated as good, while one study (Haidari et al. 2020) was rated as fair. A sensitivity analysis of three studies (Haidari et al. 2020; Vahedpoor et al. 2017; Vahedpoor et al. 2018) involving 157 patients with baseline mean 25(OH)D levels in the deficiency range indicated a potential modest effect of VIDS in reducing serum CRP levels, though this effect was not statistically significant (SMD, 95% CI: -0.14, -0.46 to 0.17) (see **Figure 5, panel B**). No heterogeneity was observed in either meta-analysis.

3.2.5 Effect of vitamin D supplementation on tumor necrosis factor-α

The combined results from four studies (Haidari et al. 2020; Hopkins et al. 2011; Mohseni et al. 2019; Naderi et al. 2022) involving 156 patients with cancer or precancerous lesions indicated that 8 to 24 weeks of VIDS had a substantial effect in reducing TNF-α serum levels (SMD, 95% CI: -1.65, -3.07 to -0.24) (see **Figure 6, panel A**). In this meta-analysis, the quality assessment of the included studies showed that two studies (Haidari et al. 2020; Mohseni et al. 2019) were of fair quality, one study (Hopkins et al. 2011) was of good quality, and one study (Naderi et al. 2022) was of poor quality. Significant heterogeneity was observed in this analysis ($I^2 = 93\%$, $p < 0.01$). Therefore, a sensitivity analysis was performed, including only studies with daily dosage regimens of VIDS (total participants = 63). This sensitivity analysis showed a substantial effect in reducing serum TNF-α levels without heterogeneity (SMD, 95% CI: -0.85, -1.37 to -0.33) (see **Figure 6, panel B**).

3.2.6 Effect of vitamin D supplementation on interleukin-6

The meta-analysis of four studies (El-Bassiouny et al. 2022; Haidari et al. 2020; Hopkins et al. 2011; Naderi et al. 2022) examining the effect of VIDS on IL-6 serum levels in 204 patients with cancer or precancerous lesions suggested a substantial decrease in IL-6 levels, although this finding was not statistically significant (SMD, 95% CI: -0.83, -1.78 to 0.13) (see **Figure 7, panel A**). The duration of VIDS ranged from 8 to 24 weeks. Among the included studies, two (El-Bassiouny et al. 2022; Naderi et al. 2022) were of poor quality, and there was considerable heterogeneity observed ($I^2 = 89\%$, $p < 0.01$), with lower effects noted in the higher quality

studies. A subsequent sensitivity analysis of two studies (Haidari et al. 2020; Hopkins et al. 2011) of good and fair quality, involving a total of 84 participants, indicated a small, non-significant reduction in IL-6 levels (SMD, 95% CI: -0.21, -0.64 to 0.22) with no observed heterogeneity ($I^2 = 0\%$, $p = 0.95$) (see **Figure 7, panel B**). It is notable that baseline mean serum 25(OH)D levels were in the normal range in these two studies.

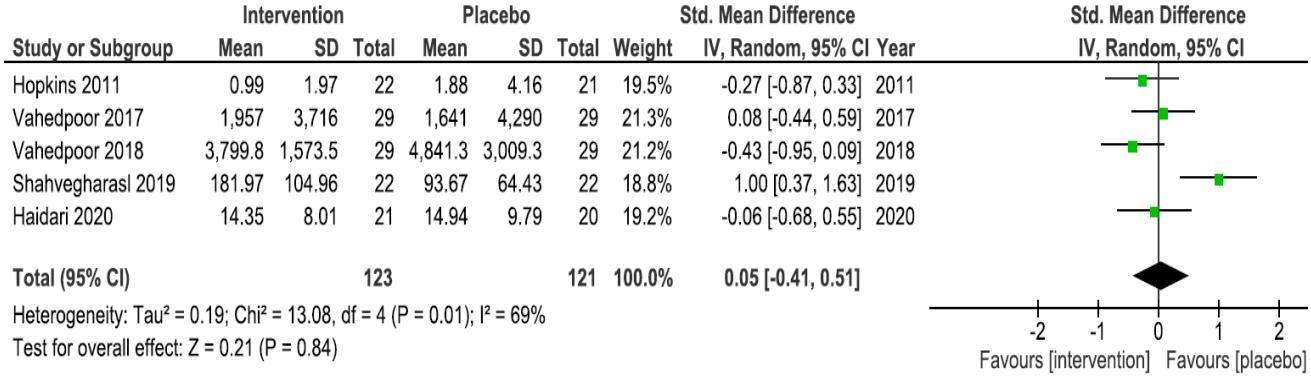
3.2.7 Effect of vitamin D supplementation on interleukin-10

The combined results from two studies (Hopkins et al. 2011; Naderi et al. 2022) involving 63 patients with cancer or precancerous lesions and baseline mean 25(OH)D levels in the normal range showed no effect of daily VIDS dosage regimens for 12 to 24 weeks on serum IL-10 levels (SMD, 95% CI: 0.00, -0.50 to 0.49) (see **Figure 8**). In this meta-analysis, one study (Hopkins et al. 2011) was of good quality, while the other (Naderi et al. 2022) was of poor quality. No heterogeneity was observed ($I^2 = 0\%$, $p = 0.74$).

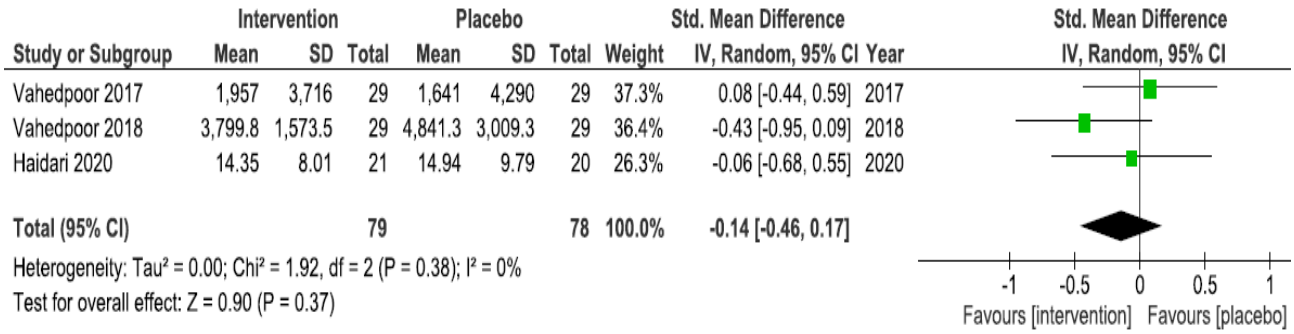
3.2.8 Effects of vitamin D supplementation with co-supplements on inflammatory biomarkers

Meta-analyses showed no significant effect of co-supplementation of VIDS with Omega-3-fatty acids/Calcium on serum levels of CRP (panel A), TNF-α (panel B) and IL-6 (panel C) in patients with cancer/precancer conditions (see **Figure 9**).

Panel A: Effect of VIDS on CRP for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 244).



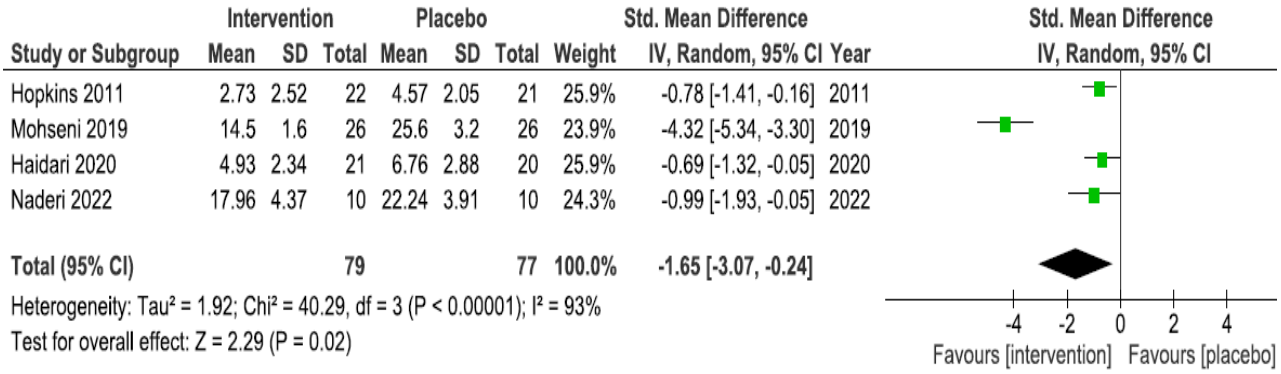
Panel B: Sensitivity analysis (n = 157).



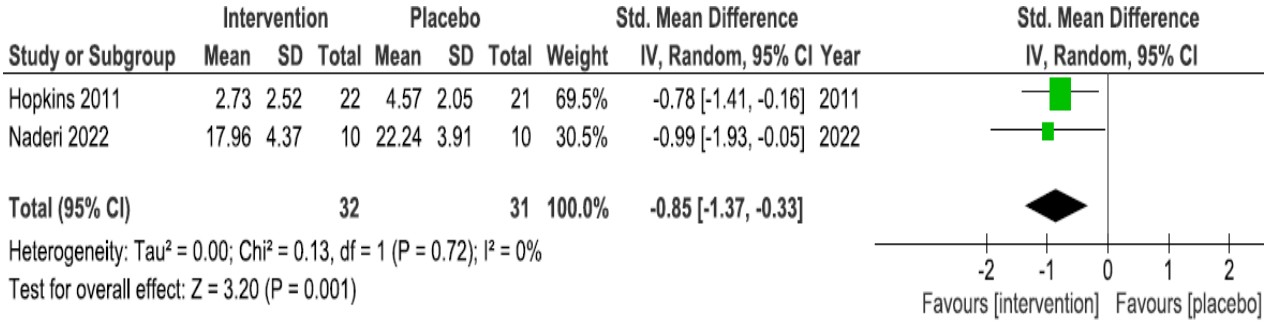
Notes: CRP-C-reactive protein; n-number of study participants; VIDS-vitamin D_3 supplement.

Figure 5. Meta-analyses of studies on the effect of vitamin D supplementation on serum levels of C-reactive protein in patients with cancer/precancer conditions.

Panel A: Effect of VIDS on TNF-α for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 156).



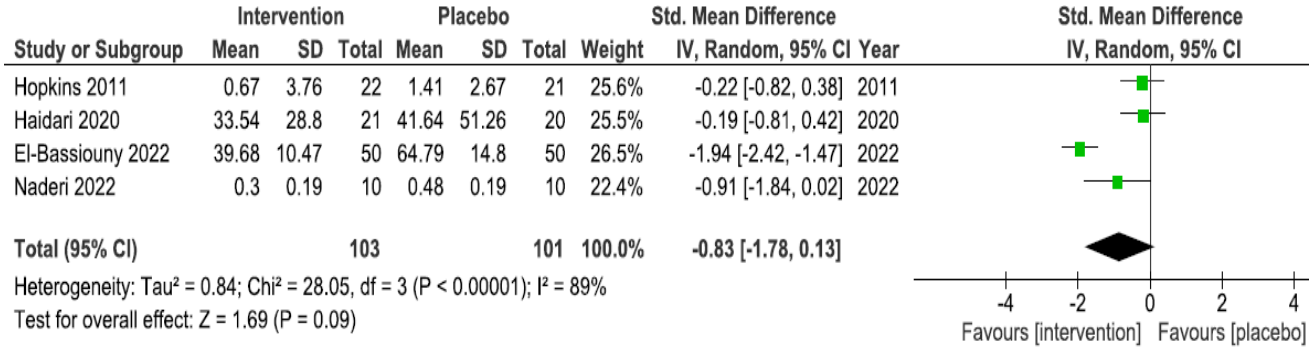
Panel B: Sensitivity analysis (n = 84).



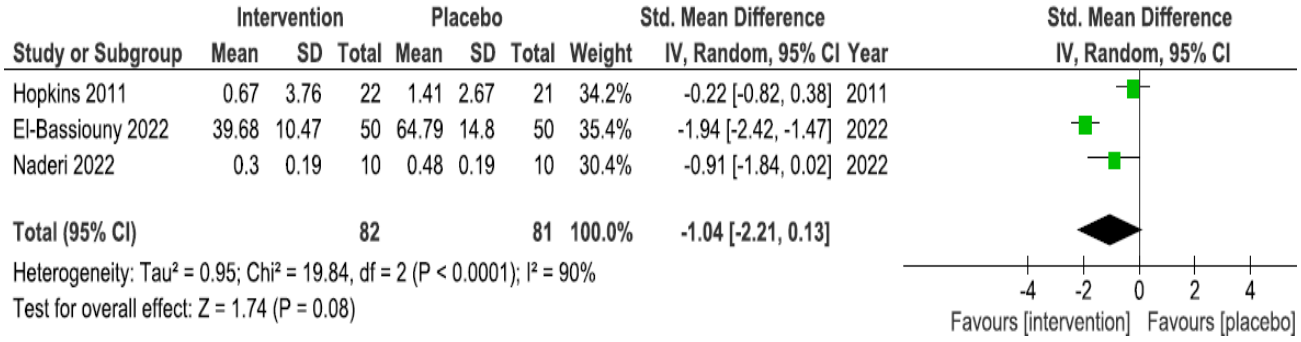
Notes: TNF-α-tumor necrosis factor alpha; n-number of study participants; VIDS-vitamin D_3 supplement.

Figure 6. Meta-analyses of studies on the effect of vitamin D supplementation on serum levels of tumor necrosis factor-alpha in patients with cancer/precancer conditions.

Panel A: Effect of VIDS on IL-6 for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 204).

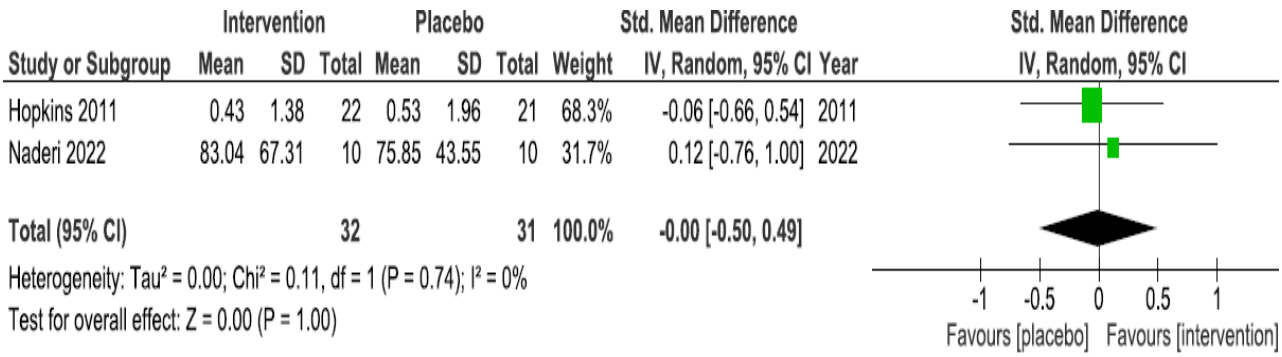


Panel B: Sensitivity analysis (n = 163).



Notes: IL-interleukin; n-number of study participants; VIDS-vitamin D_3 supplement.

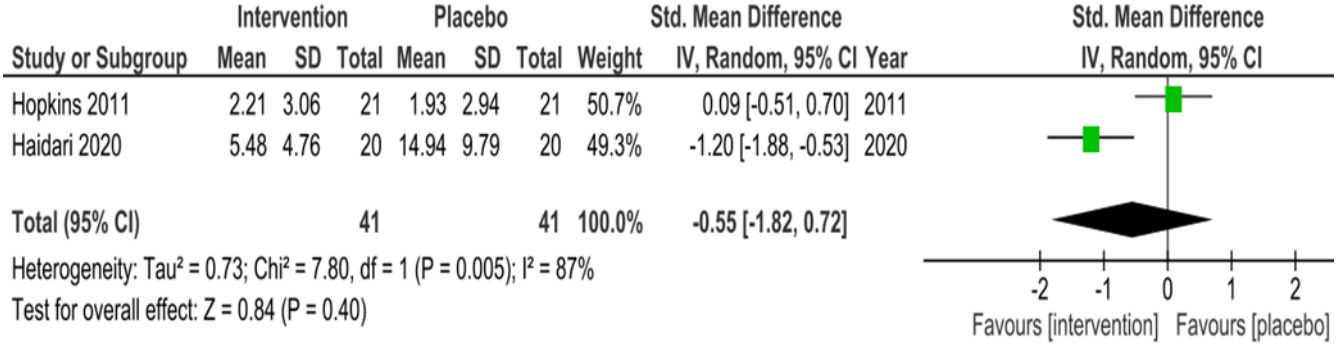
Figure 7. Meta-analyses of studies on the effect of vitamin D supplementation on serum levels of interleukin-6 in patients with cancer/precancer conditions.



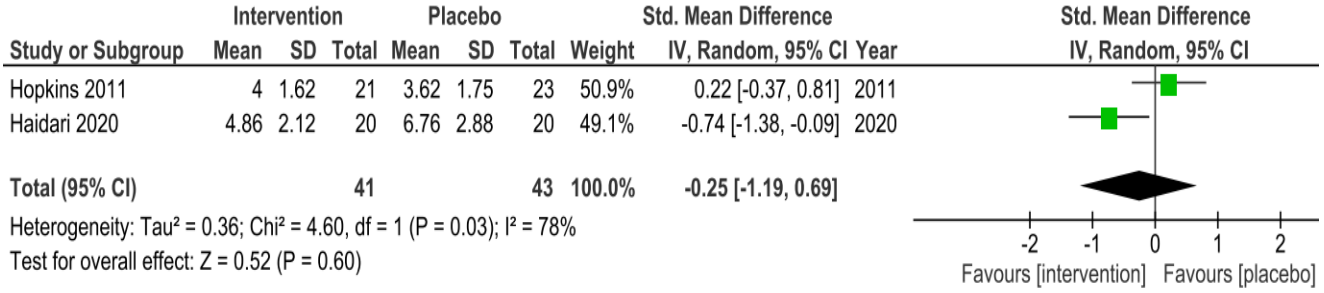
Notes: IL-interleukin; n-number of study participants; VIDS-vitamin D_3 supplement.

Figure 8. Meta-analyses of studies on the effect of vitamin D supplementation serum levels of interleukin-10 in patients with cancer/precancer conditions (n = 63).

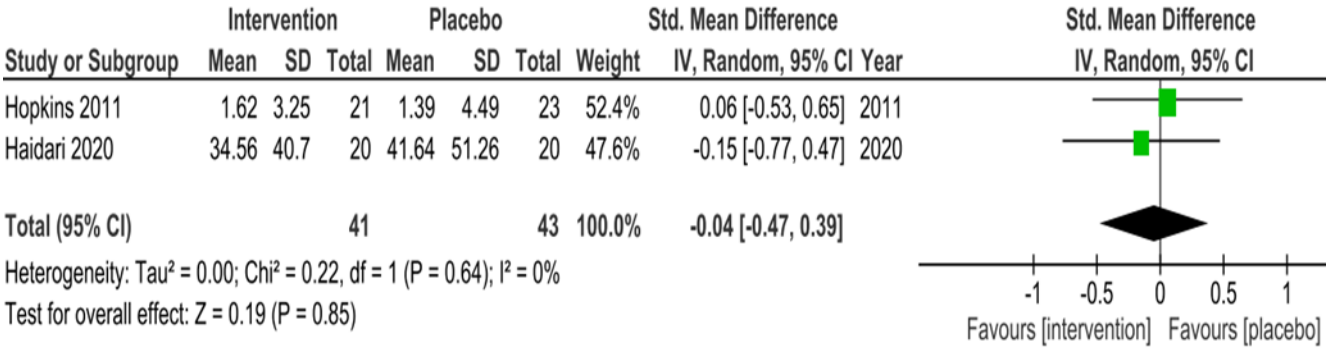
Panel A: Effect of VIDS + Omega-3-fatty acids/Calcium on CRP for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 82).



Panel B: Effect of VIDS + Omega-3-fatty acids/Calcium on TNF-α for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 84).



Panel C: Effect of VIDS + Omega-3-fatty acids/Calcium on IL-6 for patients with cancer/precancer conditions after 8 - 24 weeks intervention (n = 84).



Notes: CRP-c reactive protein; IL-interleukin; TNF-α-tumor necrosis factor alpha; n-number of study participants; VIDS-vitamin D_3 supplement.

Figure 9. Meta-analyses of studies on the effect of co-supplementation of vitamin D supplementation with Omega-3-fatty acids/Calcium on serum levels of C-reactive protein (panel A), tumor necrosis factor-alpha (panel B) and interleukin-6 (panel C).

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3.3 Anti-inflammatory effects of personalized vitamin D supplementation among colorectal cancer patients: randomized trial.

3.3.1 Distribution of patient characteristics at baseline

Patient characteristics are presented in **Table 12**. The age distribution of included patients was similar in the placebo and VIDS groups, with median age of 61 years (IQR 56-68) and 60 years (IQR 55-69), respectively. In both arms, there were more male than female patients. The distribution of CRC stages at diagnosis was similar for stage I -III between groups, while more patients were diagnosed with stage IV in the intervention than in the placebo group (10% vs. 4.6%, respectively). The BMI among patients in both arms was similar, with median of 27.2 kg/m² (IQR 24.0-29.4) in the placebo group and 26.5 kg/m² (IQR 24.5-29.5) in the intervention group. The median 25(OH)D concentration was slightly higher in the intervention group compared to the placebo group (24 nmol/L vs. 20 nmol/L). The median serum levels of log2 normalized IL-6 were slightly elevated in the placebo group compared to the treatment group (3.28 vs. 2.94 pg/ml, respectively) while IFN-γ levels were similar across groups (7.71 pg/ml in the placebo group and 7.80 pg/ml in the intervention group). MMP-1 levels were also comparable, at 15.41 pg/ml and 15.47 pg/ml in the placebo and intervention groups, respectively. The normal distribution plots for the main outcome biomarkers at baseline and at the end of trial stratified by treatment group are shown in **Figures 10 - 12**.

Table 12. Baseline Characteristics at Recruitment in the VICTORIA trial.

Baseline Characteristic	**Placebo, n = 65**[1]	**Treatment, n = 61**[1]
Age [Median; IQR]	61 (56, 68)	60 (55, 69)
Sex Female	17 (26%)	22 (36%)
Male	48 (74%)	39 (64%)
CRC Stage at Diagnosis		
I	19 (29%)	17 (28%)
II	17 (26%)	21 (35%)
III	22 (34%)	14 (23%)
IV	3 (4.6%)	6 (10%)
Unknown	4 (6.2%)	2 (3.3%)
Time Since Surgery		
0-1 month	3 (4.7%)	5 (8.3%)
1-3 months	23 (36%)	25 (42%)
3-6 months	8 (13%)	8 (13%)
6-9 months	10 (16%)	14 (23%)
9-12 months	13 (20%)	5 (8.3%)
>12 months	7 (11%)	3 (5.0%)
Previous Chemotherapy	35 (54%)	33 (54%)
Previous Radiotherapy	20 (31%)	8 (13%)
Diabetes	10 (16%)	12 (20%)
History of CVD[2]	0 (0%)	3 (4.9%)
Hypertension	35 (57%)	31 (51%)
Body Mass Index (kg/m^2) [Median; IQR]	27.2 (24.0, 29.4)	26.5 (24.5, 29.5)
<25	22 (34%)	19 (31%)
25-30	27 (42%)	29 (48%)
>30	16 (25%)	13 (21%)
Alcohol Consumption[3]	59 (94%)	55 (92%)

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Baseline Characteristic	**Placebo, n = 65[1]**	**Treatment, n = 61[1]**
Physical Activity[4]		
Low	33 (54%)	28 (46%)
Adequate	28 (46%)	33 (54%)
25(OH)D nmol/l [Median; IQR]	20 (12, 28)	24 (15, 35)
IL-6 pg/ml [Median; IQR] *	3.28 (2.68, 3.89)	2.94 (2.65, 3.82)
IFN-γ pg/ml [Median; IQR] *	7.71 (7.07, 8.65)	7.80 (7.14, 8.50)
MMP-1 pg/ml [Median; IQR] *	15.41 (15.02, 15.82)	15.47 (15.04, 15.82)
Surgery	65 (100%)	60 (98%)
Time Since Chemotherapy		
0-1month	4 (11%)	7 (21%)
1-3months	9 (26%)	11 (33%)
3-6months	14 (40%)	9 (27%)
6-9months	6 (17%)	4 (12%)
9-12months	0 (0%)	2 (6.1%)
>12 months	2 (5.7%)	0 (0%)
Time Since Radiotherapy		
0-1month	0 (0%)	0 (0%)
1-3months	2 (10%)	1 (13%)
3-6months	4 (20%)	0 (0%)
6-9months	6 (30%)	4 (50%)
9-12months	4 (20%)	1 (13%)
>12 months	4 (20%)	2 (25%)
Planned Chemo/Radiotherapy in the next 3 months	4 (6.2%)	6 (9.8%)
Time Spent in School		
<9yrs	24 (37%)	24 (39%)
>11yrs	12 (18%)	13 (21%)
10-11yrs	28 (43%)	24 (39%)
Other	1 (1.5%)	0 (0%)

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Baseline Characteristic	**Placebo, n = 65[1]**	**Treatment, n = 61[1]**
History of CHF	3 (4.8%)	2 (3.3%)
History of Stroke	0 (0%)	3 (4.9%)
Chronic Obstructive Pulmonary Disease	7 (11%)	4 (6.6%)
Asthma	3 (4.8%)	2 (3.3%)
Renal Failure	3 (4.8%)	1 (1.7%)
Arthropathy	17 (27%)	14 (23%)
Diarrhea in the past week		
None	28 (44%)	35 (57%)
A little	18 (29%)	17 (28%)
Moderate	14 (22%)	4 (6.6%)
A lot	3 (4.8%)	5 (8.2%)
Smoking Status		
Never	24 (39%)	26 (43%)
Former	34 (55%)	24 (39%)
Current	4 (6%)	11 (18%)
Red Meat Consumption		
Never	3 (4.9%)	2 (3.3%)
Up to 3 times a month	10 (16%)	14 (23%)
1-3 days a week	36 (59%)	33 (54%)
4-6 days per week	8 (13%)	10 (16%)
Daily	4 (6.6%)	2 (3.3%)
Stoma	15 (24%)	8 (13%)
Subjective Pain Burden		
1	21 (32%)	25 (41%)
2	24 (37%)	25 (41%)
3	15 (23%)	11 (18%)
4	5 (7.7%)	0 (0%)
Subjective Exhaustion Burden		

Baseline Characteristic	Placebo, n = 65[1]	Treatment, n = 61[1]
1	11 (17%)	5 (8.2%)
continued on next page		
2	16 (25%)	21 (34%)
3	21 (32%)	24 (39%)
4	17 (26%)	11 (18%)
Global Quality of Life		
1	1 (1.5%)	0 (0%)
2	10 (15%)	5 (8.2%)
3	8 (12%)	12 (20%)
4	15 (23%)	16 (26%)
5	20 (31%)	18 (30%)
6	11 (17%)	9 (15%)
7	0 (0%)	1 (1.6%)

*Values were Log2 transformed

[1]n (%) unless otherwise stated; [2]CVD was defined as having diagnosed of Myocardial Infarction, or Stroke or Congestive Heart Failure; [3]During the year before the CRC diagnosis; [4]During the year before the CRC diagnosis; physical activity was assessed with the Rapid Assessment of Physical Activity questionnaire (Topolski et al. 2006). However, we used the definition of the Healthy Lifestyle Score for healthy physical activity, which was as follows: at least 150 min of moderate-intensity or 75 min of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate-intensity physical activity are needed to meet the recommendations of healthy physical activity (Erben et al. 2019).

Abbreviations: 25 (OH)D, 25-hydroxyvitamin D; CHF, congestive heart failure; CRC, colorectal cancer; CVD, cardiovascular disease; IFN-γ, interferon gamma; IL-6, interleukin 6; IQR, interquartile range; MMP-1, matrix metalloproteinase-1

Missing: CRC Stage at Diagnosis (n = 1), Time Since Surgery (n = 2), Previous Radiotherapy (n = 1), Diabetes (n = 3), History of CVD (n = 4), Hypertension (n = 4), Alcohol Consumption (n = 3), Physical Activity (n = 4), Time since chemotherapy (n = 58), Time since radiotherapy (n = 98), CHF (n = 3), Stroke (n = 4), Chronic obstructive pulmonary disease (n = 3), Asthma (n = 3), Renal failure (n = 4), Arthropathy (n = 3), Diarrhea (n = 2), Smoking (n = 3), Red meat consumption (n = 4), Stoma (n = 2).

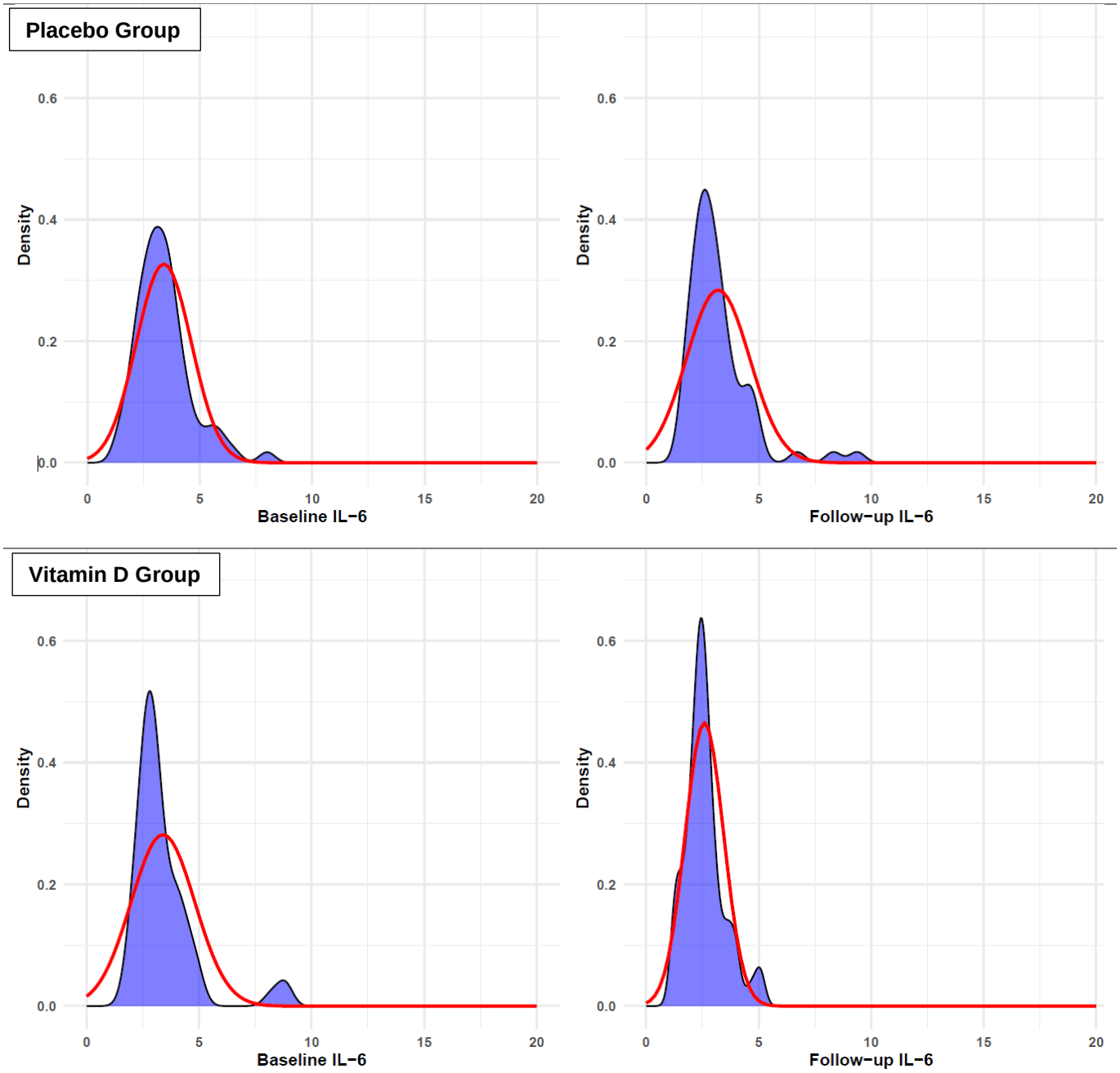


Figure 10. Distribution patterns of log2 interleukin-6 (pg/ml) for placebo and intervention groups at baseline and end of trial.

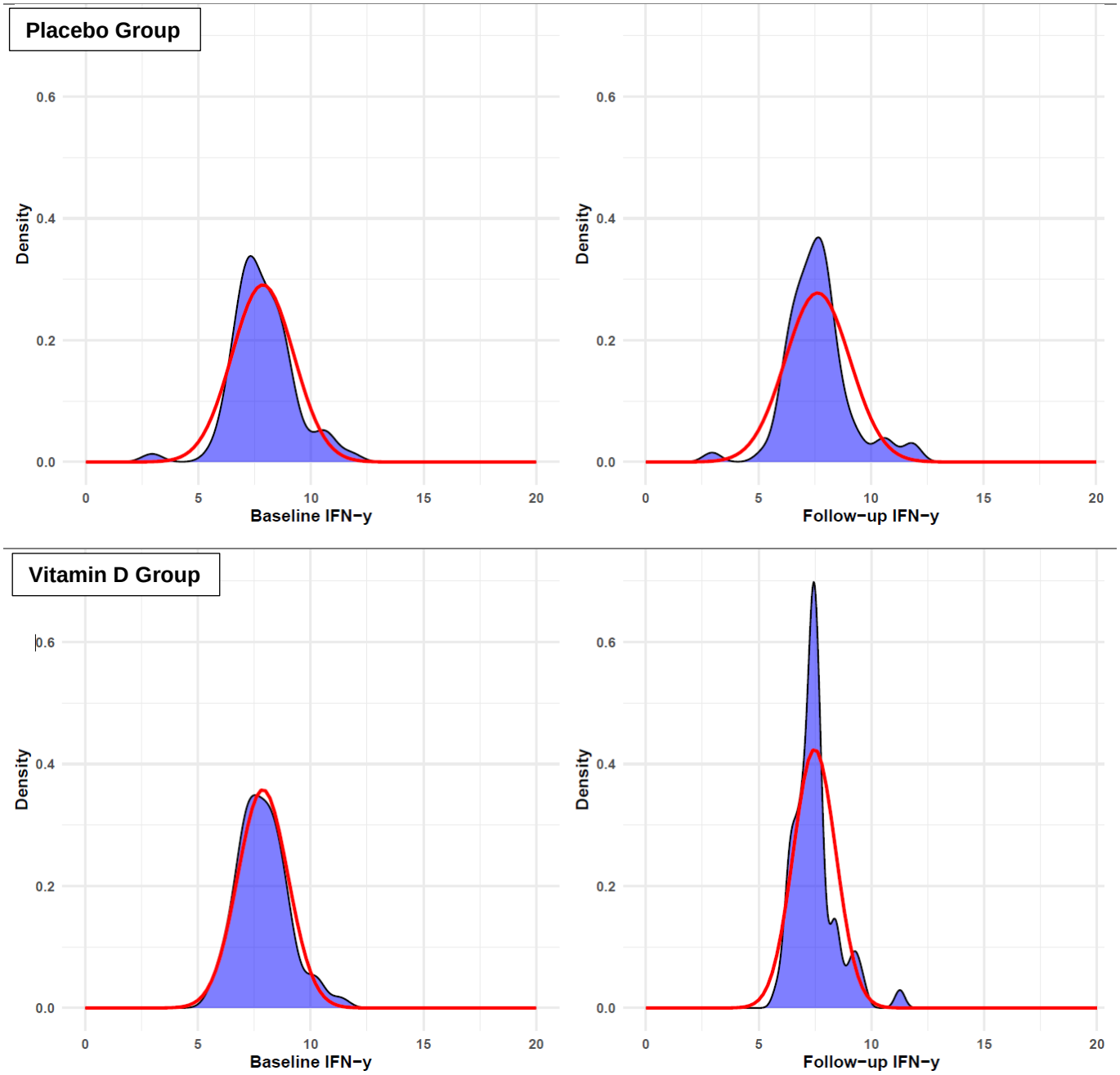


Figure 11. Distribution patterns of log2 interferon-gamma (pg/ml) for placebo and intervention groups at baseline and end of trial.

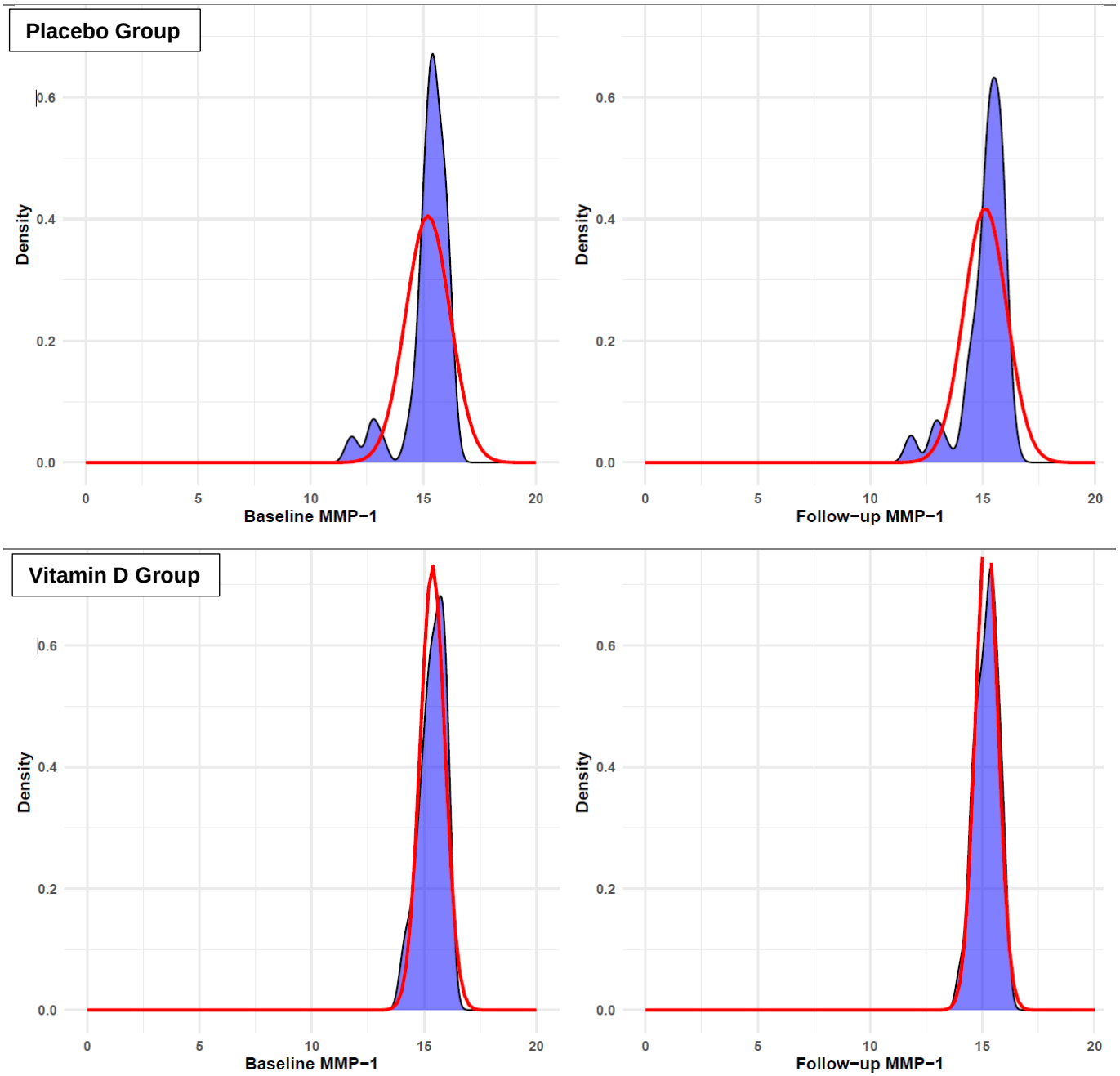


Figure 12. Distribution patterns of log2 matrix metalloproteinase-1 (pg/ml) for placebo and intervention groups at baseline and end of trial.

3.3.2 Serum 25(OH)D concentrations and prevalence of serum vitamin D inadequacy at different follow-up times

The changes in mean serum 25(OH)D levels from BL to FU2 are graphically presented in **Figure 13** and tabulated in **Table 13**. At BL, the mean serum 25(OH)D level was 22.1 nmol/L in the placebo group and 25.7 nmol/L in the intervention group. At FU1 the mean serum 25(OH)D level increased to 34.9 nmol/L in the placebo group, while in the intervention group it increased significantly to 69.6 nmol/L. Overall, the placebo group showed a total increase of 15.6 nmol/L (71%) in serum 25(OH)D levels from BL to FU2, whereas the intervention group demonstrated a significant overall increase of 49.4 nmol/L (192%). The prevalence of serum vitamin D inadequacy, defined as 25(OH)D levels less than 50 nmol/L (Ross et al, 2011), was 100% in the placebo group and 98.4% in the intervention group at BL (see **Table 14**). At FU1, the prevalence of vitamin D inadequacy in the placebo group decreased to 87.5%, while in the intervention group, it was reduced to 8.3%, indicating a significant correction of vitamin D inadequacy. Similar prevalence patterns to those observed at FU1 were also observed at FU2, with an overall 90% decrease in vitamin D inadequacy in the intervention group.

3.3.3 Changes in inflammatory biomarker serum levels at the end of trial

The differences in mean serum levels of IL-6, IFN-γ, and MMP-1 in the placebo and intervention groups at FU2 are graphically depicted in **Figure 14** with further details in **Table 15**. A significant difference was observed for IL-6 levels between the placebo and intervention group with 33.4% lower IL-6 serum levels in the intervention group (95%CI: 13 - 50%). However, these differences were not statistically significant for IFN-γ (10.1% lower in the intervention group, 95%CI: -33.9 to 20.5%) and MMP-1 (5.8% higher in the intervention group, 95%CI: -11.5 to 27.2%). In the PP analysis, results remained more or less similar to those reported in the ITT analysis (see **Table 16**).

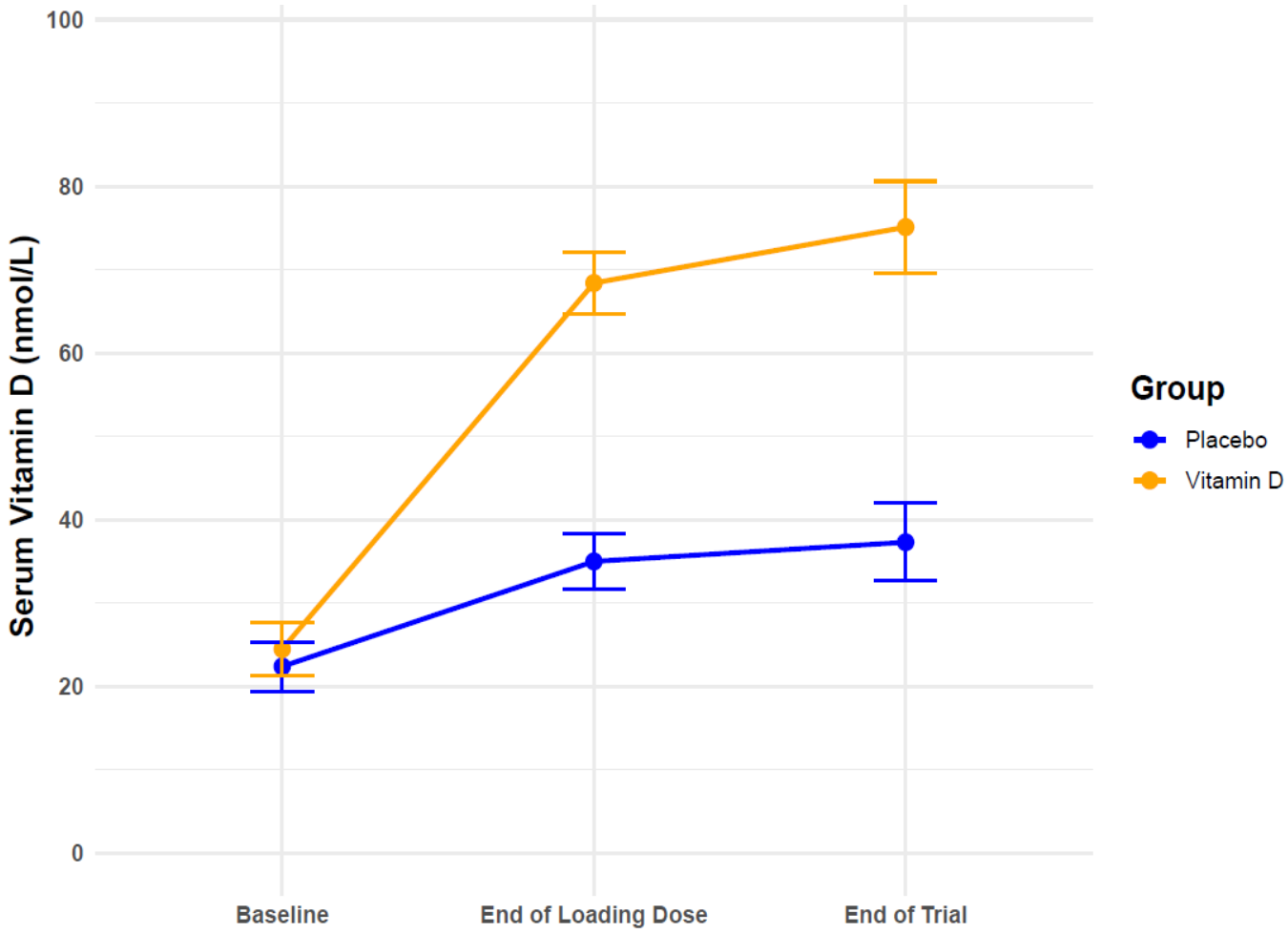


Figure 13. Change in serum vitamin D concentrations at different follow-up times.

Table 13. Serum 25(OH)D concentration at different follow-up time-points: Intention-To-Treat Analysis.

Timepoint	**Placebo** (n = 65)		**Treatment** (n = 61)	
	Mean (95% CI)	Change (95% CI)	Mean (95% CI)	Change (95% CI)
BL	22.1 (19.3, 25.0)	-	25.7 (22.5, 28.9)	-
FU1	34.9 (31.7, 38.1)	12.8 (9.1, 16.4)[1]	69.6 (65.8, 73.4)	44.2 (39.5, 49.0)[1]
FU2	37.7 (33.2, 42.3)	2.6 (-0.9, 6.0)[2]	75.1 (70.1, 80.2)	5.8 (1.0, 10.6)[2]
Overall Change (95%CI)	15.6 (10.5, 20.7)[3]		49.4 (43.3, 55.5)[3]	

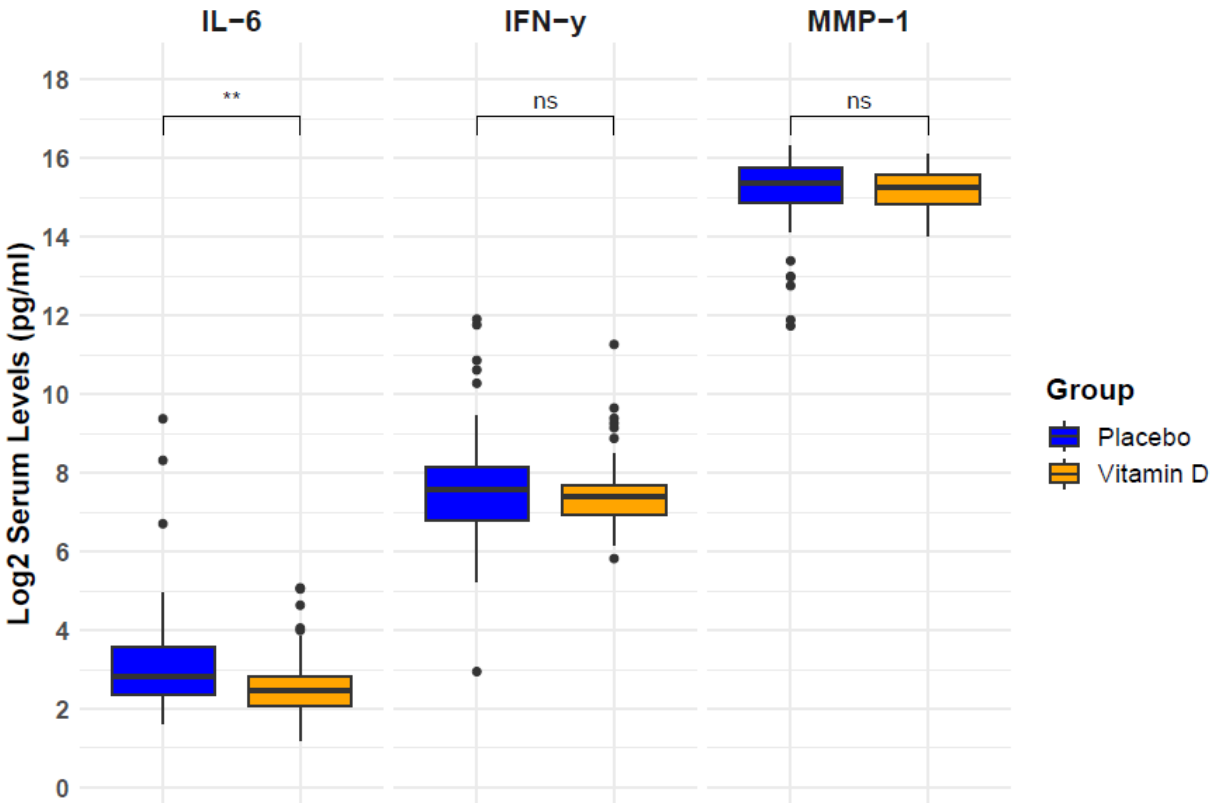
Notes: Serum 25(OH)D values are in nmol/L; [1]Difference between FU1 and BL mean values; [2]Difference between FU2 and FU1 mean values; [3]Difference between FU2 and BL mean values.
Abbreviations: BL, baseline; CI, confidence interval; FU1, end of rehabilitation; FU2, end of trial

Table 14. Prevalence of vitamin D inadequacy [25(OH)D levels < 50 nmol/L] at different follow-up times: Intention-To-Treat Analysis.

Timepoint	**Placebo** (n = 65)	**Treatment** (n = 61)
	Proportion (95% CI)	Proportion (95% CI)
BL	100.0 (94.5 – 100.0)	98.4 (91.2 – 100.0)
FU1	87.5 (76.8 – 94.4)	8.3 (2.8 – 18.4)
FU2	76.9 (64.8 – 86.5)	8.2 (2.7 – 18.1)

Notes: Proportions are presented as percentages
Abbreviations: BL, baseline; CI, confidence interval; FU1, end of rehabilitation; FU2, end of trial

The results of the ITT multivariable linear regression analysis for estimating the changes in serum concentrations of IL-6, IFN-γ, and MMP-1 due to VIDS are presented in **Table 17**. The estimated percentage change for IL-6 in the intervention compared to the placebo group was -39.3% (95% CI, -54.9 to -18.2%), with a p-value of 0.001. However, for IFN-γ and MMP-1 these changes were not statistically significant [-6.7%; (95% CI, -30.3 to 27.5%) and -5.4%; (95% CI, -12.9 to 3.5%), respectively]. In the PP and sensitivity analyses, similar results were observed as for the ITT analysis, although with a slightly more pronounced effect of VIDS on IL-6 (see **Table 18** and **19**). In the exploratory analyses including the remaining 69 biomarkers, VIDS showed promising effects in reducing CUB domain-containing protein 1 (CDCP1), C-X-C motif chemokine (CXCL) 11, and CXCL 6 compared to placebo (estimated change in the intervention group: -11.1%, p = 0.03; -17.1%, p = 0.04; and -13.5%, p = 0.02, respectively) (see **Table 20**). For the main analysis, no violation of linear regression assumptions was deemed unsatisfactory or needing any further investigation (see **Figures 15 - 17**).



Abbreviations: IFN-y, interferon gamma; IL-6, interleukin-6; MMP-1; ns, non-significant

Figure 14. Differences in mean biomarker levels between placebo and intervention groups at the end of trial (Intention-To-Treat, n = 126).

Table 15. Differences in mean biomarker levels between placebo and intervention groups at the end of trial: Intention-To-Treat Analysis.

Treatment Group	Biomarker		
	IL-6	**INF-γ**	**MMP-1**
Placebo (n = 65)	3.18 (1.40)	7.63 (1.44)	15.11 (0.95)
Intervention (n = 61)	2.59 (0.86)	7.48 (0.94)	15.19 (0.50)
Log2 Difference (95%CI)	**-0.59 (-1.00, -0.19)**	-0.15 (-0.58, 0.27)	0.08 (-0.18, 0.35)
% Difference[1]	**-33.4 (-50.0, -13.0)**	-10.1 (-33.9, 20.5)	5.8 (-11.5, 27.2)

Notes: Serum biomarker values are in pg/mL and were $\log_2$ transformed; mean values are presented with their respective standard deviations in parentheses; Bold figures are statistically significant.
[1]Calculated from the formula ($2^{\log 2 \text{ difference}}$ - 1) x 100%.
Abbreviations: CI, confidence interval; IFN-γ, interferon-gamma; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1

Table 16. Differences in mean biomarker levels between placebo and intervention groups at the end of trial (Per Protocol, n = 120).

Treatment Group	Biomarker		
	IL-6	**INF-γ**	**MMP-1**
Placebo (n = 63)	3.19 (1.43)	7.66 (1.45)	15.15 (0.86)
Intervention (n = 57)	2.53 (0.82)	7.49 (0.96)	15.19 (0.51)
Log2 Difference (95%CI)	**-0.66 (-1.07, -0.24)**	-0.17 (-0.60, 0.28)	0.04 (-0.21, 0.30)
% Actual Difference[1]	**-35.0 (-52.0, -15.0)**	-11.0 (-34.0, 21.0)	3.0 (-13.0, 23.0)

Notes: Serum biomarker values are in pg/mL and were $\log_2$ transformed; Mean values are presented with their respective standard deviations in parentheses.

[1]Calculated from the formula $(2^{\log 2 \text{ difference}} - 1) \times 100\%$.

Abbreviations: CI, confidence interval; IFN-γ, interferon-gamma; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1.

Table 17. Linear regression estimates of the change in inflammatory biomarker levels due to vitamin D supplementation at the end of trial (Intention to-Treat, n = 126).

Biomarker[1]	Model 1				Model 2			
	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]
IL-6	**-0.59 (-1.01, -0.18)**	0.21	**0.005**	**-33.6 (-50.3, -11.7)**	**-0.72 (-1.15, -0.29)**	0.22	**0.001**	**-39.3 (-54.9, -18.2)**
IFN-γ	-0.15 (-0.59, 0.28)	0.22	0.479	-9.9 (-33.6, 21.4)	-0.10 (-0.52, 0.35)	0.22	0.692	-6.7 (-30.3, 27.5)
MMP-1	0.09 (-0.19, 0.36)	0.14	0.535	6.4 (-12.3, 28.3)	-0.08 (-0.20, 0.05)	0.06	0.227	-5.4 (-12.9, 3.5)

[1]Biomarkers values were $\log_2$ transformed; Bold figures are statistically significant after adjustment for type-1 error (FWER) using Bonferroni correction with α-threshold of 0.0166. Model 1, univariable; Model 2, adjusted for baseline concentration of the respective inflammatory biomarker (continuous), baseline age (continuous), sex, baseline serum 25(OH)D (continuous), BMI (continuous), cancer stage (I, II, III, or IV), time since surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), previous chemotherapy and previous radiotherapy.

[2]Calculated from the formula $(2^{\beta} - 1) \times 100\%$

Abbreviations: CI, confidence interval; IFN-γ, interferon-gamma; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1; SE, standard error

Table 18. Linear regression estimates of the change in inflammatory biomarker levels due to vitamin D supplementation at the end of trial (Per Protocol, n = 120).

Biomarker[1]	Model 1				Model 2			
	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]
IL-6	**-0.66 (-1.08, -0.23)**	0.22	**0.003**	**-36.7 (-52.7, -14.7)**	**-0.71 (-1.16, -0.26)**	0.23	**0.002**	**-38.9 (-55.2, -16.5)**
IFN-γ	-0.16 (-0.61, 0.29)	0.23	0.476	-10.5 (-34.5, 22.3)	-0.19 (-0.57, 0.33)	0.23	0.602	-12.3 (-32.6, 25.7)
MMP-1	0.05 (-0.21, 0.30)	0.13	0.727	3.5 (-13.5, 23.1)	-0.08 (-0.21, 0.05)	0.06	0.212	-5.4 (-13.5, 3.5)

[1]Biomarkers values were $\log_2$ transformed; Bold figures are statistically significant after adjustment for type-1 error (FWER) using Bonferroni correction with α-threshold of 0.0166. Model 1, univariable; Model 2, adjusted for baseline concentration of the respective inflammatory biomarker (continuous), baseline age (continuous), sex, baseline serum 25(OH)D (continuous), BMI (continuous), cancer stage (I, II, III, or IV), time since surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), previous chemotherapy and previous radiotherapy; [2]Calculated from the formula $(2^{\beta} - 1) \times 100\%$

Abbreviations: CI, confidence interval; IFN-γ, interferon-gamma; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1; SE, standard error

Table 19. Sensitivity analysis: Linear regression estimates of the change in inflammatory biomarker levels due to vitamin D supplementation at the end of trial excluding patient samples with Quality Control Warnings (Per Protocol, n = 113).

Biomarker[1]	Model 1				Model 2			
	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]	β-coefficient (95% CI)	SE	P-value	% Change (95% CI)[2]
IL-6	**-0.65 (-1.10, -0.19)**	0.23	**0.006**	**-36.3 (-53.3, -12.3)**	**-0.79 (-1.27, -0.31)**	0.24	**0.002**	**-42.2 (-58.5, -19.3)**
IFN-γ	-0.15 (-0.64, 0.33)	0.24	0.525	-9.9 (-35.8, 25.7)	-0.09 (-0.58, 0.40)	0.25	0.722	-6.0 (-33.1, 32.0)
MMP-1	0.07 (-0.21, 0.35)	0.14	0.621	5.0 (-13.5, 27.5)	-0.06 (-0.20, 0.07)	0.07	0.342	-4.1 (-12.9, 5.0)

[1]Biomarkers values were $\log_2$ transformed; Bold figures are statistically significant after adjustment for type-1 error (FWER) using Bonferroni correction with α-threshold of 0.0166. Model 1, univariable; Model 2, adjusted for baseline concentration of the respective inflammatory biomarker (continuous), baseline age (continuous), sex, baseline serum 25(OH)D (continuous), BMI (continuous), cancer stage (I, II, III, or IV), time since surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), previous chemotherapy and previous radiotherapy; [2]Calculated from the formula $(2^{\beta} - 1) \times 100\%$

Abbreviations: CI, confidence interval; IFN-γ, interferon-gamma; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1; SE, standard error

Table 20. Exploratory linear regression estimates of the effects of vitamin D supplementation on 69 inflammatory biomarkers at the end of trial (Intention-To-Treat Analysis, n = 126).

Abbreviation	Biomarker name	β (s.e)	P-value
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1	-0.15 (0.22)	0.484
ADA	Adenosine Deaminase	-0.03 (0.11)	0.799
AXIN1	Axin-1	-0.11 (0.16)	0.503
CASP-8	Caspase-8	-0.07 (0.20)	0.743
CCL11	Eotaxin	-0.12 (0.06)	0.053
CCL19	C-C motif chemokine 19	0.05 (0.09)	0.583
CCL20	C-C motif chemokine 20	-0.26 (0.20)	0.196
CCL23	C-C motif chemokine 23	0.04 (0.07)	0.496
CCL25	C-C motif chemokine 25	0.02 (0.07)	0.738
CCL28	C-C motif chemokine 28	-0.13 (0.07)	0.062
CCL3	C-C motif chemokine 3	-0.20 (0.13)	0.128
CCL4	C-C motif chemokine 4	-0.03 (0.10)	0.776
CD244	Natural killer cell receptor 2B4	-0.04 (0.06)	0.480
CD40	CD40L receptor	-0.02 (0.07)	0.820
CD5	T-cell surface glycoprotein CD5	-0.10 (0.06)	0.087
CD6	T cell surface glycoprotein CD6 isoform	-0.08 (0.09)	0.403
CD8A	T-cell surface glycoprotein CD8 alpha chain	-0.06 (0.09)	0.536
CDCP1	**CUB domain-containing protein 1**	**-0.17 (0.08)**	**0.034**
CSF-1	Macrophage colony-stimulating factor 1	-0.06 (0.03)	0.080
CST5	Cystatin D	-0.04 (0.06)	0.573
CX3CL1	Fractalkine	-0.04 (0.07)	0.509
CXCL1	C-X-C motif chemokine 1	0.02 (0.10)	0.874
CXCL10	C-X-C motif chemokine 10	-0.10 (0.23)	0.662
CXCL11	**C-X-C motif chemokine 11**	**-0.27 (0.13)**	**0.042**
CXCL5	C-X-C motif chemokine 5	-0.08 (0.09)	0.359
CXCL6	**C-X-C motif chemokine 6**	**-0.21 (0.09)**	**0.023**
CXCL9	C-X-C motif chemokine 9	-0.02 (0.13)	0.850
DNER	Delta and Notch-like epidermal growth factor-related receptor	-0.03 (0.04)	0.532
EN-RAGE	Protein S100-A12	0.02 (0.21)	0.918
FGF-19	Fibroblast growth factor 19	-0.01 (0.21)	0.943
FGF-21	Fibroblast growth factor 21	-0.10 (0.22)	0.655
FGF-5	Fibroblast growth factor 5	-0.06 (0.05)	0.292
Flt3L	Fms-related tyrosine kinase 3 ligand	-0.06 (0.06)	0.313
HGF	Hepatocyte growth factor	-0.02 (0.07)	0.766
IL-10	Interleukin-10	0.03 (0.09)	0.721
IL-10RA	Interleukin-10 receptor subunit alpha	-0.02 (0.06)	0.800
IL-10RB	Interleukin-10 receptor subunit beta	-0.02 (0.04)	0.567
IL-12B	Interleukin-12 subunit beta	-0.02 (0.08)	0.760
IL-15RA	Interleukin-15 receptor subunit alpha	0.01 (0.05)	0.912
IL-17C	Interleukin-17C	0.07 (0.20)	0.711
IL-18	Interleukin-18	0.00 (0.09)	0.994
IL-18R1	Interleukin-18 receptor 1	0.02 (0.08)	0.789

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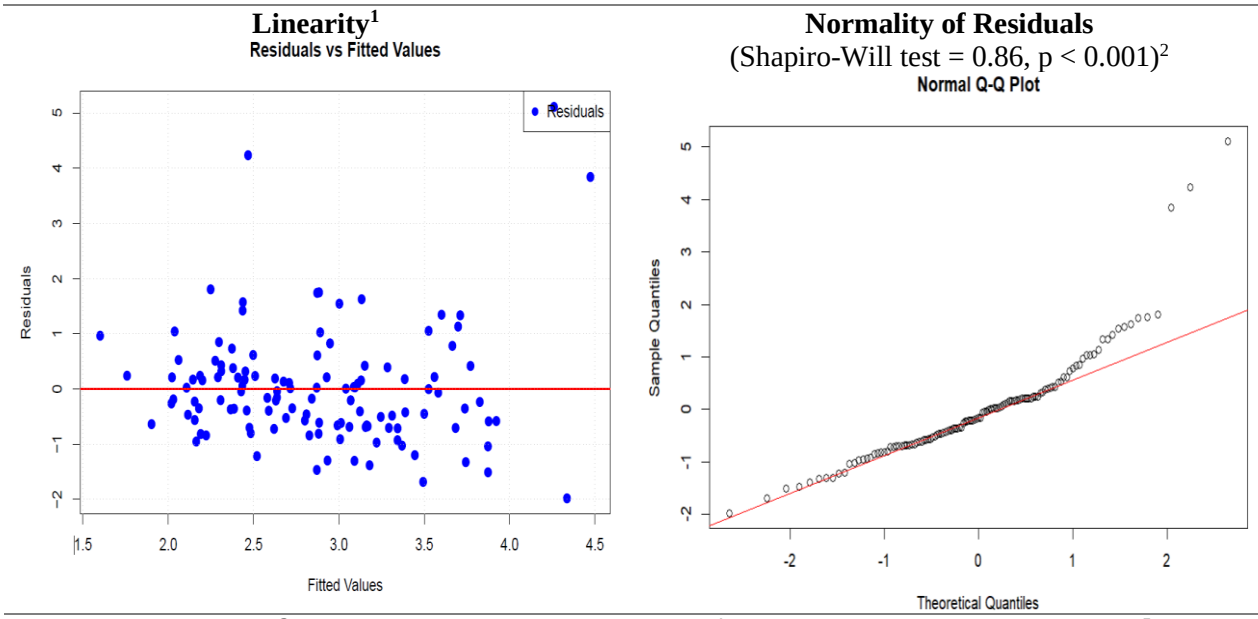
Abbreviation	Biomarker name	β (s.e)	P-value
IL-7	Interleukin-7	0.04 (0.11)	0.718
IL-8	Interleukin-8	0.11 (0.28)	0.701
LAP TGF-beta-1	Latency-associated peptide transforming growth factor beta-1	0.01 (0.07)	0.920
LIFR	Leukemia inhibitory factor receptor	-0.05 (0.05)	0.297
MCP-1	Monocyte chemotactic protein 1	-0.08 (0.07)	0.298
MCP-2	Monocyte chemotactic protein 2	-0.02 (0.07)	0.830
MCP-3	Monocyte chemotactic protein 3	-0.09 (0.23)	0.692
MCP-4	Monocyte chemotactic protein 4	-0.11 (0.09)	0.226
MMP-10	Matrix metalloproteinase-10	-0.04 (0.09)	0.682
OPG	Osteoprotegerin	-0.10 (0.05)	0.058
OSM	Oncostatin-M	0.13 (0.18)	0.457
PD-L1	Programmed cell death 1 ligand 1	-0.03 (0.06)	0.614
SCF	Stem cell factor	-0.04 (0.07)	0.591
SIRT2	SIR2-like protein 2	-0.10 (0.22)	0.638
SLAMF1	Signaling lymphocytic activation molecule	0.00 (0.07)	0.976
ST1A1	Sulfotransferase 1A1	-0.02 (0.15)	0.887
STAMBP	STAM-binding protein	-0.01 (0.16)	0.954
TGF-alpha	Transforming growth factor alpha	0.14 (0.11)	0.202
TNF	Tumor necrosis factor	-0.03 (0.08)	0.660
TNFB	TNF-beta	-0.02 (0.06)	0.667
TNFRSF9	Tumor necrosis factor receptor superfamily member 9	-0.01 (0.06)	0.834
TNFSF14	Tumor necrosis factor ligand superfamily member 14	0.04 (0.13)	0.740
TRAIL	TNF-related apoptosis-inducing ligand	-0.01 (0.06)	0.868
TRANCE	TNF-related activation-induced cytokine	0.12 (0.11)	0.311
TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12	0.01 (0.06)	0.880
uPA	Urokinase-type plasminogen activator	-0.05 (0.05)	0.359
VEGF-A	Vascular endothelial growth factor-A	-0.05 (0.08)	0.564

[1]Biomarkers values were $\log_2$ transformed; Biomarkers in bold have p-values < 0.05 after adjustment for baseline concentration of the respective inflammatory biomarker (continuous), baseline age (continuous), sex, baseline serum 25(OH)D (continuous), BMI (continuous), cancer stage (I, II, III, or IV), time since surgery (No surgery, 0-1, 2-3, 4-6, 7-9, 10-12, >12 months), previous chemotherapy and previous radiotherapy.
Abbreviations: s.e, standard error.

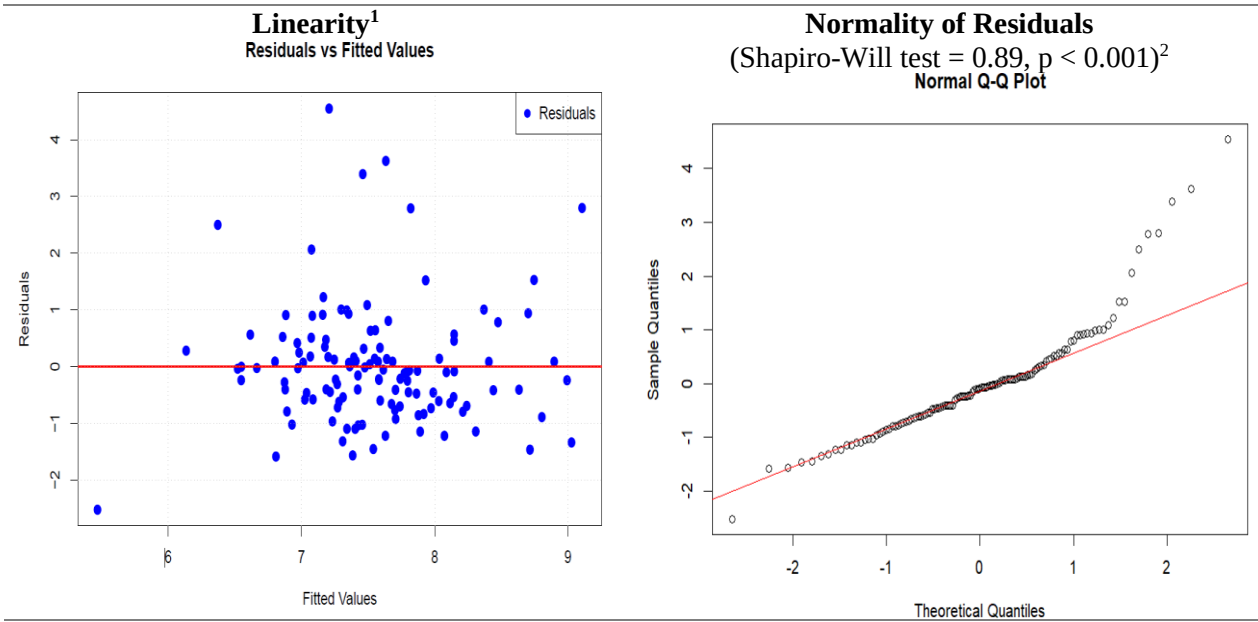


Correlations[3]
Autocorrelation = -0.02; Durbin-Watson statistic = 2.03; P = 0.96

Homoscedasticity[4]
Breusch-Pagan test = 19.59; df = 13; p = 0.11

Multicollinearity[5]
Variance Inflation Factors (VIF) range from 1.13 – 2.85

Figure 15. Test for linear regression assumptions for estimating interleukin-6 change (Intention-To-Treat analysis).

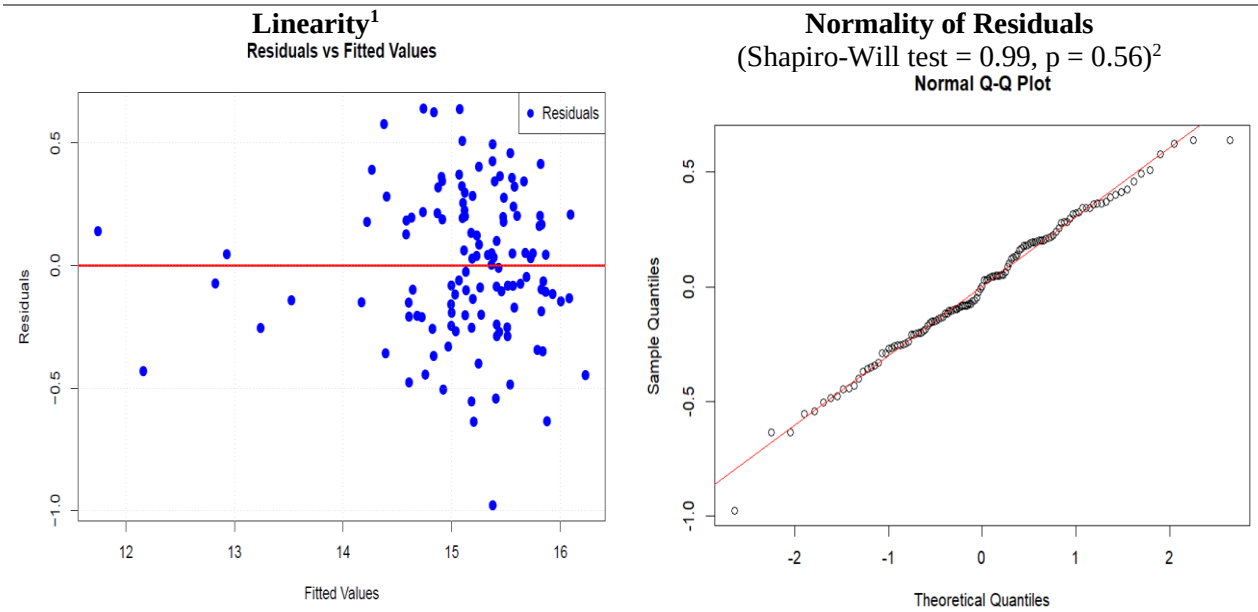


Correlations[3]
Autocorrelation = -0.16; Durbin-Watson statistic = 2.30; P = 0.07

Homoscedasticity[4]
Breusch-Pagan test = 7.36; df = 13; p-value = 0.88

Multicollinearity[5]
Variance Inflation Factors (VIF) range from 1.08 – 2.91

Figure 16. Test for linear regression assumptions for estimating interferon-gamma change (Intention-To-Treat analysis).



Correlations[3]	Homoscedasticity[4]	Multicollinearity[5]
Autocorrelation = -0.06; Durbin-Watson statistic = 2.10; P = 0.69	Breusch-Pagan test = 15.73; df = 13; p-value = 0.26	Variance Inflation Factors (VIF) range from 1.08 – 2.83

Figure 17. Test for linear regression assumptions for estimating matrix metalloproteinase-1 change (Intention-To-Treat analysis).

[1]Linearity Visual Inspection: Plot residuals vs. fitted values. If the plot shows a random pattern, linearity is likely satisfied.

[2]Normality of Residuals: Residuals should be approximately normally distributed. Q-Q Plot: Compare the distribution of residuals to a normal distribution. Shapiro-Wilk Test: Formal test for normality. The Shapiro-Wilk test is used to determine whether a sample comes from a normally distributed population. H_0: The data is normally distributed. H_1: The data is not normally distributed.

[3]The Durbin-Watson test is used to detect the presence of autocorrelation (serial correlation) in the residuals of a regression analysis. The Durbin-Watson statistic ranges from 0 to 4. A value around 2 suggests no autocorrelation. A value less than 2 indicates positive autocorrelation. A value greater than 2 indicates negative autocorrelation. H_0: There is no autocorrelation in the residuals. H_1: There is autocorrelation in the residuals.

[4]Breusch-Pagan Statistic: quantifies the degree of heteroscedasticity detected in the residuals. H_0: Homoscedasticity (the residuals have constant variance). H_1: Heteroscedasticity (the residuals do not have constant variance).

[5]Variance Inflation Factor (VIF) is a measure of how much the variance of a regression coefficient is inflated due to multicollinearity among the predictor variables. VIF = 1: There is no multicollinearity between the predictor variable and the other predictor variables in the model. 1 < VIF < 5: Moderate multicollinearity exists, but it is generally not severe enough to require corrective measures. VIF ≥ 5: High multicollinearity is present. This may indicate that the predictor variable is highly collinear with other predictor variables, which can lead to unreliable estimates of regression coefficients. VIF ≥ 10: Very high multicollinearity exists, often considered a sign that the model has severe multicollinearity issues, and corrective measures are typically required.

4 Discussion

4.1 Vitamin D Status, *Cdx2* Genotype, and Colorectal Cancer Survival: Population-Based Patient Cohort.

In this large cohort of CRC patients, those with deficient vitamin D status exhibited significantly poorer survival compared to patients with insufficient or sufficient vitamin D levels. This association was particularly evident in the majority of patients carrying the GG genotype of rs11568820 (*Cdx2*), whereas no clear pattern emerged among those with the AA/AG genotype. However, tests for interaction between vitamin D status and genotype did not achieve statistical significance.

4.1.1 Vitamin D status and colorectal cancer survival

My results demonstrated significant associations between vitamin D status and survival outcomes, independent of other established prognostic factors such as the stage at diagnosis. The findings indicate that patients with serum 25(OH)D levels in the vitamin D deficiency range (<30 nmol/L) had substantially worse survival compared to those with higher concentrations, aligning with previous observational studies (Bao et al. 2020; Maalmi et al. 2018; Vaughan-Shaw et al. 2020b; Zhou et al. 2021). In my study, these associations were consistently observed across all four major survival outcomes assessed. Although observational studies do not establish causality, the consistency of these findings with a recent meta-analysis of RCTs is notable. The meta-analysis reported a 30% lower risk for CSS and progression-free survival (PFS) outcomes with VIDS among CRC patients (Vaughan-Shaw et al. 2020a).

The exact mechanism by which vitamin D improves survival in CRC patients remains unclear. However, mechanistic studies suggest that calcitriol, the most active form of vitamin D, acts through VDRs expressed on human cells to regulate the transcription of genes involved in metastasis (Huang et al. 2022), angiogenesis, cell differentiation, apoptosis, and DNA repair (Latacz et al. 2020). Additionally, calcitriol may influence cancer development and progression through immune-inflammatory modulation (Chen et al. 2022b; Liu et al. 2018).

4.1.2 VDR *Cdx2* locus genotypes and colorectal cancer survival

My findings of null associations between the *Cdx2* genotype and survival outcomes in CRC patients align with previous reports of no significant associations between VDR polymorphisms rs731236 (Taq1), rs2228570 (Fok1), *Cdx2*, and rs1989969 (VDR-5132) with OS and CSS in a smaller, partially overlapping sample of CRC patients (Perna et al. 2013). While there have been mixed results regarding the association of *Cdx2* with CRC incidence (Bentley et al. 2012; Flügge et al. 2007; Ochs-Balcom et al. 2008; Slattery et al. 2009; Theodoratou et al. 2008), studies on prognostic outcomes are limited. A 2016 meta-analysis found that the G allele of the *Cdx2* gene was associated with a 12% higher risk for CRC (Serrano et al. 2016). The protective role of the *Cdx2* A-allele has been suggested by its association with a lower risk of fractures in ethnic groups with higher A-allele frequencies. The A-allele frequency was highest among individuals of African descent, followed by Asian and Caucasian groups (74%, 43%, and 19%, respectively) (Fang et al. 2003). These findings suggest a potential interaction between *Cdx2* and vitamin D status in cancer development and progression (Gnagnarella et al. 2021). Additionally, a study by Ochs-Balcom et al. reported a strong association between *Cdx2* and colon cancer risk, particularly in individuals with low BMI or waist circumference, suggesting a modifying effect of adiposity (Ochs-Balcom et al. 2008). However, my study did not observe any effect modification by BMI on the association between *Cdx2* and survival outcomes.

The effects of vitamin D are mediated by the VDR, a member of the nuclear receptor superfamily involved in regulating numerous transcription genes. As a result, cellular responses to vitamin D depend on the expression levels of the VDR (Ferrer-Mayorga et al. 2017). Recent research has indicated that CRC patients with low serum VDR expression levels have a poorer prognosis compared to those with higher levels (Shi et al. 2020). Additionally, serum VDR expression levels are significantly lower in CRC patients than in the general population (Al-Ghafari et al. 2020). Future prognostic studies should consider both VDR genotypes and serum expression levels.

4.1.3 Joint associations of vitamin D status and VDR *Cdx2* locus genotypes with colorectal cancer survival

Although the interaction tests between vitamin D status and *Cdx2* genotype in relation to survival did not achieve statistical significance in my study, the pattern observed—a strong inverse

association between vitamin D levels and mortality in individuals with the GG genotype and no such association in those with the AA/AG genotype—aligns well with findings from two slightly smaller UK CRC patient cohorts (n = 687 and n = 1848, respectively) (Vaughan-Shaw et al. 2020b). The seemingly weaker association between vitamin D status and survival in my entire cohort, and among those with the GG genotype, could be due to differences in vitamin D status categorization (standard categories in my study versus tertiles in the UK studies) and a more comprehensive adjustment for confounders in my study (adjusting for 10 covariates, including chemotherapy use, smoking, and physical activity).

The *Cdx2* SNP is situated at the 5′ end promoter region of the VDR gene, where it plays a crucial role in calcium regulation. A previous study of 261 Japanese women reported that the G-allele reduces VDR transcription by eliminating the *Cdx2* transcription binding site, while the A-allele is thought to upregulate VDR transcription (Arai et al. 2001). Consistent with the UK cohorts, my study—likely the largest to investigate the joint associations of vitamin D status and *Cdx2* genotype with survival outcomes in CRC patients—does not support any survival advantage for those with the AA/AG genotype within these Caucasian populations.

4.1.4 Strengths and limitations

The strengths of my study include the large sample size of patients recruited from over 20 clinics providing CRC surgery within a defined study region. The study features comprehensive follow-up regarding four common survival outcomes, thorough ascertainment of clinical and lifestyle factors, and adjustment for potential confounders. However, residual confounding by unmeasured or imperfectly measured variables cannot be entirely ruled out, and causality cannot be established in this observational study. Additionally, my study predominantly included patients of Caucasian origin, so the results may not be generalizable to populations with different ancestries.

4.2 Effects of vitamin D supplementation on inflammatory response in patients with cancer and precancerous lesions: Systematic review and meta-analysis of randomized trials.

To the best of my knowledge, this is the first systematic review and meta-analysis aimed at evaluating the potential anti-inflammatory effects of VIDS in adults with cancer or precancerous lesions based on RCT evidence. My study demonstrated significant reductions in serum TNF-alpha levels. Additionally, the meta-analyses suggested a potentially large effect on IL-6 levels and a potentially small effect on CRP levels with VIDS, though these estimates were not statistically significant. No differences in IL-10 serum levels were observed after VIDS.

The role of vitamin D in modulating inflammatory processes is mediated by the regulation of VDR gene expression in various human cells (Liu et al. 2018). Mechanistic studies suggest that vitamin D may downregulate the expression of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) and inhibit immune-cell-mediated inflammatory responses (E et al. 2021). Therefore, VIDS may potentially reduce tumor-promoting inflammatory cytokines such as CRP, TNF-α, and IL-6 in cancers like colorectal, prostate, breast, pancreatic, and liver, where these markers are highly expressed (Liu et al. 2018).

In my meta-analysis, a small, non-significant effect of VIDS was observed in reducing CRP serum levels in patients with vitamin D deficiency. Similarly, small but significant CRP reductions after VIDS have been reported in patients with rheumatoid arthritis and vitamin D deficiency (Chandrashekara and Patted 2017). Higher, but still safe, doses of VIDS and long-term treatment might be necessary to achieve larger effects (Terzić et al. 2010). Conversely, a recent meta-analysis of RCTs showed a significant effect of daily VIDS in reducing serum levels of high-sensitivity CRP, but no effect on TNF-α and IL-6, in patients with type 2 diabetes mellitus (Yu et al. 2018).

There was a significant reduction in serum TNF-α levels with VIDS in patients with cancer or precancerous lesions. A sensitivity analysis focusing on studies with daily oral dosage regimens of VIDS provided a more precise effect estimate for reducing TNF-α serum levels. Daily dosage regimens may offer advantages over bolus doses (Mazess et al. 2021). Consistent with my findings, treatment of prostate cancer (PCa) cell lines with calcitriol has demonstrated downstream inhibition of TNF-α production (Nonn et al. 2006). However, large and sustained suppression of

TNF-α may require higher vitamin D doses, as shown in a study reporting dose-dependent suppression of TNF-α by vitamin D in Mycobacterium tuberculosis-infected mononuclear cells (Khoo et al. 2011).

My meta-analysis also showed a large, though statistically non-significant, effect of VIDS in reducing IL-6 serum levels in patients with cancer or precancerous lesions. Calcitriol has been shown to downregulate IL-6 expression in both normal colon and colorectal cancer cells (Liu et al. 2018; van Harten-Gerritsen et al. 2015). An in vitro study by Nonn and colleagues also showed that vitamin D treatment inhibited TNF-α-stimulated IL-6 production in both normal and PCa cells (Nonn et al. 2006). However, a meta-analysis of RCTs in healthy, obese, and overweight adults found no effect of VIDS on serum IL-6, suggesting that healthy individuals may not benefit from the anti-inflammatory effects of VIDS (Jamka et al. 2016).

My results indicated no effect of VIDS on serum IL-10, an anti-inflammatory cytokine. Similarly, treatment of human colon cancer cell lines with vitamin D has shown strong effects on TNF-α and IL-6 levels, but only a weak effect in increasing IL-10 levels (Bessler and Djaldetti 2012). In an RCT by Naderi and colleagues, IL-10 gene expression increased significantly more with high-dose (4,000 IU/day) VIDS and yoga co-intervention than with low-dose (2,000 IU/day) VIDS and yoga in breast cancer patients (Naderi et al. 2022). These findings suggest that high-dose VIDS may have clinically significant IL-10-mediated anti-inflammatory effects in cancer patients. However, more evidence is needed to determine the VIDS dosages required to achieve changes in IL-10 levels.

4.2.1 Potential sources of heterogeneity

Variations in intervention parameters such as dosage, duration, and compliance rates could contribute to heterogeneity. My study included individual trials with patients having different mean baseline vitamin D status. Some studies have demonstrated benefits in achieving sufficient serum 25(OH)D levels in deficient populations through large single bolus doses, while others have shown benefits with daily low doses of VIDS (de Medeiros Cavalcante et al. 2015; Haidari et al. 2020; Mohseni et al. 2019; Tripkovic et al. 2012). However, higher doses pose a greater risk of hypercalcemic toxicity (Leyssens et al. 2013). The European expert panel recommends large loading doses of 6000 IU/day for 4-12 weeks for patients at high risk of 25(OH)D deficiency, followed by maintenance doses of 800-2000 IU/day to achieve therapeutic serum levels of 30-50

ng/mL (Pludowski et al. 2022). Heterogeneity might also arise from differences in geography, study design or quality, sample sizes, age, sex, race or ethnicity, VDR gene polymorphisms, obesity, and cancer site and stage (Azab et al. 2014; Hopkins et al. 2011; Irani et al. 2017; Krishnan et al. 2012; Mohseni et al. 2019).

4.2.2 Limitations

Most of the trials included in my study involved patients with a sufficient mean baseline vitamin D status, who may not derive significant benefits from VIDS. Additionally, the considerable heterogeneity observed in many of the meta-analyses complicates the ability to generalize the findings to a specific patient group. Due to the limited number of included studies, my research could not explore all potential sources of heterogeneity. Similarly, publication bias could not be systematically assessed because of the low number of RCTs. Overall, both the limited number of studies and the small sample sizes within those studies restricted the ability to draw strong conclusions regarding the effects of VIDS on the inflammatory response in the target population.

4.3 Anti-inflammatory effects of personalized vitamin D supplementation among colorectal cancer patients: randomized trial.

In this double-blind, placebo-controlled randomized clinical trial, I assessed the impact of tailored VIDS on inflammatory markers in 126 CRC patients who had low initial serum 25(OH)D levels (<60 nmol/L). The results revealed that patients administered an initial personalized vitamin D loading dose, followed by 2000 IU of VIDS daily for 12 weeks exhibited significant elevations in serum 25(OH)D and substantial decreases in IL-6 levels compared to those in the placebo group. While reductions in IFN-γ and MMP-1 were observed, these changes did not reach statistical significance. Additionally, exploratory analyses indicated that VIDS may have beneficial effects in lowering serum levels of CDCP1, CXCL11, and CXCL6, suggesting potential avenues for further investigation.

The findings of this trial are consistent with my previous meta-analysis of RCTs, which confirmed the anti-inflammatory properties of VIDS in individuals with cancer or pre-cancerous conditions, resulting in reduced levels of TNF-α, IL-6, and CRP (Gwenzi et al. 2023a). However, the RCTs

included in the meta-analysis might not have fully captured the true potential of VIDS due to several methodological limitations. These include the application of uniform VIDS doses without accounting for critical variables such as initial vitamin D status, BMI, and the specific supplementation regimen (bolus vs. daily) (Brenner 2023). Notably, supplementation appears to be most beneficial for individuals deficient in vitamin D, and there is a pronounced sequestration of 25(OH)D in obese individuals compared to their non-obese counterparts (Brenner et al. 2017; Lee et al. 2009; Vashi et al. 2011). Further, emerging evidence suggests superior outcomes with intermittent dosing of vitamin D_3 compared to bolus dosing in ameliorating vitamin D deficiency (Ketha et al. 2018; Mazess et al. 2021). The RCT, on which may analyses are based, rigorously addressed these methodological shortcomings to enhance the reliability and applicability of the findings.

Calcitriol, the biologically active metabolite of vitamin D, exerts its anti-inflammatory effects via the VDR, which regulates vitamin D-responsive gene expression across a variety of human cell types (Liu et al. 2018). Calcitriol is a potent hormone that influences the transcription of more than 200 genes, thereby directly or indirectly affecting cellular processes such as immune responses (Holick 2010; Liu et al. 2018). Specifically, calcitriol is known to suppress the activity of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells, a key regulator of inflammation, and can also mitigate immune-cell-mediated inflammatory responses. Consequently, VIDS holds potential clinical value in attenuating inflammation-driven tumorigenesis by elevating circulating calcitriol levels. This is particularly relevant for cancers such as CRC where inflammatory cytokines like IL-6, TNF-α, and CRP are prominently elevated (Liu et al. 2018).

My findings reveal a significant 39% decrease in IL-6 levels, a principal pro-inflammatory cytokine positively associated with neoplastic proliferation, higher tumor grade, and high mortality rates in CRC patients (van Harten-Gerritsen et al. 2015). Similar to these findings, my prior meta-analysis indicated a considerable reduction in IL-6 serum concentrations following VIDS in patients across various cancers and pre-cancerous conditions, although this reduction did not achieve statistical significance (Gwenzi et al. 2023a). The relationship between elevated IL-6 levels and advanced disease stages, increased recurrence risk, and poor overall survival in CRC patients has been substantiated by multiple clinical studies, and attributed to the pro-tumorigenic role of IL-6 mediated through the Janus Kinase/Signal Transducer and Activator of Transcription

3 (JAK/STAT3) signaling pathway (Cheng et al. 2023; Feng et al. 2023; Huang et al. 2022; Lin et al. 2020). Targeting the IL-6/JAK/STAT3 signaling axis has emerged as a viable therapeutic approach in CRC management (Wang et al. 2015), offering potential for directly suppressing cancer cell proliferation and enhancing antitumor immunity (Johnson et al. 2018). Consequently, several therapeutic strategies have been developed that target the IL-6/JAK/STAT3 pathway for the treatment of CRC (Waldner et al. 2012). Specifically, the FDA-approved humanized monoclonal anti-IL-6R antibody Tocilizumab has been shown to disrupt JAK/STAT3 pathway activation, thereby augmenting the efficacy of chemotherapeutic agents (Maryam et al. 2023). Given that elevated circulating IL-6 is linked with adverse clinical outcomes in CRC (Knüpfer and Preiss 2010; van Harten-Gerritsen et al. 2015), interventions such as personalized VIDS that reduce IL-6 could play a critical role in modulating both inflammation and tumor progression (Vaughan-Shaw et al. 2020a), potentially enhancing HRQoL (Martínez-Alonso et al. 2016).

While my study observed trends suggesting that personalized VIDS might decrease levels of IFN-γ and MMP-1 in CRC patients, these reductions did not achieve statistical significance. Mechanistic research proposes that calcitriol could modulate immune responses in CRC by repressing IFN-γ gene transcription in T cells, thereby diminishing IFN-γ production (Byers et al. 2012; Cippitelli and Santoni 1998). Furthermore, in vitro experiments have shown that vitamin D can reduce IFN-γ output by peripheral blood mononuclear cells (Ragab et al. 2016). IFN-γ plays a crucial role in macrophage activation and the induction of Class II major histocompatibility complex molecules, possessing both immune regulatory (Kosmidis et al. 2018) and antitumor effects (Liu et al. 2017). Additionally, genetic variations in IFN-γ and its receptor subunits are strongly linked to CRC risk and patient survival post-diagnosis (Wang et al. 2015). Despite these connections, personalized VIDS showed only a small and non-significant impact on IFN-γ levels in my analysis.

Regarding MMP-1, evidence from multiple studies has established that its elevated expression in CRC tissue correlates with poorer prognosis and increased metastatic risk (Murray et al. 1996; Sunami et al. 2000; Yu et al. 2021). Although the influence of VIDS on MMP-1 in CRC remains unexplored, studies in other contexts, such as uterine fibroids, indicate that calcitriol can downregulate the expression and activity of specific MMPs, including MMP-2 and MMP-9 (Halder et al. 2013). My exploratory analysis revealed potential reductions in CDCP1, CXCL11,

and CXCL6 due to VIDS. To date, no research has specifically investigated the effects of VIDS on these inflammatory biomarkers in CRC. This identifies a crucial gap in the literature, highlighting the need for further studies to evaluate the potential associations between these biomarkers and CRC prognosis.

4.3.1 Clinical implications and future research

The clinical implications of my findings are considerable, especially considering the high prevalence of vitamin D inadequacy in CRC patients, which in my study was effectively mitigated by the end of the trial in the VIDS group. Implementing routine screening and correction of vitamin D inadequacy in clinical settings could be beneficial for CRC patients, given the association between low vitamin D levels and adverse clinical outcomes. In addition to the pleiotropic benefits of vitamin D including bone and muscle health, patients with CRC might derive significant benefits from the anti-inflammatory properties of VIDS as a supportive therapy post-treatment. VIDS presents a potentially cost-effective option, considering the vitamin D safety profile (Kuznia et al. 2022), affordability, and wide availability. Carefully designed and adequately powered future RCTs should validate my findings. Extended follow-up periods are crucial to assess the long-term clinical impacts of my observations and to evaluate whether reductions in inflammatory markers lead to enhanced survival rates or low disease recurrence in CRC patients. Additionally, tailored strategies to optimize VIDS for obese CRC patients are imperative, addressing the unique pharmacokinetic challenges presented by this subgroup.

4.3.2 Strengths and limitations

This study has several strengths, including the careful selection of CRC patients with low serum 25(OH)D levels, coupled with a robust randomized trial design. Additionally, rigorous adjustment for potential confounders such as age, sex, cancer stage, BMI, and prior treatments was conducted. The adoption of personalized dosing strategies enabled precise correction of vitamin D deficiencies. Importantly, the choice of vitamin D_3, recognized as the most effective form of vitamin D, over vitamin D_2, optimizes the treatment efficacy (Balachandar et al. 2021). In my study, I carefully selected outcome measures known to be prognostic indicators for CRC patients, enhancing the relevance and utility of my findings.

Nevertheless, there are several limitations that warrant mention. First, the homogeneity of the study population, consisting predominantly of Caucasian individuals, may restrict the generalizability of the results to more diverse populations. Additionally, it is important to note that I was not able to determine the statistical power of the study a priori, as the original design was focused primarily on exploring the effects of personalized VIDS on cancer-related fatigue. This limitation may affect the interpretability and broad applicability of my findings. More than 20% of biomarkers were excluded from my analyses because of high rates of assays below the lower limit of detection. These biomarkers may need to be investigated in future studies.

5 Conclusions

The aim of my dissertation was to evaluate the potential of VIDS to improve the prognosis of CRC patients, particularly focusing on its role in inflammatory modulation. To address this, I integrated findings from several sub-projects exploring various related aspects.

Emerging evidence suggests that the prognostic value of vitamin D status for CRC patients might be confined to those with the GG genotype of *Cdx2*, a functional polymorphism of the VDR gene. I aimed to validate these findings in a cohort of 2819 CRC patients. Post-operative serum 25(OH)D levels were measured using mass spectrometry, and *Cdx2* genotyping was performed from blood or buccal swabs using standard methods. Joint associations of vitamin D status and *Cdx2* with OS, CSS, RFS, and DFS were assessed using Cox regression. For patients with the GG genotype, the adjusted HRs (95% CI) for sufficient versus deficient vitamin D status were 0.63 (0.50–0.78) for OS, 0.68 (0.50–0.90) for CSS, 0.66 (0.51–0.86) for RFS, and 0.62 (0.50–0.77) for DFS. These associations were weaker and not statistically significant for the AA/AG genotype. Vitamin D deficiency is an independent predictor of poorer survival, particularly for GG *Cdx2* carriers, suggesting that VIDS should be evaluated in RCTs.

Inflammation plays a key role in tumor development and progression, and calcitriol has potential tumor-suppressing effects through inflammatory modulation. I conducted a systematic review and meta-analysis of RCTs to evaluate the effects of VIDS on serum inflammatory biomarkers in patients with cancer or precancerous lesions. I searched PubMed, Web of Science, and Cochrane databases until November 2022. The effects of VIDS were estimated from pooled SMDs with their 95% CIs for inflammatory biomarker follow-up levels between intervention and control groups. A meta-analysis of eight RCTs (total of 592 patients) showed that VIDS significantly lowered serum TNF-α levels (SMD [95% CI]: -1.65 [-3.07; -0.24]). VIDS also resulted in non-significant reductions in serum levels of IL-6 (SMD [95% CI]: -0.83, [-1.78; 0.13]) and CRP (SMD [95% CI]: -0.09, [-0.35; 0.16]), while IL-10 levels were unchanged (SMD [95% CI]: 0.00, [-0.50; 0.49]). These results indicate a significant reduction in TNF-α levels by VIDS for patients with cancer or precancerous lesions, suggesting potential benefits in suppressing tumor-promoting inflammatory responses.

In my final project, I assessed the efficacy of personalized VIDS in reducing pro-inflammatory biomarkers in CRC patients with low vitamin D status. In a multi-center randomized, double-blind, placebo-controlled trial, 126 patients treated for CRC within the past 12 months and presenting with serum 25(OH)D levels < 60 nmol/L were recruited from nine German rehabilitation clinics. Randomization was computer-generated, with participants assigned to receive either a personalized loading dose of VIDS or a placebo, followed by a 12-week maintenance dose of 2000 IU/day or placebo. The primary analysis was conducted on an intention-to-treat basis. Primary outcomes included changes in serum IL-6, IFN-γ, and MMP-1, estimated through multivariable linear regression at the trial's conclusion. Between September 23, 2020, and July 19, 2023, patients were randomized (65 in the placebo and 61 in the intervention group). One adverse event was reported in the intervention group (1.6% of patients). The VIDS group showed a 39.3% reduction in IL-6 levels compared to the placebo group (% Change, 95% CI: -39.3, -54.9 to -18.2; $p < 0.01$). Changes in IFN-γ and MMP-1 due to VIDS were not statistically significant (-6.7%; $p = 0.69$ and -5.4%; $p = 0.69$, respectively). In CRC patients, VIDS may help correct low serum 25(OH)D levels and reduce serum IL-6, a pro-inflammatory biomarker associated with poor cancer outcomes. VIDS has potential as a supportive therapy in managing cancer-related inflammation and improving CRC outcomes.

In summary, given the high prevalence of vitamin D inadequacy in operable CRC patients and the association between low 25(OH)D levels and adverse clinical outcomes, routine screening and personalized VIDS for correcting vitamin D inadequacy may be a promising approach to enhance prognosis in clinical settings. Beyond its well-established benefits for bone and muscle health, CRC patients undergoing surgery might gain significant additional benefits from VIDS as a supportive anti-inflammatory therapy. Considering the high financial costs of CRC care, VIDS may be a particularly cost-effective option due to its safety, affordability, and availability. By elucidating the connection between vitamin D, systemic inflammation, and CRC prognosis, this dissertation could pave the way for developing novel therapeutic and tertiary prevention strategies to improve patient outcomes.

6 Summary

6.1 English summary

Low vitamin D status, measured by serum 25-hydroxyvitamin D [25(OH)D], is common in the post-operative period among CRC patients and is associated with poor long-term prognosis. Moreover, elevated post-operative systemic inflammation is strongly linked to long term adverse outcomes in CRC patients. Pre-clinical and to some extent clinical evidence suggest that calcitriol, the most active form of vitamin D, can modulate immune-inflammatory response. I explored the potential role of vitamin D supplementation (VIDS) in modulating inflammatory response towards improving the prognosis of CRC patients undergoing surgery.

I assessed the prognostic value of post-operative vitamin D status on long-term CRC survival outcomes and examined the role of the vitamin D receptor *Cdx2* genotype in a cohort of 2819 CRC patients. Patients with deficient vitamin D status [25(OH)D < 30nmol/L] had significantly shorter survival than those with insufficient or sufficient status [25(OH)D ≥ 30nmol/L]. These associations were particularly evident in patients with the GG genotype of *Cdx2*, but not in those with the AA/AG genotype. These results suggest that post-operative vitamin D status is a potentially modifiable prognostic factor among CRC patients, especially for carriers of the GG *Cdx2* genotype. Future randomized clinical trials (RCTs) should assess the efficacy of tailored VIDS to improve vitamin D status as well as clinical outcomes among CRC patients with low 25(OH)D. Such interventions should also evaluate the efficacy of VIDS among patient subgroups by vitamin D *Cdx2* genotype.

In a follow-up project, I conducted a meta-analysis of RCTs involving 592 patients with cancer or precancerous lesions to evaluate the effects of VIDS on systemic inflammatory biomarkers. The results showed a significant reduction in serum tumor necrosis factor-alpha levels. VIDS also showed potentially large effects on reducing serum interleukin-6 (IL-6) and small effects on reducing C-reactive protein levels, although these were not statistically significant. Despite the limited number of small and variable-quality studies, the results support the hypothesis that VIDS may provide anti-inflammatory benefits to patients with cancer or precancerous lesions. Further high-quality RCTs are needed, with larger patient numbers and tailored VIDS dosage regimens over extended intervention periods. The design of such future studies should also take into account

factors that determine VIDS efficacy, such as baseline vitamin D status and potential interactions with genetic and clinical factors.

Finally, I assessed the effects of personalized VIDS on post-operative systemic inflammatory biomarkers in CRC patients with low vitamin D status in a RCT. In 126 CRC patients with serum 25(OH)D levels <60 nmol/L, an initial personalized loading dose, followed by 2000 IU of VIDS daily for 12 weeks showed significant increases in serum 25(OH)D and substantial decreases in serum IL-6 levels compared to the placebo group. Although reductions in interferon-gamma and matrix metalloproteinase-1 were observed, they were not statistically significant. Additional exploratory analyses suggested that VIDS might lower serum levels of pro-inflammatory biomarkers CUB domain-containing protein 1, C-X-C motif chemokine 11, and C-X-C motif chemokine 6, warranting further investigation.

Overall, personalized VIDS can correct vitamin D deficiency and attenuate pro-inflammatory responses in CRC patients with low serum 25(OH)D levels. Given the high prevalence of vitamin D inadequacy among operable CRC patients and its association with poor clinical outcomes, routine clinical screening for vitamin D inadequacy and personalized VIDS may be a promising approach to improve patient outcomes. Besides bone and muscle health, VIDS can provide additional benefits as a supportive anti-inflammatory therapy for CRC patients undergoing surgery. Ultimately, personalized VIDS could enhance long-term prognosis and quality of life, offering a cost-effective, safe, and widely available option. In addition, this dissertation highlights the connection between vitamin D, systemic inflammation, and CRC prognosis, paving the way for possible new therapeutic and preventive strategies to improve patient prognosis.

6.2 Deutsche Zusammenfassung

Ein niedriger Vitamin-D-Status, der anhand von 25-Hydroxyvitamin D [25(OH)D] im Serum gemessen wird, ist bei Darmkrebs-Patienten in der postoperativen Phase häufig anzutreffen und wird mit einer schlechten Langzeitprognose in Verbindung gebracht. Darüber hinaus steht eine erhöhte postoperative systemische Entzündung in engem Zusammenhang mit einer schlechten Langzeitprognose bei Darmkrebs-Patienten. Präklinische und zu einem gewissem Grad auch klinische Evidenz legen nahe, dass Calcitriol, die aktivste Form von Vitamin D, die Immunantwort auf Entzündungen modulieren kann. Ich untersuchte die mögliche Rolle einer Vitamin-D-Supplementierung (VIDS) bei der Modulation der Entzündungsreaktion zur Verbesserung der Prognose von Darmkrebs-Patienten, die sich einer Operation unterzogen.

Ich bewertete den prognostischen Wert des postoperativen Vitamin-D-Status für das langfristige Überleben von Darmkrebs und untersuchte die Rolle des Genotyps des Vitamin-D-Rezeptors *Cdx2* in einer Kohorte von 2819 Darmkrebspatienten. Patienten mit mangelhaftem Vitamin-D-Status [25(OH)D < 30nmol/L] hatten ein deutlich kürzeres Überleben als Patienten mit unzureichendem oder ausreichendem Status [25(OH)D ≥ 30nmol/L]. Diese Assoziationen waren besonders deutlich bei Patienten mit dem GG-Genotyp von *Cdx2*, aber nicht bei denen mit dem AA/AG-Genotyp. Diese Ergebnisse deuten darauf hin, dass der postoperative Vitamin-D-Status ein potenziell modifizierbarer prognostischer Faktor bei CRC-Patienten ist, insbesondere bei Trägern des GG-*Cdx2*-Genotyps. Künftige randomisierte klinische Studien (RCTs) sollten die Wirksamkeit maßgeschneiderter VIDS zur Verbesserung des Vitamin-D-Status sowie der klinischen Ergebnisse bei Darmkrebs-Patienten mit niedrigem 25(OH)D untersuchen. Solche Interventionen sollten auch die Wirksamkeit von VIDS bei Patientenuntergruppen nach Vitamin-D-*Cdx2*-Genotyp untersuchen.

In einem Folgeprojekt führte ich eine Meta-Analyse von RCTs mit 592 Patienten mit Krebs oder Krebsvorstufen durch, um die Auswirkungen von VIDS auf systemische Entzündungsbiomarker zu untersuchen. Die Ergebnisse zeigten eine signifikante Senkung der Serumspiegel des Tumor-Nekrose-Faktors-alpha. VIDS zeigte auch potenziell große Auswirkungen auf die Senkung des Serum-Interleukin-6 (IL-6) und geringe Auswirkungen auf die Senkung des C-reaktiven Proteins, obwohl diese statistisch nicht signifikant waren. Trotz der begrenzten Anzahl kleiner Studien von unterschiedlicher Qualität stützen die Ergebnisse die Hypothese, dass VIDS bei Patienten mit

Krebs oder Krebsvorstufen entzündungshemmend wirken kann. Weitere qualitativ hochwertige RCTs mit einer größeren Patientenzahl und maßgeschneiderten VIDS-Dosierungsschemata über längere Interventionszeiträume sind erforderlich. Bei der Planung solcher zukünftiger Studien sollten auch Faktoren berücksichtigt werden, die für die Wirksamkeit von VIDS ausschlaggebend sind, wie zum Beispiel der Vitamin-D-Status im Ausgangszustand und mögliche Wechselwirkungen mit genetischen und klinischen Faktoren.

Schließlich habe ich die Auswirkungen von personalisiertem VIDS auf postoperative systemische Entzündungsbiomarker bei Darmkrebs-Patienten mit niedrigem Vitamin-D-Status in einem RCT untersucht. Bei 126 Darmkrebs-Patienten mit einem Serum-25(OH)D-Spiegel <60 nmol/L führte eine initiale personalisierte Vitamin D Aufsättigung, gefolgt von der täglichen Gabe von 2000 IE VIDS über einen Zeitraum von 12 Wochen zu einem signifikanten Anstieg des Serum-25(OH)D-Spiegels und zu einer deutlichen Senkung des Serum-IL-6-Spiegels im Vergleich zur Placebogruppe. Obwohl eine Verringerung von Interferon-gamma und Matrix-Metalloproteinase-1 beobachtet wurde, war sie statistisch nicht signifikant. Zusätzliche explorative Analysen deuteten darauf hin, dass VIDS die Serumspiegel der proinflammatorischen Biomarker CUB domain-containing protein 1, C-X-C motif chemokine 11 und C-X-C motif chemokine 6 senken könnte, was weitere Untersuchungen rechtfertigt.

Insgesamt kann personalisierte VIDS bei CRC-Patienten mit niedrigem 25(OH)D-Serumspiegel einen Vitamin-D-Mangel korrigieren und proinflammatorische Reaktionen abschwächen. In Anbetracht der hohen Prävalenz von Vitamin-D-Mangel bei operablen Darmkrebs-Patienten und des Zusammenhangs mit schlechten klinischen Ergebnissen könnte ein routinemäßiges klinisches Screening auf Vitamin-D-Mangel und personalisierte VIDS ein viel versprechender Ansatz sein, um die Ergebnisse der Patienten zu verbessern. Neben der Knochen- und Muskelgesundheit kann VIDS als unterstützende entzündungshemmende Therapie für Darmkrebspatienten, die sich einer Operation unterziehen, zusätzliche Vorteile bieten. Letztendlich könnte personalisierte VIDS die Langzeitprognose und die Lebensqualität verbessern und eine kosteneffiziente, sichere und allgemein verfügbare Option darstellen. Darüber hinaus unterstreicht diese Dissertation den Zusammenhang zwischen Vitamin D, systemischer Entzündung und Darmkrebs-Prognose und ebnet den Weg für mögliche neue therapeutische und präventive Strategien zur Verbesserung der Patientenprognose.

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8 Own publications and contributions

The results presented in this dissertation are either published in peer-reviewed scientific journals or are currently being prepared for submission.

8.1 First authored, peer-reviewed publications:

1. Gwenzi T, Schrotz-King P, Anker SC, Schöttker B, Hoffmeister M, Brenner H. **Post-operative C-reactive protein as a strong independent predictor of long-term colorectal cancer outcomes: consistent findings from two large patient cohorts.** ESMO Open. 2024 Apr;9(4):102982. doi: 10.1016/j.esmoop.2024.102982. Epub 2024 Apr 12. PMID: 38613909; PMCID: PMC11033061.
2. Gwenzi T, Brenner H. **Reply - Letter to the Editor - Patients with cancer and precancerous lesions: Systematic review and meta-analysis of randomized trials**. Clin Nutr. 2024 Apr;43(4):1076. doi: 10.1016/j.clnu.2023.12.015. Epub 2023 Dec 20. PMID: 38142213.
3. Gwenzi T, Zhu A, Schrotz-King P, Schöttker B, Hoffmeister M, Edelmann D, Brenner H. **Prognostic Value of Post-Operative C-Reactive Protein-Based Inflammatory Biomarkers in Colorectal Cancer Patients: Systematic Review and Meta-Analysis**. Clin Epidemiol. 2023 Jun 27;15:795-809. doi: 10.2147/CLEP.S415171. PMID: 37396024; PMCID: PMC10314753.
4. Gwenzi T, Schrotz-King P, Schöttker B, Hoffmeister M, Brenner H. **Vitamin D Status, Cdx2 Genotype, and Colorectal Cancer Survival: Population-Based Patient Cohort. Nutrients.** 2023 Jun 12;15(12):2717. doi: 10.3390/nu15122717. PMID: 37375621; PMCID: PMC10305330.
5. Gwenzi T, Zhu A, Schrotz-King P, Schöttker B, Hoffmeister M, Brenner H. **Effects of vitamin D supplementation on inflammatory response in patients with cancer and precancerous lesions: Systematic review and meta-analysis of randomized trials.** Clin Nutr. 2023 Jul;42(7):1142-1150. doi: 10.1016/j.clnu.2023.05.009. Epub 2023 May 17. PMID: 37244755.

Section 1 of the dissertation is based on **publication 1**. Sections 1, 2, 3, 4, 5 and 6 of the dissertation are based on **publication 2.** Section 1 of the dissertation is based on **publication 3**.

Sections 1, 2, 3, 4, 5 and 6 of the dissertation are based on **publication 4**. Sections 1, 2, 3, 4, 5 and 6 of the dissertation are based on **publication 5**.

My own contributions to **publication 1** were: Conceptualization; Study design; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. My own contributions to **publication 2** were: Writing - original draft, review and editing of final manuscript. My own contributions to **publication 3** were: Conceptualization; Study design; Development of the searching strategy, Study selection; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. Data extraction and Quality assessment were carried out together with my co-doctoral fellow Anna Zhu. My own contributions to **publication 4** were: Conceptualization; Study design; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. My own contributions to **publication 5** were: Conceptualization; Study design; Development of the searching strategy, Study selection; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. Data extraction and Quality assessment were carried out together with my co-doctoral fellow Anna Zhu. For all publications, my supervisor Prof. Dr. Hermann Brenner was involved in all stages from Conceptualization to Editing of the manuscripts.

The original data used for **publication 1** was from two studies: the DACHS study, which was designed and led by Prof. Dr. Hermann Brenner, Prof. Dr. Jenny Chang-Claude, and Prof. Dr. Michael Hoffmeister, and the UK Biobank which is being run and managed by the Wellcome Trust based in the United Kingdom. The original data used for **publication 4** was from the German DACHS study, which was designed and led by Prof. Dr. Hermann Brenner, Prof. Dr. Jenny Chang-Claude, and Prof. Dr. Michael Hoffmeister.

8.2 First authored, accepted for peer-reviewed publications:

6. Gwenzi T, Schrotz-King P, Anker SC, Schöttker B, Hoffmeister M, Brenner H. **Prognostic value of post-operative iron biomarkers in colorectal cancer: population-based patient cohort.** British Journal of Cancer. 2024 Aug 27. doi: 10.1038/s41416-024-02814-4. Online ahead of print.

My own contributions to **publication 6** were: Conceptualization; Study design; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. My supervisor Prof. Dr. Hermann Brenner was involved in all stages from Conceptualization to Editing of the manuscripts. The original data used for the publication was from the German DACHS study, which was designed and led by Prof. Dr. Hermann Brenner, Prof. Dr. Jenny Chang-Claude, and Prof. Dr. Michael Hoffmeister.

8.3 Papers in preparation for submission:

7. Gwenzi T, Weber ANR, Trares K, Vlaski T, Slavic M, Sha S, Edelmann D, Rammensee H-G, Küster B, Caspari R, Bilsing B, Fischer H, Czock D, Schöttker B, Brenner H. **Anti-inflammatory effects of personalized vitamin D_3 supplementation among colorectal cancer patients: randomized trial.**
8. Gwenzi T, Wankhede D, Yuan T, Fan Z, Schrotz-King P, Anker SC, Schöttker B, Hoffmeister M, Brenner H. **The Combined Prognostic Value of Post-operative C-Reactive Protein Levels and Tumor Immune Cell Score in Patients with Colorectal Cancer**.

Sections 1, 2, 3, 4, 5 and 6 of the dissertation are based on **publication 7**. My own contributions to **publications 7 and 8** were: Conceptualization; Study design; Data analysis; Interpretation; Writing - original draft, review and editing of final manuscript. For all publications, my supervisor Prof. Dr. Hermann Brenner was involved in all stages from Conceptualization to Editing of the manuscripts. The original data used for **publication 7** was from the German VICTORIA trial, which was designed and led by Prof. Dr. Hermann Brenner and PD. Dr. Ben Schöttker. The original data used for **publication 8** was from the German DACHS study, which was designed and led by Prof. Dr. Hermann Brenner, Prof. Dr. Jenny Chang-Claude, and Prof. Dr. Michael Hoffmeister.

8.4 Co-author publication(s):

9. Sha S, Gwenzi T, Chen LJ, Brenner H, Schöttker B. **About the associations of vitamin D deficiency and biomarkers of systemic inflammatory response with all-cause and**

cause-specific mortality in a general population sample of almost 400,000 UK Biobank participants. Eur J Epidemiol. 2023 Sep;38(9):957-971. doi: 10.1007/s10654-023-01023-2. Epub 2023 Jun 21. PMID: 37340242; PMCID: PMC10501954.

My own contributions to **publication 9** were: Writing - original draft, review and editing of final manuscript. The original data used for the publication was from the UK Biobank which is being run and managed by the Wellcome Trust based in the United Kingdom.

8.5 Poster and oral presentations at scientific conferences:

Joint International Symposium Vitamin D in Prevention and Therapy and Biologic Effects of Light, May 8-10, 2024, Homburg/Saar, Germany

Oral Presentation: “Effects of Vitamin D Supplementation on Inflammatory Response in Patients with Cancer and Precancerous Lesions: Systematic Review and Meta-analysis of Randomized Trials”

16th International PhD Cancer Conference, July 5-7, 2023, Cambridge, United Kingdom

Poster: “Post-Operative C-reactive Protein as a Strong Independent Predictor of Long-term Colorectal Cancer Survival: Consistent Findings from Two Large Patient Cohorts”

9 Appendix

Table 1. List of biomarkers measured with Olink Proseek® Multiplex Inflammation I96x96 kits.

Abbreviation	Biomarker name
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
ADA	Adenosine Deaminase
ARTN	Artemin
AXIN1	Axin-1
Beta-NGF	Beta-nerve growth factor
CASP-8	Caspase-8
CCL11	Eotaxin
CCL19	C-C motif chemokine 19
CCL20	C-C motif chemokine 20
CCL23	C-C motif chemokine 23
CCL25	C-C motif chemokine 25
CCL28	C-C motif chemokine 28
CCL3	C-C motif chemokine 3
CCL4	C-C motif chemokine 4
CD244	Natural killer cell receptor 2B4
CD40	CD40L receptor
CD5	T-cell surface glycoprotein CD5
CD6	T cell surface glycoprotein CD6 isoform
CD8A	T-cell surface glycoprotein CD8 alpha chain
CDCP1	CUB domain-containing protein 1
CSF-1	Macrophage colony-stimulating factor 1
CST5	Cystatin D
CX3CL1	Fractalkine
CXCL1	C-X-C motif chemokine 1
CXCL10	C-X-C motif chemokine 10
CXCL11	C-X-C motif chemokine 11
CXCL5	C-X-C motif chemokine 5
CXCL6	C-X-C motif chemokine 6
CXCL9	C-X-C motif chemokine 9
DNER	Delta and Notch-like epidermal growth factor-related receptor
EN-RAGE	Protein S100-A12
FGF-19	Fibroblast growth factor 19
FGF-21	Fibroblast growth factor 21
FGF-23	Fibroblast growth factor 23
FGF-5	Fibroblast growth factor 5
Flt3L	Fms-related tyrosine kinase 3 ligand
GDNF	Glial cell line-derived neurotrophic factor

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Abbreviation	Biomarker name
HGF	Hepatocyte growth factor
IFN_gamma	Interferon gamma
IL1_alpha	Interleukin-1 alpha
IL-10	Interleukin-10
IL-10RA	Interleukin-10 receptor subunit alpha
IL-10RB	Interleukin-10 receptor subunit beta
IL-12B	Interleukin-12 subunit beta
IL13	Interleukin-13
IL-15RA	Interleukin-15 receptor subunit alpha
IL-17A	Interleukin-17A
IL-17C	Interleukin-17C
IL-18	Interleukin-18
IL-18R1	Interleukin-18 receptor 1
IL2	Interleukin-2
IL20	Interleukin-20
IL-20RA	Interleukin-20 receptor subunit alpha
IL22-RA1	Interleukin-22 receptor subunit alpha-1
IL24	Interleukin-24
IL2RB	Interleukin-2 receptor subunit beta
IL33	Interleukin-33
IL4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
LAP TGF-beta-1	Latency-associated peptide transforming growth factor beta-1
LIF	Leukemia inhibitory factor
LIFR	Leukemia inhibitory factor receptor
MCP-1	Monocyte chemotactic protein 1
MCP-2	Monocyte chemotactic protein 2
MCP-3	Monocyte chemotactic protein 3
MCP-4	Monocyte chemotactic protein 4
MMP-1	Matrix metalloproteinase-1
MMP-10	Matrix metalloproteinase-10
NRTN	Neurturin
NT-3	Neurotrophin-3
OPG	Osteoprotegerin
OSM	Oncostatin-M
PD-L1	Programmed cell death 1 ligand 1
SCF	Stem cell factor

continued on next page

Abbreviation	Biomarker name
SIRT2	SIR2-like protein 2
SLAMF1	Signaling lymphocytic activation molecule
ST1A1	Sulfotransferase 1A1
STAMBP	STAM-binding protein
TGF-alpha	Transforming growth factor alpha
TNF	Tumor necrosis factor
TNFB	TNF-beta
TNFRSF9	Tumor necrosis factor receptor superfamily member 9
TNFSF14	Tumor necrosis factor ligand superfamily member 14
TRAIL	TNF-related apoptosis-inducing ligand
TRANCE	TNF-related activation-induced cytokine
TSLP	Thymic stromal lymphopoietin
TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12
uPA	Urokinase-type plasminogen activator
VEGF-A	Vascular endothelial growth factor-A

10 Curriculum Vitae

PERSONAL INFORMATION

Name and first name: Tafirenyika Gwenzi

Date of birth: 21 July 1983

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SCHOOL AND UNIVERSITY CAREER

09.2018 – 07.2020 Master of Medical Sciences in Global Health, Gothenburg University, Gothenburg, Sweden

08.2003 – 07.2007 Bachelor of Pharmacy with Honors, University of Zimbabwe, Harare, Zimbabwe

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PROFESSIONAL CAREER

Since 07.2021 PhD Researcher and Student, Division of Preventive Oncology and Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany

01.2012 – 12.2017 Provincial Coordinator and Senior Lecturer, School of Pharmacy, University of Zimbabwe, Gweru Campus, Zimbabwe

05.2015 – 08.2018 Managing Director and Senior Pharmacist, Spring Pharmacy, Gweru, Zimbabwe

01.2012 – 04.2015 Branch Manager and Senior Pharmacist, Medix Group of Pharmacies, Gweru, Zimbabwe

01.2010 – 12.2011 Branch Manager and Pharmacist, Your Pharmacy, Gweru, Zimbabwe

01.2008 – 12.2009 Provincial Hospital Pharmacist, Ministry of Health and Child Care, Gweru, Zimbabwe

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I am also grateful to PD Dr. Ben Schöttker, who together with Prof. Dr. med. Hermann Brenner designed and led the VICTORIA trial, and provided critical review of my scientific publications.

I am thankful to Prof. Dr. Michael Hoffmeister, who together with Prof. Dr. med. Hermann Brenner designed and led the DACHS study and also gave me great assistance in reviewing my scientific manuscripts for publication.

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I would also like to thank my colleagues, Dr. Anna Zhu, Dr. Petra Schrotz-King, and colleagues from the Division of Preventive Oncology at the German Cancer Research Center for their valuable contributions to my scientific publications.

Finally, I would like to thank my family and friends for all the support I needed for my academic journey.

12 EIDESSTATTLICHE VERSICHERUNG

1. Bei der eingereichten Dissertation zu dem Thema

   „The Potential of Vitamin D Supplementation to Enhance Prognosis of Colorectal Cancer Patients: Role of Vitamin D on Inflammatory Modulation"

   handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

3. Die Arbeit oder Teile davon habe ich bislang nicht an einer Hochschule des In- oder Auslands als Bestandteil einer Prüfungs- oder Qualifikationsleistung vorgelegt.

4. Die Richtigkeit der vorstehenden Erklärungen bestätige ich.

5. Die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt. Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Ort und Datum Unterschrift