CLINCIAL STUDY REPORT

'Palliative-D'

Vitamin D supplementation to palliative cancer patients – A double blind, randomised controlled trial

Product:	Detremin
Substance:	Cholecalciferol
EudraCT Number:	*2017-000268-14*
Sponsor:	Linda Björkhem-Bergman
Coordinating Investigator	Linda Björkhem-Bergman
Principal Investigators:	Linda Björkhem-Bergman
Phase	II (Therapeutic exploratory)

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2. SUMMARY OF TRIAL PROTOCOL

PROTOCOL IDENTITY AND OBJECTIVES	
EudraCT Number	*2017-000268-14*
Ethical Permission	Dnr 2017/405-31/1, Approval date 7th 2017
Protocol Title	Vitamin D supplementation to palliative cancer patients – A double blinded, randomised controlled trial. Acronym: "Palliative-D"
Trial Objectives	To test the hypothesis that vitamin D treatment during 12 weeks to palliative cancer patients can decrease opioid consumption, fatigue and infectious burden and increase quality of life.
INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)	
Test Product	Detremin, MA holder Renapharma
Pharmaceutical Form	Oil
Route of Administration	Oral route
METHODOLOGY	
Trial Design	Double-blinded, parallel randomised and placebo controlled trial
Dose/Duration	4000 IU/day for 12 weeks
Primary Endpoint	The decline of opioid-consumption during 12 weeks in the vitamin D group compared to the placebo group, based on 4 measurements with 4 weeks intervals.
Secondary end point	1. Decline in antibiotic consumption 2. Improvement in quality of life 3. Improvement in fatigue 4. Vitamin D levels in serum after 12 weeks 5. Association between change in opioid dose and genetic polymorphism in genes involved in the effect and metabolism of vitamin D in the body.
Efficacy Parameters	Opioid dose, translated to fentanyl per hour measured at baseline and at week 4, 8 and 12. Antibiotic consumption expressed as % of days with antibiotics during the last 4 weeks measured at baseline and at week 4, 8 and 12. Quality of Life measured with EORTC-QLQ-C15-PAL and ESAS at screening and after 12 weeks. Fatigue measured with EORTC-QLQ-C15-PAL at screening and after 12 weeks. 25-hydroxyvitamin D levels in blood at screening and after 12 weeks.
Safety Parameters	S-calcium and S-creatinine will be controlled in all subjects at screening, at week 4, 8 and 12.
Power	We estimate "the least clinically meaningful effect" to be a 20% decline in opioid dose compared to the placebo. To obtain this result with 80% power we estimate the number of patients to be 127 in each arm i.e. 254 patients in total. This includes a drop-out of 25% per group.
Statistical Analyse plan	The primary endpoint will be analysed using linear regression, using bias corrected and accelerated bootstrap confidence intervals, while controlling for baseline measure of opoid dose (similar to ANCOVA). Adjustment for other background variables - such as vitamin D-level at baseline, gender and age - will be made in a secondary analysis to gain efficiency. The continuous secondary endpoints will be analysed using the same method as the primary endpoint .
POPULATION OF TRIAL SUBJECTS	
Number of Subjects	254 (127 vitamin D and 127 placebo)

Description of Trial Subjects	Palliative Cancer Patients (any cancer form) with a life expectancy of more than 3 months. Three study Sites: ASIH Stockholm Södra and Stockholms Sjukhem
TRIAL TIMETABLE	
First Subject In	Nov 2017
Last Subject In	March 2020
Last Subject Out	June 2020
End of Trial:	May 2021

3. SUMMARY OF STUDY REPORT

Purpose: To test the hypothesis that correction of vitamin D deficiency reduces opioid use in cancer patients admitted to palliative care. Secondary objectives included effects on antibiotic use, 25-hydroxyvitamin D levels(25-OHD), fatigue and Quality of Life(QoL).

Patients and Methods: A multicentre randomized, placebo-controlled, double-blind trial in three home-based palliative care facilities in Stockholm, Sweden was performed. Adult patients with advanced cancer with expected survival time ≥3 months and 25-OHD<50 nmol/L were randomized to vitamin D3 4000 IU/day or placebo for 12 weeks. The primary endpoint was the difference of long-acting opioid use (fentanyl ug/h) between the groups during 12 weeks, based on 4 time-points, with adjustment for base-line opioids. Secondary outcomes were changes in antibiotic use, 25-OHD, fatigue and QoL after 12 weeks.

Results: The study was performed during Nov 2017 and June 2020. 530 patients were screened, and 244 patients were randomized (intention-to-treat, ITT). 150 patients completed all 12 weeks (per-protocol, PP) of vitamin D (n=67) and placebo (n=83). The major reason for drop-out was death due to cancer.

There was no significant difference between the treatment arms in the ITT-analysis, beta -0.60 (95%CI -1.21; 0.02; p=0.06). In the PP-analysis, the vitamin D-group had significantly less increase of opioid doses compared to the placebo-group; beta -0.56 (95%CI -1.07; -0.05; p=0.03), i.e. 0.56 µg lower fentanyl/h and week with vitamin D treatment. Vitamin D treatment reduced fatigue assessed with Edmonton Symptom Assessment Scale; -1.1 points after 12 weeks (p<0.01). Antibiotic use or QoL did not differ significantly between the groups.

There were no significant differences between treatment arms for any of the outcomes after 4 och 8 weeks.

Vitamin D receptor genotype (TaqI and Fok1) did not affect the opioid doses.

The treatment was safe and well-tolerated. There were two incidences of mild hypercalcemia in the vitamin D group and two in the placebo-group.

Conclusion: Correction of vitamin D deficiency may have positive effects on opioid use and fatigue in advanced cancer patients, but only in those with a survival time more than 12 weeks.

Trial registration: Clinicaltrial.gov: NCT03038516.

4. ABBREVIATIONS

25 OHD	25 hydroxvitamin D
AE	Adverse Event
ADR	Adverse Drug Reaction
AMP	Antimicrobial Peptides
ESAS	Edmonton Symptom Assessment Scale
CI	Confidence Interval
CRF	Case Report Form
EORTC-QLQ-C15-PAL	EORTC Quality of Life Questionnaire for Palliative patients
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Products
MPA	Medicinal Product Agency
QoL	Quality of Life
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
VDR	Vitamin D receptor

5. ADMINISTRATIVE INFORMATION

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6. BACKGROUND

Vitamin D is a steroid hormone that maintains calcium homeostasis and skeletal health.[1] More recently, vitamin D has been investigated for effects on the immune, cardiovascular and nervous systems.[2,3] 25-hydroxyvitamin D (25-OHD) is used to assess an individual's vitamin D status in routine clinical settings.[1] Levels of 25-OHD lower than 50 nmol/L are considered insufficient, and below 20 nmol/L as severe insufficiency.[4] Vitamin D induces the synthesis of antimicrobial peptides on mucosal surfaces, on the skin and in immune cells, supporting the immune response to infections.[5] Vitamin D also reduces cytokine release, dampens inflammatory T-cell responses and has been shown to downregulate prostaglandin synthesis.[6-9] These findings provide a possible mechanistic explanation for effects of vitamin D supplementation regarding infections and pain.

Previous randomized controlled trials have shown that vitamin D supplementation reduced antibiotic use and respiratory tract infections.[10-12]

Vitamin D supplementation has been studied in clinical cohorts experiencing different types of pain, including musculoskeletal pain, migraine as well as visceral and neuropathic pain, with both positive and negative results.[3,13,14] A recent study showed that patients with vitamin D deficiency (25-OHD< 25 nmol/L) who had undergone surgery were at increased risk for higher opioid use compared to those with normal vitamin D levels.[15] Previous trials have shown that vitamin D supplementation is beneficial only in patients with low levels of 25-OHD at baseline.[13,16] Furthermore, patients diagnosed with cancer have lower 25-OHD levels than healthy controls from the same latitude.[17-21] Interestingly, vitamin D supplementation has been associated with decreased cancer mortality.[22]

The effect of vitamin D has been reported to be affected by the genotype of the vitamin D receptor (VDR). Especially the single nucleotide polymorphisms TaqI and Fok1 have been reported to have an impact. Previous studies have shown that CC carriers of TaqI have a 4 times increased risk of muscle pain when treated with statins.[23] CC carriers probably have a reduced expression of VDR and thus an increased risk of symptoms such as vitamin D deficiency, especially if levels of vitamin D in circulation are low. Previous studies have also shown that CC carriers benefit more from vitamin D treatment, e.g. to reduce infections.[24] For Fok1 CC carriers have a better function of VDR than TT-carriers according to several reports.[25-27]

A cross-sectional observational study at our palliative care facility revealed that lower levels of 25-OHD were associated with prescription of higher doses of opioids.[17] Based on this observation we performed a pilot study, where 39 patients with vitamin D deficiency were supplemented with vitamin D_3 oil drops (4000 IU/day) for 12 weeks.[28] Compared to matched, untreated patients from the observational cohort, these patients received lower opioid doses after one month, and had fewer days on antibiotic treatment after three months. QoL assessed with Edmonton Symptom Assessment Scale (ESAS) [29] improved significantly in the intervention group.[28] To test whether these results could be reproduced in a randomized and placebo-controlled setting we designed the 'Palliative-D'-study.

7. METHOD

7.1 Study design

'Palliative-D' was a multicenter, double-blind parallel group, 1:1, randomized, placebo-controlled trial performed at three palliative care facilities in Stockholm, Sweden; ASIH Stockholm Södra, ASIH Stockholm Norr and ASIH Stockholms Sjukhem.

The average number of enrolled patients on any given day at ASIH Stockholm Södra is 380, at Stockholms sjukhem 240 and at ASIH Stockholm Norr 280. More than half of the enrolled patients are diagnosed with cancer. Patients are on average admitted for four months and include patients who receive advanced home care from a period of a few days to those who are admitted for periods longer than one year. All three facilities also operate palliative wards.
In 'Palliative-D', 328 patients were recruited from ASIH Stockholm Södra, 159 from Stockholms Sjukhem, and 44 from ASIH Stockholm Norr.

During the trial period, two amendments were made to the original design:

- Jan 2018: The exclusion criteria were changed from exclusion of patients on any type of vitamin D treatment or supplementation to allowing recruitment of patients on daily doses of vitamin D up to 400 IE/day. The change was made since large number of patients met this exclusion criterion and since this dose was not considered to affect the endpoints. 2) We added eGFR < 30 ml/h as an exclusion criterion for safety reasons.
- January 2019: A third site (ASIH Stockholm Norr) was added to increase inclusion rate. The duration of the trial was also extended from end of 2019 to June 2020 to enable the recruitment of all planned patients.

7.2 Participants

Included patients were admitted to one of the three recruiting palliative care facilities, ≥18 years old, had advanced and/or metastatic cancer in palliative phase (any type of cancer), a life expectancy of at least three months as assessed by one of the three study physician and 25-OHD ≤50 nmol/L. Ongoing oncological treatment was allowed, but not with intention to cure.

Exclusion criteria at screening were 25-OHD>50 nmol/L, hypercalcemia during the past two months; eGFR < 30 ml/h; a medical history of kidney stones, sarcoidosis and/or primary hyperparathyroidism; current medication including vitamin D>400 IU/day, digoxin/digitoxin or thiazides; hypersensitivity to the study drug; participation in other clinical trials involving medication; or other reasons for not being able to complete planned procedures. Ongoing opioid treatment at screening was not required for participation in the trial.

Written informed consent was obtained from all participants before any study-related procedures were performed. Full inclusion and exclusion criteria are listed below.

7.2.1 Inclusion Criteria

1. Patients admitted to ASIH Stockholm Södra, ASIH Stockholm Norr or Stockholms Sjukhem.
2. Incurable cancer patients with any type of cancer. They could have ongoing oncological treatment but only with palliative intention. No patients with ongoing oncological treatment with curative intended treated will be included.

3. The life expectancy should be at least 3 months according to the clinical assessment of the study physician at the screening visit.
4. The patient should have no cognitive failure, being able to comprehend oral and written information about the study.
5. 25 OHD ≤ 50 nmol/L.
6. Men and women aged ≥18
7. Signed 'informed consent'

7.2.2 Exclusion Criteria

1. Ongoing treatment with vitamin D in a dose > 400 IE/ day or any ongoing treatment with active vitamin D, i.e. alfakacidiol eller calcitriol.
2. Kidney insufficiency defined as eGFR < 30 ml/min.
3. Serum level of 25-OH vitamin D >50 nmol/L
4. Known sarkoidosis
5. Treament with tiazides
6. Treatment with digoxin/digitoxin.
7. Primary hyperparathyroidism
8. Hypercalcaemia (verified by a laboratory result younger than 2 month)
9. Plans to leave the Stockholm county within 12 weeks of inclusion
10. History of kidney stones
11. Taking part of another clinical study involving drugs
12. Hypersensivity to cholecalciferol and/or any of the excipients
13. Other criteria that could jeopardize the study or its intention as judged by the investigator
14. Not being able to perform EORTC-QLQ-C15-PAL or ESAS

7.3 **Rational for design and end-points**

The primary end-point was defined as "The decline of opioid-consumption during 12 weeks in the vitamin D group compared to the placebo groups, based on 4 measurements with 4 weeks intervals" since it reflects the experience of pain for the individual patient.

Since fentanyl dose was the main outcome in the pilot-study that had shown a positive result already after one month of vitamin D treatment we choose this parameter for the primary end-points in the Palliative-D study. Fentanyl patch is the most commonly used opioid-treatment among the palliative care facilities in Stockholm and the dose is evaluated every week and adjusted accordingly. In patients treated with other opioids, a conversion table was used to assess fentanyl µg/hour.

In our different specialized home-based palliative-care facilities patients receive weekly visits by a nurse. During this visit, patients are dispensed all their non-oncological medications for the coming week. Pain is assessed at every visit (biweekly using ESAS recorded in electronic medical records, every week through oral communication). Use of short acting opioids during the past week is assessed by inspection and oral communication. If use of short-acting opioids has increased or decreased during the past week, this reported back to the responsible physician, who can adjust the dose of long-acting opioid accordingly. If needed, an extra visit is scheduled so that patients need not to wait another week for change in long-acting opioid dose. Thus, patient's long-acting opioid doses are always up-to-date, and we therefore chose to use long-acting opioid dose assessed on a single day as primary endpoint. We chose to use fentanyl ug/hour, rather than morphine equivalent daily dose, since the majority of our patients are prescribed fentanyl

patches. Fewer conversion calculations thus had to be made, minimizing bias from inaccurate conversion assessments.

In the adjusted model, adjustments were made for baseline opioid dose, age, sex and oncological treatment since we know from previous research and clinical experience that these factors may affect future change in opioid doses and pain [13,17,18,28]. We also know from our previous studies that patients with colectomy or short-bowel syndrome may have an impaired absorption of vitamin D from the gut [13,18,28], thus this variable was also adjusted for.

Secondary outcomes included days of antibiotic use during the past month, as a proxy for bacterial infections. This was one of the outcomes in the pilot-study that had shown positive effects after 3 months of vitamin D treatment [28]. This is also the measure in previous studies on vitamin D treatment evaluating effect on infections [10,17,30]. Thus, this outcome was also chosen for the Palliative-D study.

Fatigue was assessed with the "tiredness" question in the ESAS which was the Question 11 outcome used in our previous study assessing fatigue in the baseline data of the Palliative-D cohort [31]. In concordance, when fatigue was measured with EORTC QLQ-C15-PAL only the tiredness question, Q11, was used for assessing fatigue [31] and not the combined Q7 and Q11-score.

7.4 **The Edmonton Symptom Assessment Scale**

ESAS is a psychometrically validated symptom assessment instrument that exists and is used in many different permutations.[29] It is used in clinical routine to quantify symptom burden in both cancer and non-cancer patient. ESAS is an eleven-point numeric rating scale (NRS), rangning from 0 to 10, with ten different scale items. The symptoms include pain, dyspnea, loss of appetite, tiredness, drowsiness, nausea, wellbeing, anxiety and depressive symptoms. In addition, a 10th optional symptom can be added. In the Palliative-D study, the 10th optional symptom added is "quality of life." At zero, the verbal anchor is "no symptom", and at ten, the verbal anchor is "worst possible" symptom intensity. Patients are asked to assess symptoms at present. Symptom intensity scored as 1-3 as considered mild, 4-6 as moderate and 7-10 as severe. A change in one point on the 11-point scale is considered to be the minimal clinically important difference [32,33]. The version of ESAS used in study 'Palliative -D' is available in the study protocol. Fatigue was assessed with the "tiredness" question in ESAS.

7.5 **EORTC-QLQ-C15-PAL**

EORTC-QLQ-C15-PAL is a shortened version of the EORTC QLQ-C30, one of the most widely used QoL questionnaires in oncology, developed for palliative care patients. The form is comprised of 15 questions and assesses symptom / performance status during the past week [34]. Question number 11 is used to assess tiredness and is stated: "Were you tired?" The patient can score from 1-4, where 1="Not at all", 2="A little", 3="Quite a bit, 4="Very much". To assess fatigue with EORTC-QLQ-PAL15 it is sometimes suggested to use a combination of question number 11 and question number 7 using the instrument's scoring manual, generating results on a scale ranging from 0-100, where 0 is no fatigue and 100 is maximum fatigue. However, in this analysis we have decided to only use Q11 for fatigue assessment which is in accordance with our previous study on fatigue in this study cohort [31]. Quality of life is assessed by Q15 in the with EORTC-QLQ-PAL15 form: "How would you assess you total quality of life during the past

week?" on a scale of 1-7, where the verbal anchor for 1 is "very poor QoL" and the verbal anchor for 7 is "Excellent QoL".

7.6 **Patient enrolment**

Study physicians screened all newly admitted patients to the participating facilities for cancer patients in the palliative phase of their disease trajectory. Study physicians then consulted with the patient's responsible physician before contacting patients (most often over the phone). Patients who expressed interest in participating in the study were sent written information and contacted again a few days later. If they were still interested in participating, an at-home screening visit was booked. Patients who fulfilled all inclusion and did not meet any exclusion criteria after the screening visit and after lab results returned, were randomized to study drug. A sequentially numbered box with two bottles of study drug was delivered to the patient within seven days (baseline visit).

7.7 **Study drug, randomization and masking**

Patients were randomly assigned 1:1 to vitamin D3 oil drops (colour and taste matched) 4000 IU/day or placebo (oil drops) for 12 weeks, dispensed in identical, sequentially numbered bottles. Randomization was performed using a computer-generated randomization list to generate a permutated block randomization with a block size of four. Randomization was not stratified. Trial masking for patients, trial staff and care providers continued until data had been analysed. Detremin 20 000 IU/ml (cholecalciferol solved in Miglyol oil) and placebo (Miglyol oil) were prepared according to Good Manufacturing Practice by Nextpharma. Eurofins LC2, a centralized randomization unit, was responsible for labelling, blinding, and randomization. Detremin was provided from Renapharma Sweden, and placebo was from Nextpharma, France.

7.8 **Interventions**

After written informed consent was collected, patients' records were reviewed. Information on age, sex, antibiotic treatment during the past 30 days, current dose of long-acting opioid at the day of screening, ongoing oncological treatment and type of cancer was retrieved. Blood samples were collected for analysis of 25-OHD, CRP, albumin, creatinine and calcium, and for biobanking. Patients completed the Edmonton Symptom Assessment Scale (ESAS) [29] and EORTC QLQ-C15-PAL [34].

Patients with 25-OHD ≤50 nmol/L, no hypercalcemia and eGFR>30 were eligible for randomization. We informed all patients about their baseline 25-OHD-level. Data on opioid dose and antibiotic use was collected once more at baseline.

Study visits were every 4th week (+/-7 days) in connection with regular weekly visits in patients' homes (Fig 1). Each study visit included a blood sample for analysis of albumin, calcium, creatinine and CRP, completion of ESAS and collection of data on current opioid dose and antibiotic use during the past month. At the end of study (visit 3; 12 weeks), patients were once more asked to complete EORTC QLQ-C15-PAL, and additional blood-samples were taken for measurement of 25-OHD (blinded for the study team) and for storage in a biobank.

All data was collected in an eCRF provided by Mats Hellström at Karolinska University Hospital. The study was regularly monitored by an external monitor, Annika Allqvist (RN, PhD) throughout the study period.

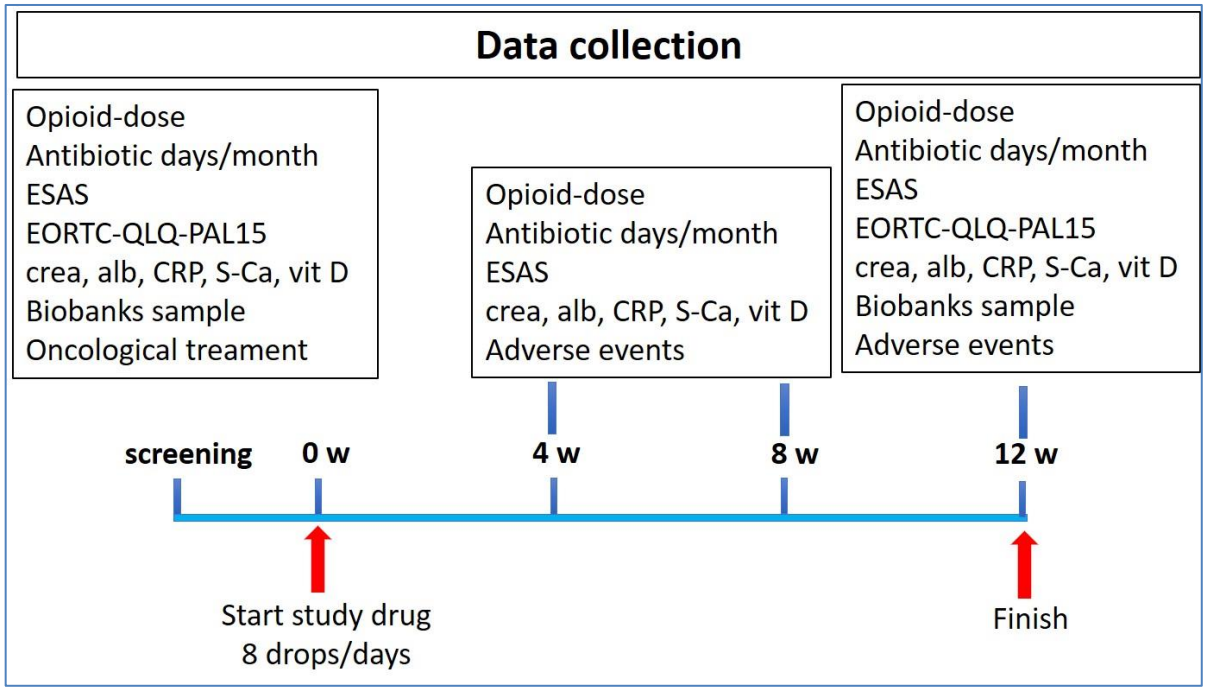

Figure 1: Schematic figure of data collection in the Palliative-D study.

7.9 **Outcomes**

7.9.1 Primary outcome

Mean difference in change in long-acting opioid dose between the treatment arms, measured as fentanyl ug/hour during 12 weeks, based on 4 time-points: 0, 4, 8 and 12 weeks, and adjusted for baseline opioid-values.
The null hypothesis was that the fentanyl dose would increase with same rate in both groups, whereas the alternative hypothesis was that the fentanyl dose would increase faster in one of the groups.
In a secondary analysis, adjustments were made also for age, sex, oncological treatment, baseline 25-OHD and colectomy.

7.9.2 Secondary outcomes

Mean difference in change on antibiotic use, fatigue and QoL assessed with ESAS and EORTC QLQ-C15-PAL, and 25-OHD-levels between the treatment arms after 12 weeks.

Antibiotic use, as a proxy for infections, was measured as the number of days with antibiotics in the previous 30 days.

Fatigue was assessed with the “tiredness” question in the ESAS form and with Question 11 in EORTC QLQ-C15-PAL.

QoL was assessed with the QoL-question in ESAS, and Question 15 in EORTC QLQ-C15-PAL.

7.10 **Exclusion criteria during study participation**

Patients were withdrawn from the study if they developed hypercalcemia (albumin -adjusted Calcium>2.60), if eGFR dropped below 30 ml/min, if they were prescribed medications that were not allowed according to the study protocol, had poor compliance, could no longer take the study drug, withdrew consent, were lost to follow-up, or reported serious or intolerable adverse events.

7.11 **Method for measurements of 25-hydroxyvitamin D levels**

Levels of 25-OHD in plasma were analyzed by chemiluminescence immunoassay (CLIA) on a LIAISON-instrument (DiaSorin Inc, Stillwater, MN, USA), detectable range 7.5-175 nmol/L, CV 2-5% at Dept of Clinical Chemistry, Karolinska University Hospital.

7.12 **Calculation of albumin adjusted calcium in plasma.**

Albumin adjusted Calcium = Calcium + 0.01 x (39 - Albumin).

7.13 **Genotyping**

Genomic DNA was isolated from 200 µl whole blood using the DNA Blood Mini kit (Qiagen, Hilden Geramany). Allelic discrimination reactions were performed using TaqMan® genotyping assays (Applied Biosystems, Foster City CA USA): C__12060045_20 for VDR Fok1 (rs222857); C___2404008_10 for VDR TaqI (rs731236). The final volume of each reaction was 10 µl consisting of 10-30 ng DNA and 2xTaqman Universal PCR Master mix (Applied Biosystems). The PCR profile consisted of 95° C for 10 minutes followed by 40 cycles of 92° C for 15 sec and 60° C for 1 minute. The fluorescence signal was measured with an ABI 7500 Sequence detector (Applied Biosystems).

Since the hypothesis was that the CC genotype of Taq1 provides an impaired VDR function, CC carriers were compared with CT/TT carriers. The hypothesis was also that the TT genotype of Fok1 provides a reduced VDR function, and thus, TT carriers were compared with CT/CC carriers.

7.14 **Monitoring of Adverse Events**

Detremin has few and mild side effects, mostly nausea and diarrhoea. Intoxication of vitamin D may lead to hypercalcemia or in worst case renal failure, but this is extremely rare. Daily doses of 4000 IU/day have not resulted in toxic 25-OHD concentrations in previous studies [10,12,30]. According to the study protocol, only GI-symptoms, increase in creatinine levels, hypercalcemia and renal failure needed to be recorded as adverse events. Data on this was collected at baseline and after 4,8 and 12 weeks.

7.15 **Procedures after end-of study**

All patients were offered a bottle of Detremin after returning the study drug and were given the option to take 4000 IU/day. After 4 weeks of treatment, routine check-up of calcium, albumin and creatinine levels was performed by the study team. After these four weeks, responsibility for continued Detremin-use was transferred from the study team to the patient's responsible physician.

7.16 **Assessment of Compliance to study drug**

We had three methods for assessing compliance:

1. Compliance was assessed by regular contact between the study nurse and participant after every follow-up visit during the intervention period.
2. Each patient was administered two bottles of study drug, and 1.5 bottles were used if patients took all planned doses during the study-period (12 weeks). At the final visit the participants were asked to return the study drug bottles for counting of bottles and oral inspection of the returned study drug.
3. In addition, 25-OHD levels at the end of study was also used as a measure of compliance.

7.17 **Statistical methods**

7.17.1 Sample size

When calculating sample size, we used results from relevant distributions in the pilot study [28] and considered 20% to be a clinically relevant effect size. Based on the pilot study, the predicted mean dose for an untreated person after 12 weeks was 125 µg/h fentanyl, which, with an effect size of 20%, would translate to a difference between group means being -25µg/h. We aimed for a targeting power of 80%, with a significance level of 0.05 (two sided) regarding primary outcome. Since some of the distributions were skewed, 10.000 Monte Carlo simulations per sample size were performed. For each of the simulations, data were generated using the distributions of the previous study, and a linear regression model using bias corrected and accelerated (BCa) bootstrap was fit. The estimated power was the proportion of rejected null hypotheses. The sample size of 190 patients resulted in an estimated power of 81.6%, and with an expected dropout rate of 25%, the estimated sample size was concluded to be 254.

7.17.2 Statistical analyses

Primary analysis was done using linear mixed effect regression using information from three time points: 4, 8 and 12 weeks, adjusting for the baseline value. The baseline values were categorized as described in Table 3. Linear mixed models with person specific random intercepts and slopes were used to control for the intra-person correlation inherent to repeated measurements. The parameter of interest was the interaction of group and time, interpreted as the average difference in weekly opioid dose change between groups. As secondary analysis adjustments were made for baseline opioid use, age, sex, oncological treatment, colectomy and baseline 25-OHD levels. Both intention-to-treat (ITT) and per-protocol (PP) analyses were performed for the primary outcome in accordance with the study protocol.

The difference in opioid doses at 12 weeks were analysed using linear regression. As with primary analysis, two models were analysed; unadjusted and adjusted. Estimation of confidence intervals (CI) was performed using normal approximation.

According to the original statistical analysis plan, the confidence intervals (CI) were to be estimated with the bias-corrected accelerated bootstrap method (Bca). However, a high proportion (20-35%) of the bootstrap replications were disregarded for several models. This was presumably due to lack of variation in the outcome, possibly causing a systematic bias. The attained results from the bootstrap-analyses were compared to the normal-approximation confidence intervals, and as the results were very similar and did not change the conclusions, the usual normal approximation CI's were used. In the comparisons, the normal approximation intervals were often wider than the Bca intervals.

All secondary outcomes were analysed at week 12 only using linear regression, as pre-specified in the protocol. Adjustments were made as for the primary outcome. Antibiotic treatment was categorized at baseline: 0, 1-7, 8-14 and 15-30 days.
Due to high death rates during follow-up, several sensitivity analyses were performed as described by Carpenter et al [35].

The number needed to treat (NNT) was calculated using the reduction of at least 12 units of fentanyl, from baseline to the end of the study at twelve weeks in the PP-population.
In addition, separate analyses of all outcomes using un-adjusted raw-data, were made at each time-point. To investigate mortality in the treatment groups, survival functions were estimated using the Kaplan-Meier estimator and log-rank test for equality.

All analyses were performed in Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.). Multiple imputation was done using using the STATA command mimix.29

7.18 **Ethical considerations**

The study protocol was published before the start of the trial[36] and registered at Clinicaltrial.gov: NCT03038516. The study was approved by the Regional Ethical Committee in Stockholm (Dnr 2017/405-31/1 and amendment 2021-00323) and was conducted according to the declaration of Helsinki. Written informed consent was obtained from all participants before any study related procedure was performed.

8. RESULTS

Palliative-D was conducted between Nov 2017 and June 2020. 530 patients were screened and 244 patients full-filled the inclusion criteria and were randomized to receive study drug (ITT-population), 121 to vitamin D_3 and 123 to placebo (Figure 1). Inclusion stopped early due to the Covid-19 pandemic, and ten patients fewer than planned were randomized. 150 patients completed all 12 weeks of vitamin D (n=67) or placebo (n=83) and constitute the PP-population (Figure 2). The major reason for drop-out was death due to cancer.

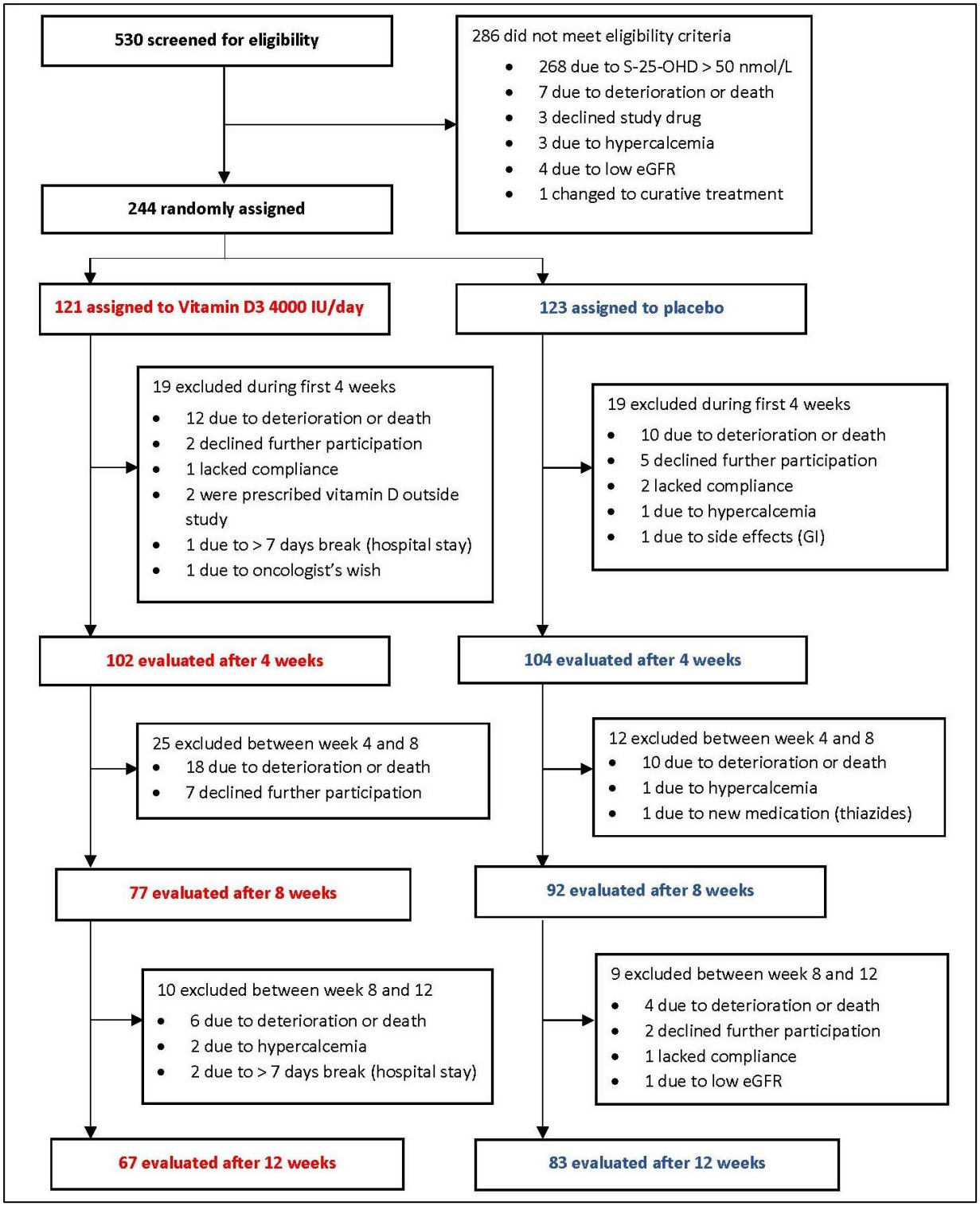

Fig 2: Flow chart of screened, included and excluded patients in the Palliative-D study.

Baseline demographics from the screened cohort (n=530) have been published in 2020.[37] Baseline demographic from the ITT and PP populations are presented in Table 1 and 2 below.

Table 1. Baseline characteristics of randomized patients in the Palliative-D study. (ITT-population)

	All (n=244)	**Vitamin D (n=121)**	**Placebo (n=123)**	**p-value**
Age, median (IQR), years	68 (61-75)	68 (61-76)	68 (61-75)	0.64
Male, n (%)	120 (49)	62 (51)	58 (47)	0.61
Female, n (%)	124 (51)	59 (49)	65 (53)	0.61
No active oncol. treatment, n (%)	78 (32)	37 (31)	41(33)	0.68
Chemotherpy, n (%)	118 (48)	58 (48)	60 (49)	0.90
Hormonal therapy, n (%)	28 (11)	18 (15)	10 (8)	0.11
Target therapy, n (%)	20 (8)	8 (7)	12 (10)	0.49
25-OHD, median (IQR), nmol/L	38 (28-45)	39 (28-45)	38 (28-45)	0.83
Fentanyl dose, median (IQR), ug/h	0 (0-25)	0 (0-37)	0 (0-25)	0.44
Prescribed long-acting opioid No. (%)	128 (52)	61 (50)	55 (45)	0.44
No. days on antibiotics, median (IQR), g/L	0 (0-3)	0 (0-1)	0 (0-3)	0.89
Albumin, median (IQR), g/L	30 (26-34)	30 (26-34)	30 (27-33)	0.88
Calcium, median (IQR), mmol/L	2.30 (2.21-2.38)	2.30 (2.19-2.36)	2.29 (2.23-2.38)	0.25
Creatinine, median (IQR), umol/L	70 (58-74)	68 (58-83)	71 (58-86)	0.54
CRP, median (IQR), mg/L	9 (3-31)	10 (3-27)	9 (3-31)	0.97
Colectomy No. (%)	31 (13)	17 (14)	14 (11)	0.57
Type of cancer, No. patients	..	..	..	
Brain	2	1	1	>0.99
Breast	26	11	15	0.53
Upper gastrointestinal	59	30	29	0.88
Lower gastrointestinal	56	28	25	0.64
Gynecological	24	12	12	>0.99
Head & Neck	1	0	1	>0.99
Hematological	6	5	1	0.12
Lung	42	19	23	0.61
Melanoma	4	2	2	>0.99
Prostate	21	13	8	0.26
Sarcoma	3	1	3	0.62
Urinary tract	3	1	5	0.21
ESAS fatigue, median (IQR)	4 (2-6)	4 (2-6)	4 (2-6)	0.88
ESAS QoL, median (IQR)	4 (2-6)	4 (2-6)	4 (2-6)	0.54
EORTC QLQ-C15-PAL, Q11 fatigue, median (IQR)	3 (2-3)	3 (2-3)	3 (2-3)	0.87

EORTC QLQ-C15-PAL Q15 QoL, median (IQR)	4 (3-5)	4 (3-5)	4 (3-5)	0.31

In the treatment group, two patients had two types of cancers (lung cancer + sarcoma, prostate cancer + upper GI cancer), and in the placebo group two patients had two types of cancer (lung cancer + urinary tract cancer, upper GI cancer + prostate cancer. CI: Confidence interval, S-25-OHD: S-25-hydroxyvitamin D, ESAS: Edmonton Symptom Assessment Scale (range 0-10), EORTC QLQ-C15-PAL: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C15 Palliative (fatigue Q 11 range 1-4, QoL Q 15 range 1-7), QoL: Quality of Life Q: Question. Mann Whitney U was used for continuous variables and Fisher's exact test for categorical variables.

Table 2: Baseline characteristics of patients who completed 12 weeks. (PP-population)

	All (n=150)	**Vitamin D (n=67)**	**Placebo (n=83)**
Age, median (IQR,)years	68 (61-75)	68 (61-76)	68 (61-75)
Male, No. (%)	74 (49)	34 (51)	40 (48)
Female, No. (%)	76 (51)	33 (49)	43 (52)
25-OHD, median (IQR), nmol/L	38 (30-45)	39 (30-46)	38 (30-45)
Fentanyl dose, median (IQR), ug/h	0 (0-12)	0 (0-25)	0 (0-12)
No. days on antibiotics, median (IQR), g/L	0 (0-2)	0 (0-0)	0 (0-3)
Albumin, median (IQR), g/L	32 (28-35)	32 (28-36)	32 (28-34)
Calcium, median (IQR), mmol/L	2.38 (2.31-2·44)	2.38 (2.29-2·43)	2.38 (2.33-2.46)
Creatinine, median (IQR), umol/L	72 (58-86)	72 (59-89)	72 (58-89)
CRP, median (IQR), mg/L	7 (2-22)	7 (1-24)	7 (2-18)
Type of cancer	..	..	..
Brain	2	1	1
Breast	14	4	10
Upper gastrointestinal	35	16	19
Lower gastrointestinal	41	21	20
Gynecological	15	7	8
Head & Neck	0	0	0
Hematological	3	2	1
Lung	21	9	12
Melanoma	0	0	0
Prostate	14	7	7
Sarcoma	3	0	3
Urinary tract	3	0	3
ESAS fatigue, median (IQR)	3 (1-5)	3 (1-5)	3 (1-5)
ESAS QoL, median (IQR)	4 (2-5)	3 (2-5)	4 (2-5)
EORTC QLQ-C15-PAL Q11 fatigue, median (IQR)	2 (2-3)	2 (2-3)	2 (2-3)
EORTC QLQ-C15-PAL Q15 QoL, median (IQR)	4 (3-5)	5 (3-5)	4 (3-5)

In the placebo group one patient had two types of cancer (upper GI cancer and prostate cancer).
S-25-OHD: S-25-hydroxyvitamin D, ESAS: Edmonton Symptom Assessment Scale (range 0-10), EORTC QLQ-C15-PAL: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C15 Palliative (fatigue range 1-4, QoL range 1-7), QoL: Quality of Life Q: Question

8.1 **Primary end-point**

244 patients were included in the ITT-analysis, which was based on 769 observations and 4 time-points over 12 weeks. Groups were well balanced at baseline (Table 1). The ITT-analysis did not show a significant difference between the slopes: beta -0.60 (95%CI -1.21; 0.02; p=0.06) and in the adjusted analysis: beta -0.59 (95%CI -1.20 to 0.03; p=0.06) (Fig 2). Beta coefficients for the adjusted variables at baseline are shown in Table 3. Sensitivity analyses of different imputation models are shown in Table 4.

In the PP-analysis (n=150), based on 450 observations, the vitamin D-group had significantly less increase of opioid doses during the study period compared to the placebo-group; beta -0.56 (95%CI -1.07; -0.05; p=0.03) in both the unadjusted and adjusted analysis, i.e. 0.56 µg less fentanyl/h and week with vitamin D treatment (Fig 2) and corresponding to 6.72 ug/h after 12 weeks. Thus, the null hypothesis that there would be no difference between the slopes could be rejected in the PP-analysis.

A separate non-longitudinal analysis performed on data after 12 weeks, also showed significantly lower opioid doses in the vitamin D arm, -7.0 µg /h (p=0.03) (Table 5).

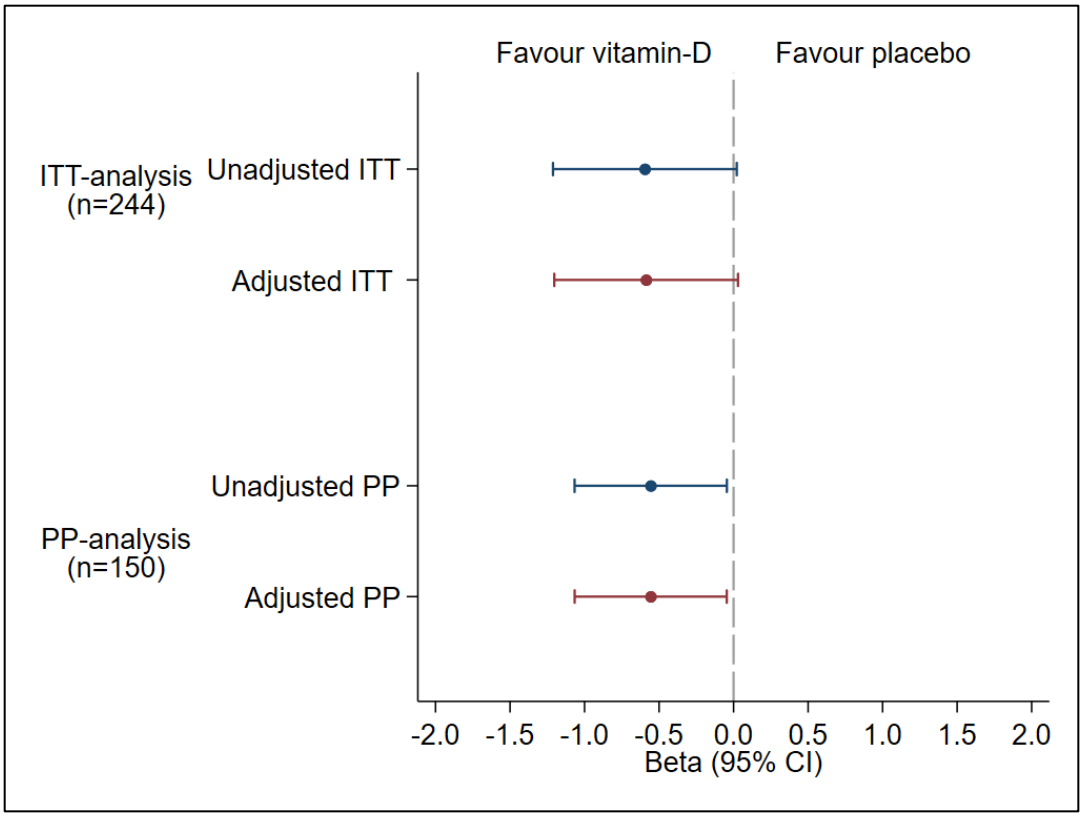

Fig 3: Main analysis: Forest plots with beta coefficient and 95% CI over the opioid use in the Palliative-D study. The intention-to-treat (ITT) analyses include all 244 randomized patients, and the Per Protocol (PP) analysis is based on the 150 patients that completed the 12 weeks study period (vitamin D 4000 IE/day n=67 and placebo n=83). In a separate ITT analysis only patients with opioid use at baseline were included (n=115). Adjustment was made for baseline in all analyses and for age, sex, oncological treatment, base-line 25-hydroxyvtaim D and colectomy in the "adjusted model".

Table 3: Beta coefficients for the adjusted variables at baseline in the longitudinal model for opioid dose (primary outcome) in the ITT-analysis. The opioid categories (fentanyl patches) listed were compared with "no opioids". The doses correspond to the available doses on the fentanyl patches except for 6 ug/h, which corresponds to 5 mg morphine per os. The oncological treatment categories were compared with "no treatment". 25-OHD=25-hydroxyvitamin D.

	Beta coefficient	p-value	95% CI
Male	1.52	0.60	-4.20 7.24
Age	0.11	0.44	-0.16 0.36
Colectomy – yes	-1.52	0.73	-7.17 10.22
25-OHD	-11.83	0.30	-34.16 10.49
Opioid dose Fentanyl (ug/h)			
6-12	15.14	<0.0001	7.0 23.3
25-37	42.7	<0.0001	33.0 52.4
50-67	62.9	<0.0001	54.5 71.3
100-175	127.7	<0.0001	111.5 143.8
250-	378.9	<0.0001	349.6 408.1
Oncological treatment			
Chemotherapy	-4.25	0.22	-11.1 2.6
Hormones	-8.31	0.09	-18.0 1.42
Target therapy	-5.79	0.33	-17.48 5.88

Table 4. Sensitivity analysis for the longitudinal linear mixed model, unadjusted ITT. Three different imputation models.

Method	**β / week**	**95% CI**	**p-value**
Worst observation carried forward	-0.31	-0.74 to 0.11	0.15
Jump to reference multiple imputation	-0.39	-1.55 to 0.77	0.51
Copy increments in reference multiple imputation	-0.46	-1.43 to 0.51	0.36

β : beta coefficient; CI: confidence interval.

8.1.1 Number needed to treat

Number needed to treat (NNT) was calculated to be 12 patients for 12 weeks to decrease the opioid-dose with at least 12 µg fentanyl/hour in one patient.

8.1.2 Self-assessed pain

Self-assessed pain did not differ between the two treatment arms during the study period, indicating that opioid-doses were adequately adjusted. The mean ESAS-score for pain in the vitamin D group was 1.8 units (SD 2.2) at baseline and 1.9 (SD 2.3) after 12 weeks. The corresponding mean scores in the placebo group were 1.9 (SD 2.2) and 1.9 (SD 1.9), respectively.

8.1.3 Subgroup analysis of the opioid users

At baseline, 50% of patients in the Vitamin D group and 55% of patients in the placebo group were prescribed long-acting opioids. For those taking opioids at baseline, the placebo group had a lower median, 25 µg/h (IQR 12-75), than the Vitamin D group, 37 µg/h (IQR 12-50). However, the placebo group had a higher mean (54.6) than the Vitamin D group (44.5), indicating that this group had higher outliers. After twelve weeks, the opioid doses of those who were taking opioids at baseline had diverged between the two groups. The median opioid dose increased in the placebo group (median 37 µg/h, mean 58.5), but decreased in the Vitamin D group (median 31 µg/h, mean 40.1).

8.2 **Secondary end-points**

After 12 weeks of treatment there was no significant difference in antibiotic use or QoL between treatment arms (Table 5, Fig 4).

The vitamin D group exhibited a significantly lower degree of fatigue compared to the placebo group after 12 weeks; -1.1 point ($p<0.01$) (Fig 4). Fatigue assessed with EORTC QLQ-C15-PAL was not significantly different between the groups ($p=0.06$) (Fig 4).

Table 5. Effect of vitamin D 4000 IU/day after 12 weeks (non-longitudinal analysis) on opioid dose, antibiotic use, fatigue and quality of life (QoL) compared to placebo in the per-protocol analysis, (n=150). Adjustments were made for baseline value in both analysis and for age, sex, oncological treatment, baseline 25-OHD and colectomy in the adjusted analysis. Effect is presented as β-coefficient representing mean difference in change from baseline values between treatment arms after 12 weeks. The scale of EORTC-QLQ-C15 PAL Q15 has been reversed so negative value of beta is an improvement in QoL, in line with all the other outcomes where negative value is improvement*p<0.05; **p<0.01.

	Unadjusted β (95% CI)	**p-value**	**Adjusted β (95% CI)**	**p-value**
Fentanyl dose/hour	-7.00 -13.22 to -0.79	***0.03**	-6.1 -12.40 to 0.21	0.058
Days on antibiotics	-0.57 -2.21 to 1.08	0.50	-0.46 -2.14 to 1.22	0.59
ESAS fatigue	-1.12 -1.88 to -0.36	****0.004**	-1.11 -1.89 to -0.33	****0.006**
EORTC QLQ-C15 PAL Q11 fatigue	-0.23 -0.49 to 0.03	0.08	-0.25 -0.52 to 0.01	0.06

ESAS QoL	-0.58 -1.32 to 0.17	0.13	-0.67 -1.42 to 0.09	0.08
EORTC QLQ-C15 PAL Q15 QoL	-0.35 -0.79 to 0.09	0.12	-0.34 -0.79 to 0.11	0.13

CI: confidence interval, ESAS: Edmonton Symptom Assessment Scale (range 0-10), EORTC QLQ-C15 PAL: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C15 Palliative (fatigue range 1-4, QoL range 1-7), QoL: Quality of Life

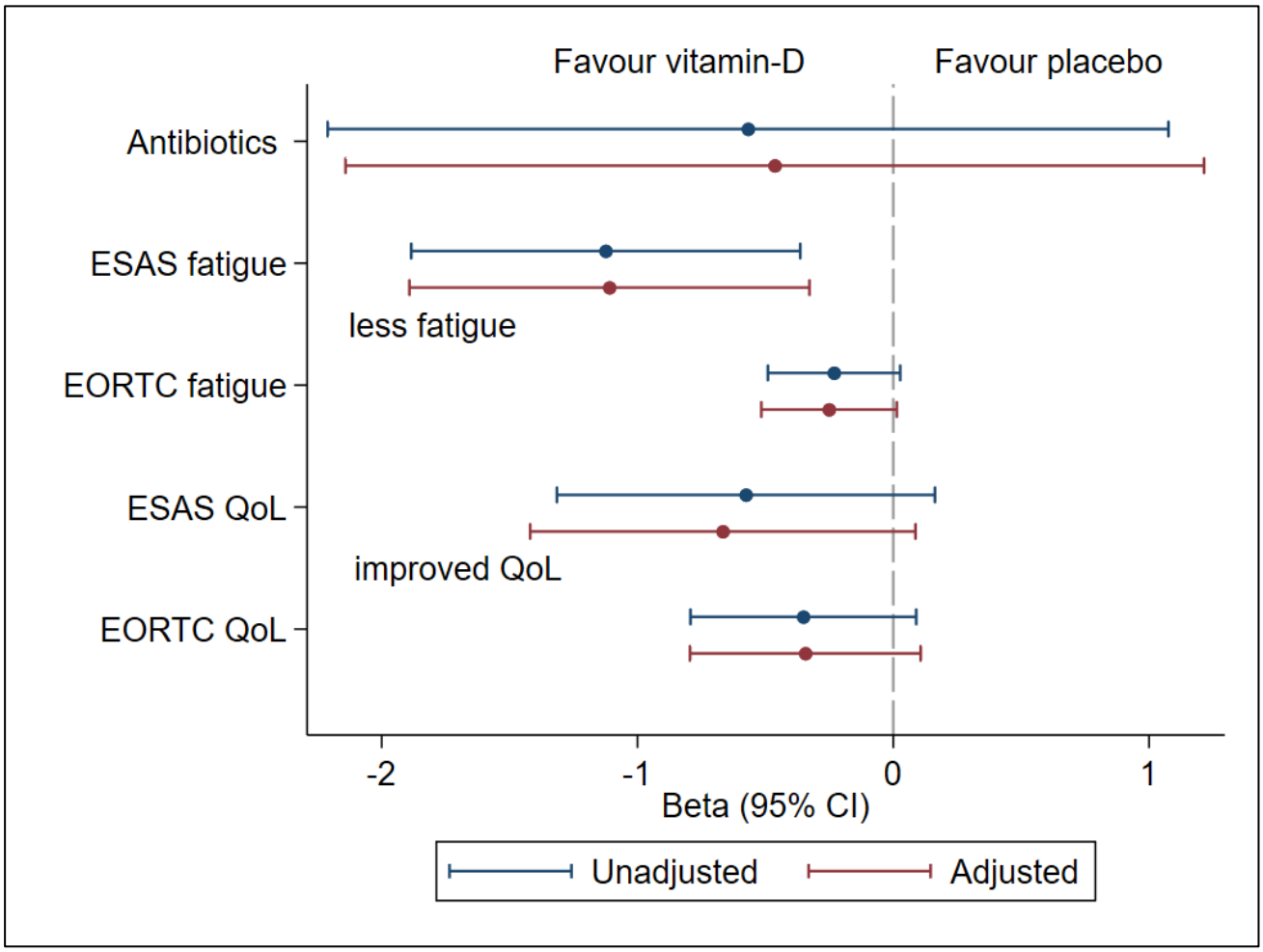

Fig 4: Secondary outcomes: Forest plot with beta coefficient and 95% CI over the antibiotic consumption, fatigue and QoL assessed with ESAS and EORTC-QLQ-PAL-C15 (EORTC) in the Palliative-D study. The analysis is based on the Per Protocol (PP) analysis of the 150 patients completing the 12 weeks study period (vitamin D 4000 IE/day n=67 and placebo n=83). Adjustment was made for baseline in all analysis and for age, sex, oncological treatment, base-line 25-hydroxyvtaim D and colectomy in the "adjusted model". The scale of EORTC-Q15 has been reversed so negative value of beta is an improvement in QoL, in line with all the other outcomes where negative value is improvement.

Vitamin D treatment increased the mean 25-OHD significantly, from 36 (±11) nmol/L to 81 (±26) nmol/L ($p<0.001$), while mean 25-OHD in the placebo group remained stable (Fig 5).

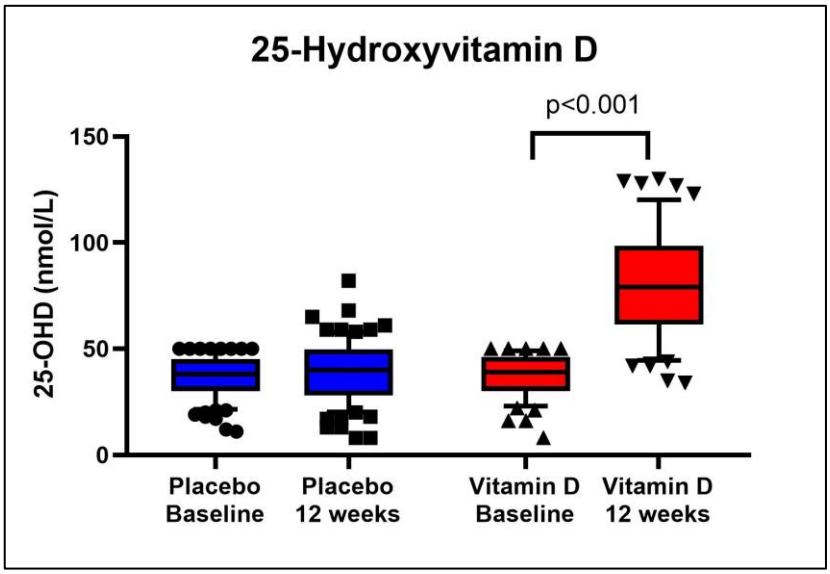

Fig 5: 25-hydroxyvitamin D levels (25-OHD) in plasma measured in the Palliative-D study at baseline and after 12 weeks of treatment with placebo (n=83) or vitamin D 4000 IE/day (n=67).

8.3 **Genotyping**

Whole blood from 435 patients were available for extraction of DNA. Genotyping of the VDR polymorphism could be successfully performed in 425 patients for TaqI and for 415 patients for Fok1. The distribution of the different genotypes are shown in Figure 6. For TaqI, 17% of patients were CC genotype and only 1.7% were TT genotypes for Fok1.

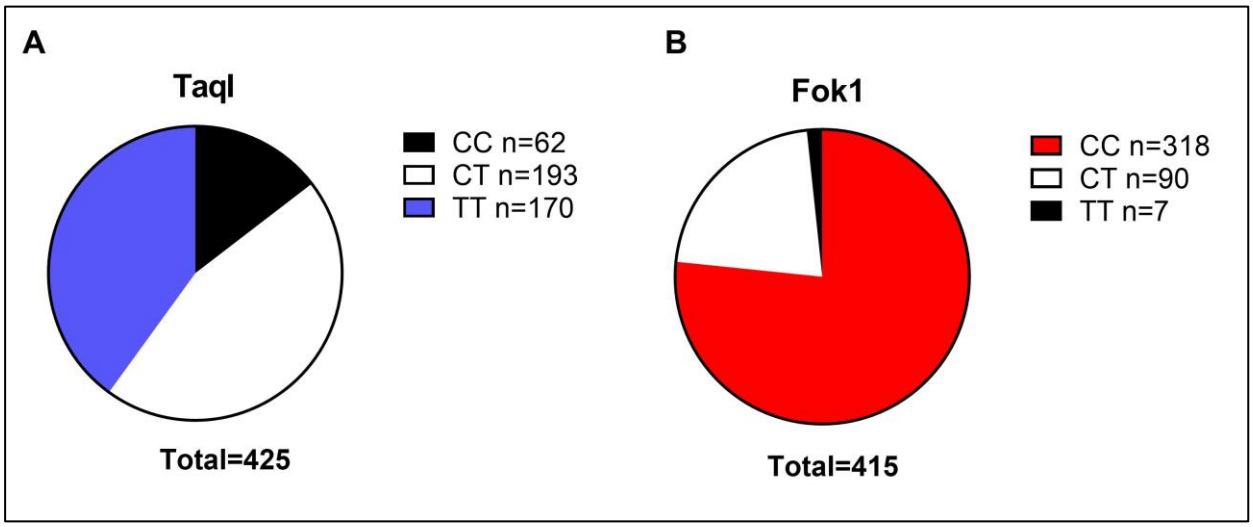

Fig 6: Distribution of CC-, CT- och TT-genotype for the two vitamin D receptor polymorphism TaqI (A) och Fok1 (B) in the Palliative cohort.

Comparison between TaqI CC carriers and CT/TT carriers showed no significant difference in opioid dose at baseline between the groups (Figure 7). Moreover, there was no difference in effect of the vitamin D treatment between the different genotypes (Fig 8).

There was no significant difference in opioid dose at baseline in Fok1 TT carriers compared to CT/CC carriers (Fig 9).

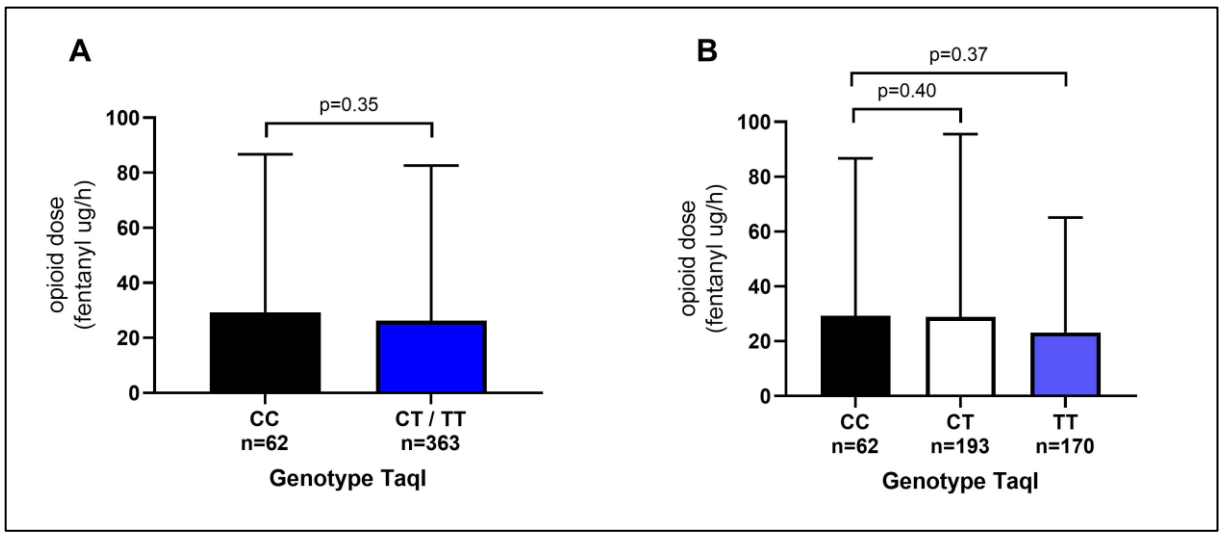

Fig 7: Comparison of TaqI genotypes and opiod dose at baseline. Column shows mean values + SD of opioid doses in fentanyl (ug/h). Statistical test was performed using Mann-Whitney U Test.

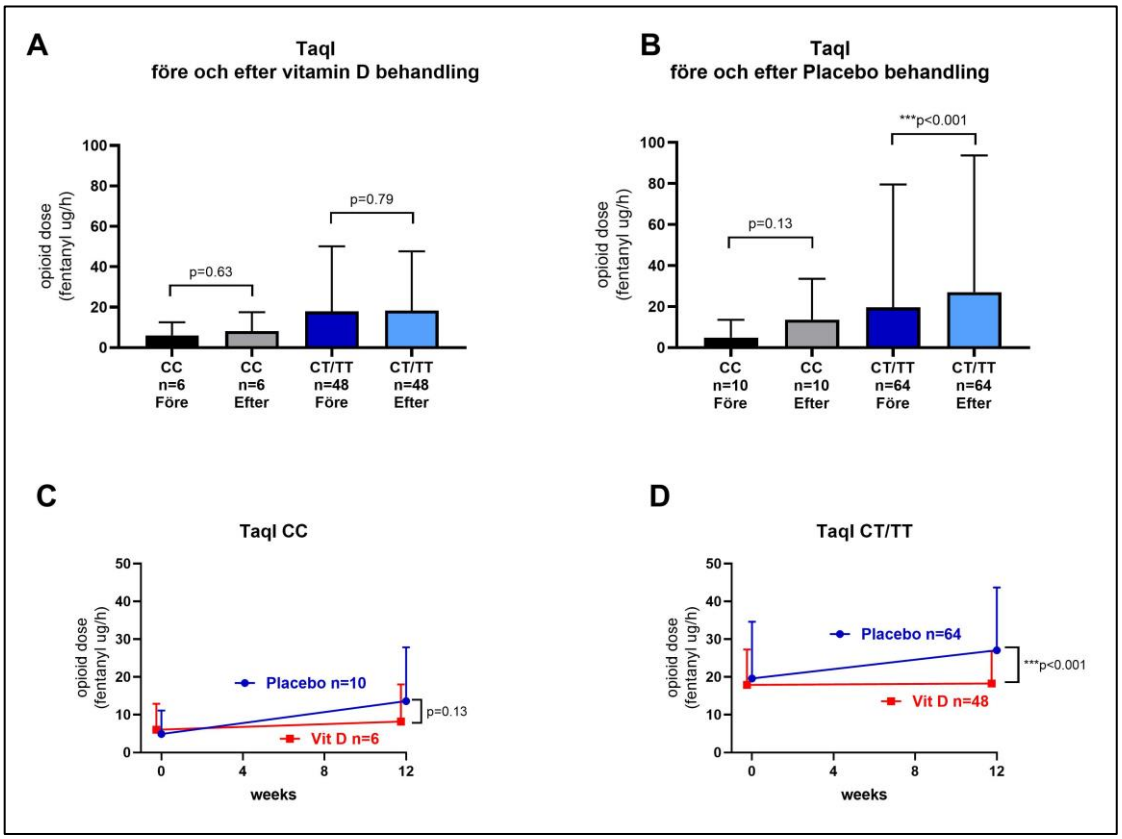

Fig 8: Change in opioid dose in patients receiving vitamin D and placebo for 12 weeks divided by genotype for VDR TaqI.

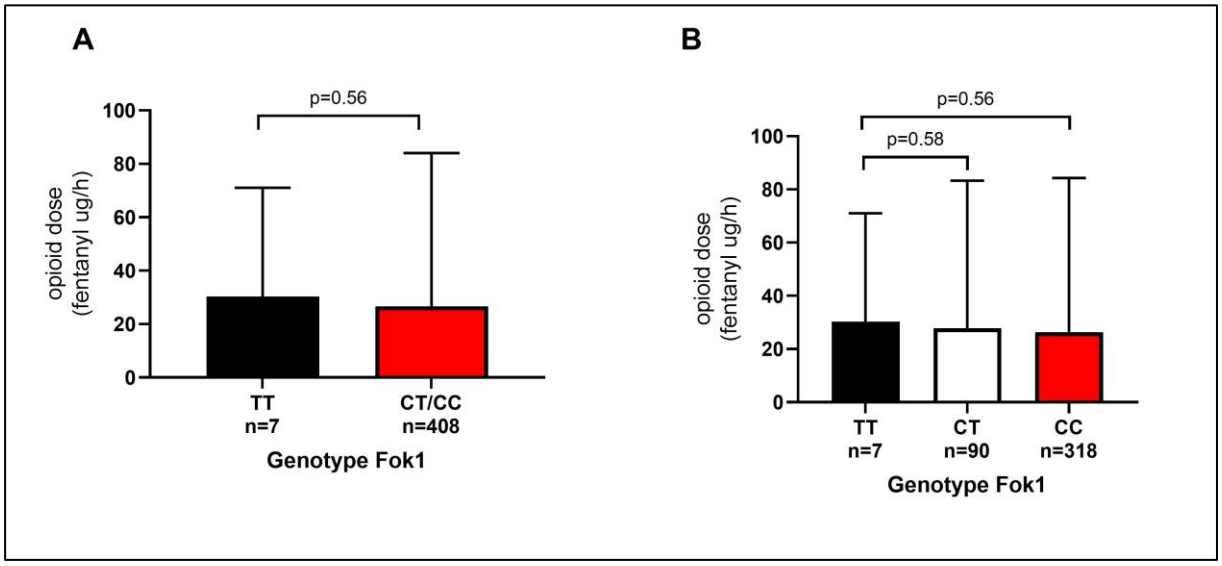

Fig 9: Comparison of Fok1 genotypes and opiod dose at baseline. Column shows mean values + SD of opioid doses in fentanyl (ug/h). Statistical test was performed using Mann-Whitney U Test.

Of the patients receiving vitamin D or placebo for 12 weeks, 124 patients could be genotyped for Fok1; 52 of those who received vitamin D and 72 of those who received placebo. The TT genotype was found only in 2 patients in the vitamin D group and in 1 patient in the placebo group. Thus, no statistical analysis could be performed between the TT-genotype and the other genotypes.

8.4 **Compliance**

In the vitamin D group, 2 out of the 67 patients who completed the study reported failure in compliance during part of the study due to a hospital stay. Their 25-OHD levels at the end of the study were 42 and 58 nmol/L. All other study participants reported sufficient compliance. Nevertheless, 6 of these participants had 25-OHD < 50 nmol/L at the end of study. A total of 45 bottles from the 67 that were distributed in the vitamin D group were returned to the study team, and 44 of the bottles showed that the participants had consumed 75-100% of the dose. One participant had consumed 50% of the dose. In 22 cases no bottles were returned. The mean 25-OHD level in the vitamin D group was 81 nmol/L; range 34-166 nmol/L (Fig 5).

In the placebo group all participants reported sufficient compliance. Bottles from 57 of 83 participants were returned to the study team and 55 had consumed 75-100% of the dose. Two participants had consumed 50% of the dose. In 26 cases no bottles were returned. The mean 25-OHD in the placebo group at end of study was 39 nmol/L, range.

8.5 **Survival and drop-out rate**

Survival was not a predefined endpoint in ‘Palliative-D’, and a Kaplan-Meier analysis was not included in the analysis plan. Since we had large drop-out rates that differed between treatment arms we conducted a post-hoc survival analysis (Fig 10). There was no difference in survival time between the two treatment arms at any timepoint, after 4 weeks (p=0.36), 8 weeks (p=0.09) or 12 weeks (p=0.08). Statistical analysis was performed by using log-rank test for equality of survivor functions. However, there was a statistically significant higher drop-out rate in the vitamin D arm between 4 and 8 weeks compared to the placebo-arm (p=0.02). This was not due to more deaths in the vitamin D group; but instead a higher rate of patients that declined further participation after 4 weeks compared to the placebo arm (Fig 1). Still, the higher drop-out rate in

the vitamin D arm, especially during the two first months, is concerning. The median survival time for all randomized patients (n=244) was 6.1 months (95% CI: 5.2 – 7.1), whereas the median survival time for those completing the study (n=150) was 8.5 months (95% CI: 7.5 – 9.9).

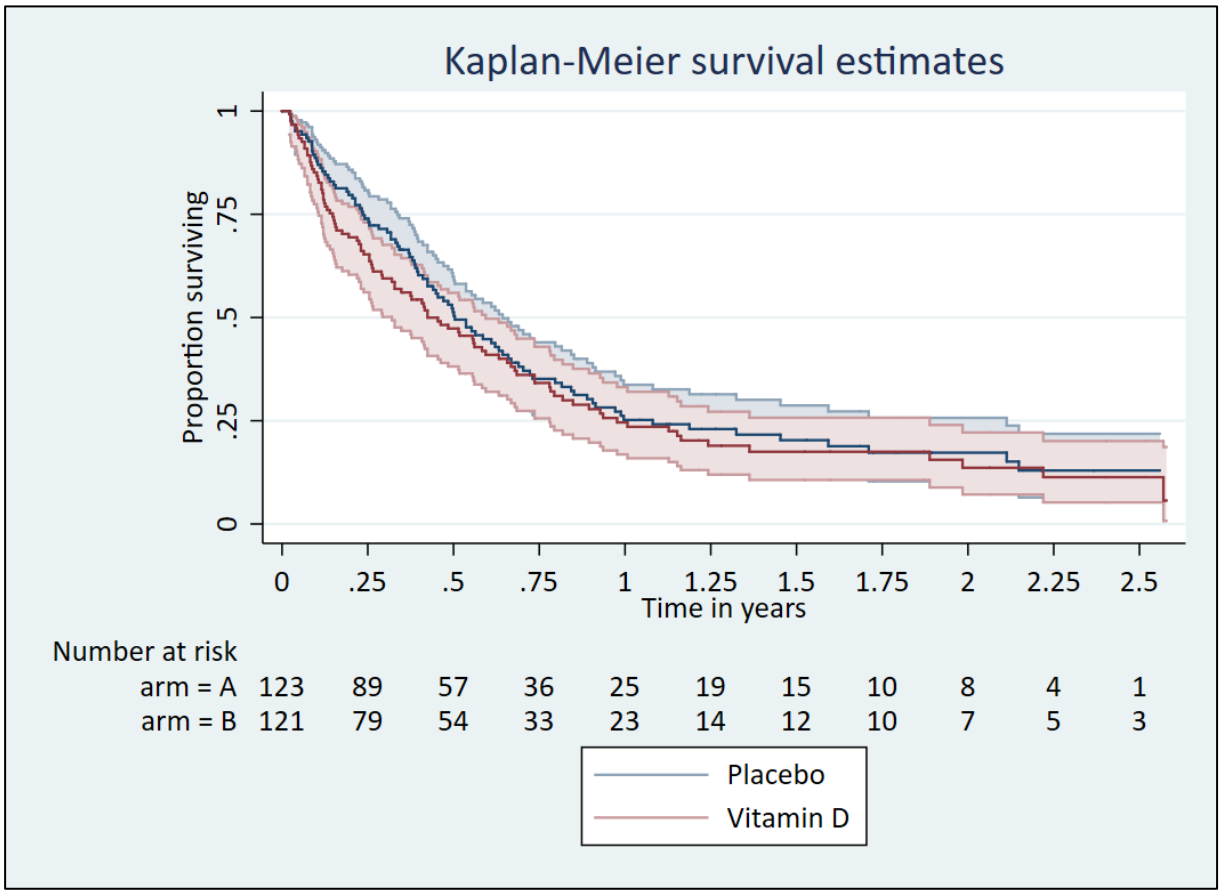

Fig 10: Kaplan-Meir plot of survival time in the Palliative D study throughout the study period (12 weeks) and follow-up for up to 2.5 years.

There were more patients who did not complete all 12 weeks in the vitamin D group (45%) than in the placebo group (33%). In both groups, baseline opioid dose was higher for patients who were excluded, compared to those who could be evaluated after 12 weeks. The baseline median opioid dose for excluded patients was 12 µg/h in both groups, but the placebo group had a wider interquartile range (IQR 0-50) than the vitamin D group (IQR 0-37). This would indicate that excluded patients from the placebo arm had higher opioid use compared to excluded patients from the Vitamin D group. This is also reflected in the mean baseline values of opioid use for excluded patients, which were higher in the placebo group (36.1) compared to the Vitamin D group (27.9).

Of those who were excluded and were taking opioids at baseline, the placebo group had higher opioid doses (median 50 µg/h (IQR 12 -75), mean 62.4) than the Vitamin D group (median 25 µg/h (IQR 18.5-50, mean: 47.1).

This would suggest that the results of the primary analysis were not due to the greater proportion of lost to follow-up in the treatment group, as there is no indication that persons who were lost to follow-up in that group had higher initial values.

8.6 **Un-adjusted raw-data at each time point,**

A separate analysis of un-adjusted raw data was performed in the ITT study population (n=244) at each time-point, i.e. after 4, 8 and 12 weeks in all randomized patients (Fig 11). This analysis showed that there was no difference between the treatment arms for opioid use, antibiotic use or QoL at any time point except for fatigue after 12 weeks (Fig 11).

The same analysis was performed in the PP-study population (n=150) also showing that the difference between the treatment arms for opioid use and fatigue was evident first after 12 weeks (Fig 12).

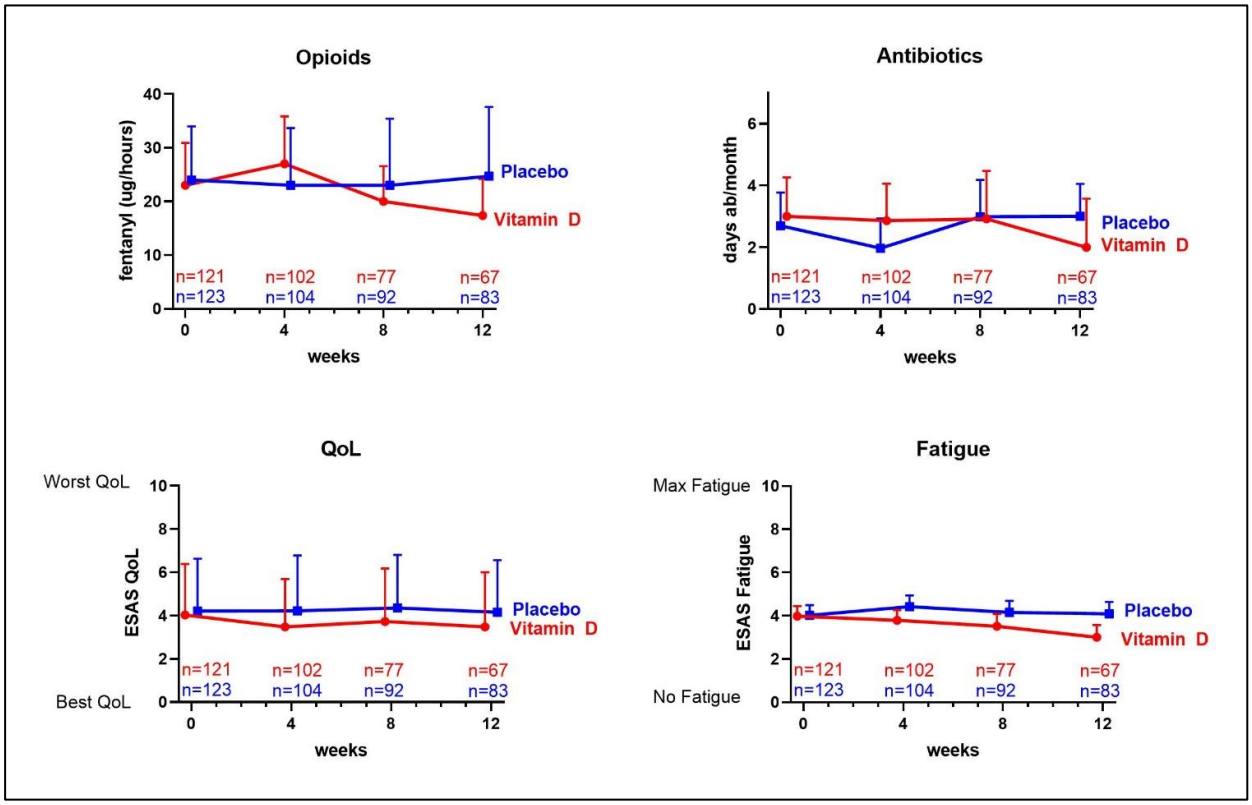

Fig 11: ITT-population: Raw-data. i.e. not adjusted for baseline. Change in opioid-doses, antibiotic consumption days of antibiotics / 30 days, Quality of Life (QoL) and fatigue were assessed with ESAS in the Palliative-D study throughout the study period in all randomized patients (n=244). The number of patients at each time point is presented. Points show mean of unadjusted raw data, + 95% CI.

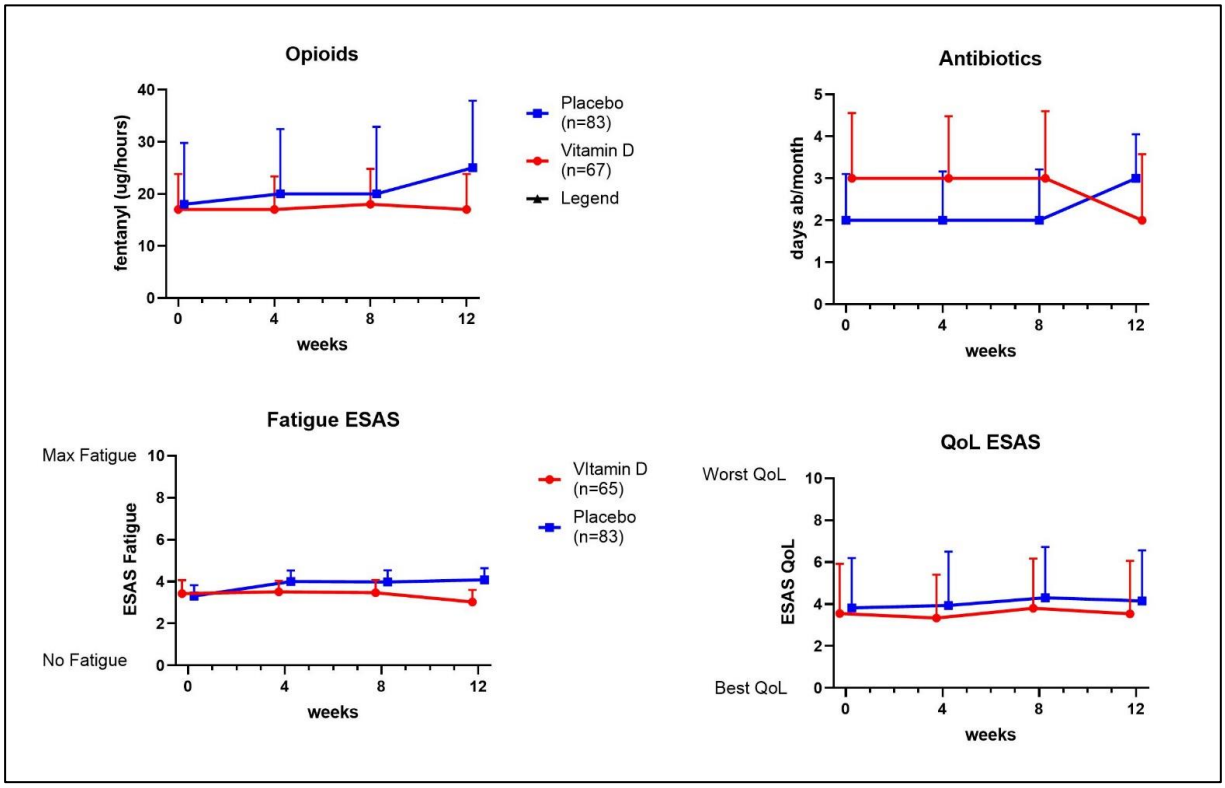

Fig 12: PP-population: Raw-data. i.e. not adjusted for baseline. Change in (A) opioid doses measured as fentanyl ug/hour throughout the study period (B) antibiotic use (days of antibiotics/30 days) (C) fatigue, and (D) QoL assessed with ESAS in the Palliative-D study in all patients completing the study. Points show mean of unadjusted raw data, +95% CI. The analysis is based on the 150 patients that completed the 12 weeks study period (vitamin D 4000 IE/day n=67 and placebo n=83)

8.7 **Lab and Safety parameters**

There was no difference in calcium (albumin adjusted), creatinine, albumin or CRP levels between the two treatment arms throughout the study. The unadjusted raw data at each time-point in the ITT- and the PP-study populations for both primary and secondary outcomes are presented in the Fig 13 and 14 below.

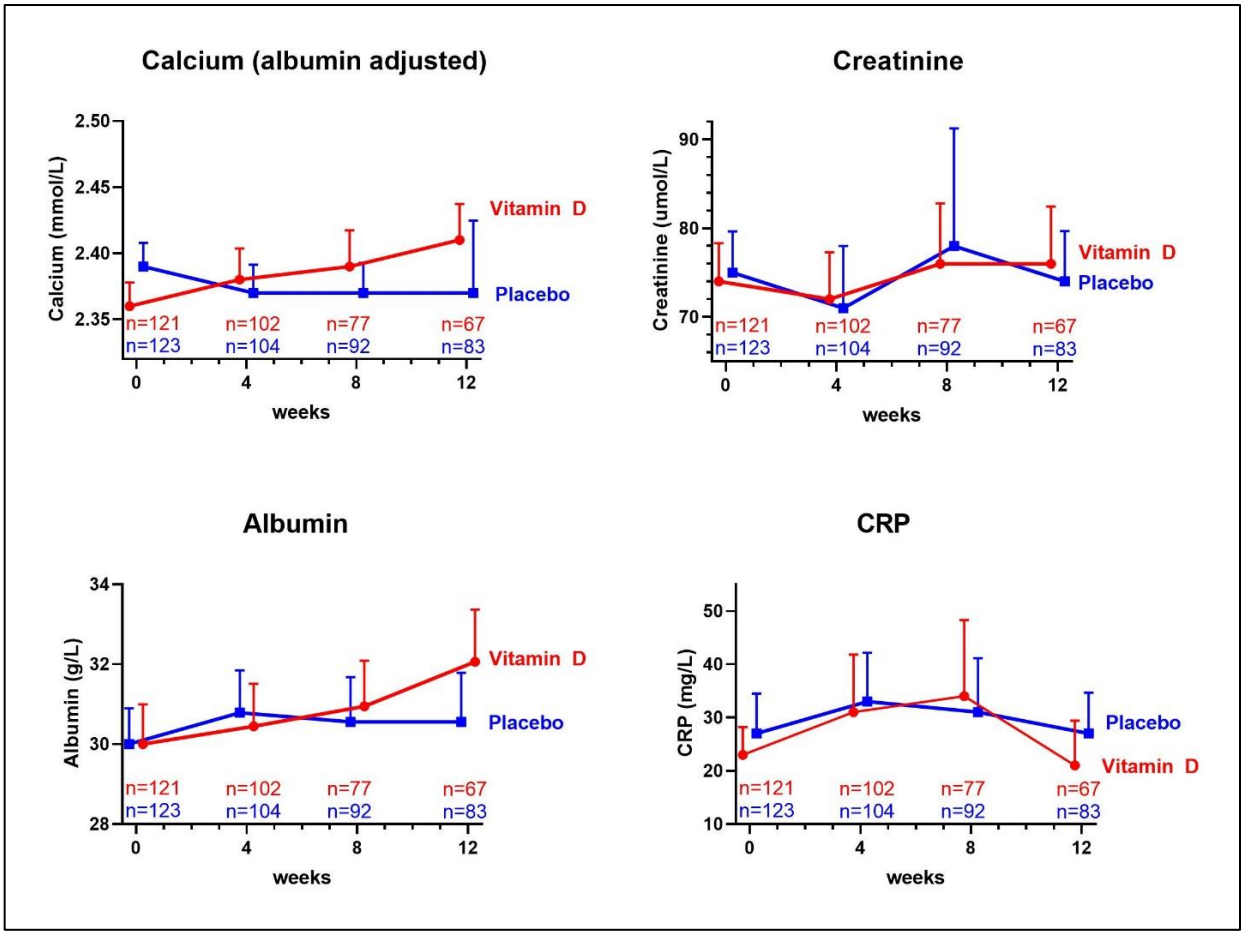

Fig 13: ITT-population: Raw-data. i.e. not adjusted for baseline. Levels of albumin adjusted calcium, C-reactive protein (CRP), albumin and creatinine in the Palliative-D study throughout the study period in all randomized patients (n=244). Number of patients still participating in the study are indicated at each time point. Points show mean of unadjusted raw data, + 95% CI.

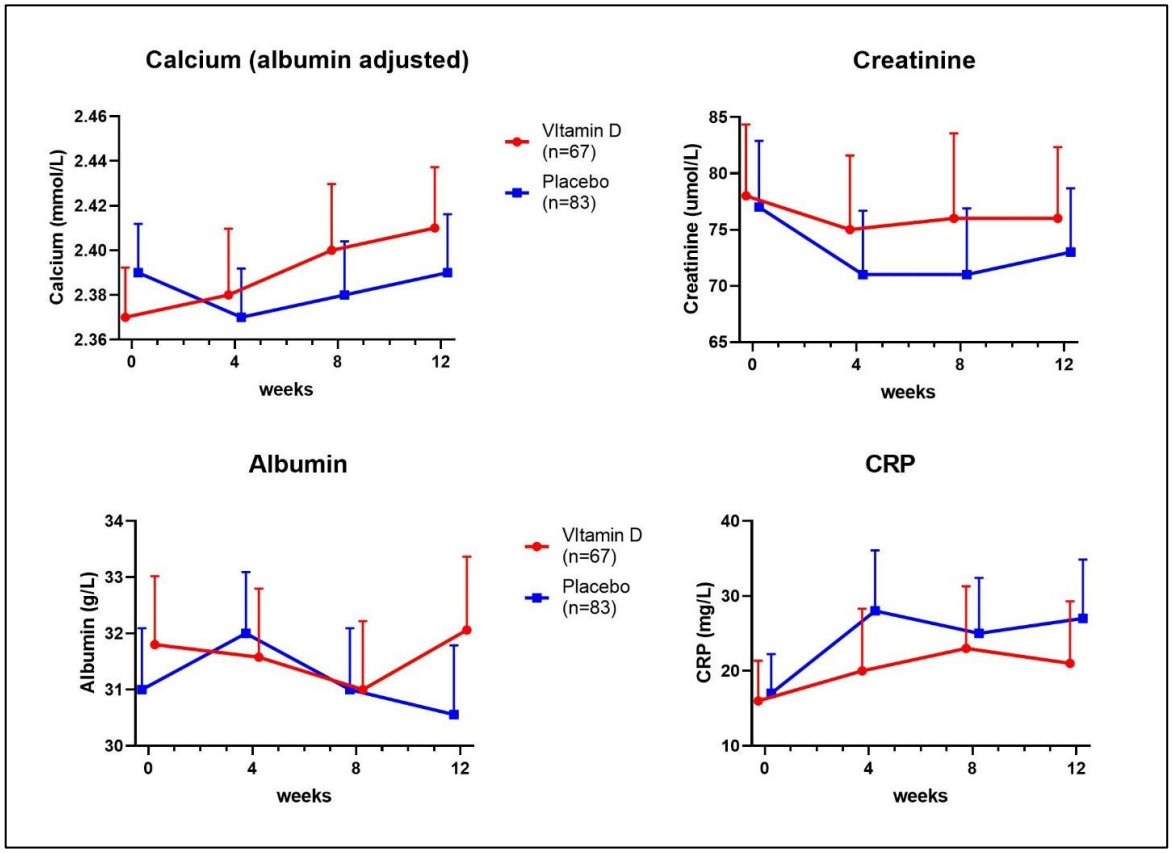

Fig 14: PP-population: Raw-data. i.e. not adjusted for baseline. Levels of albumin adjusted calcium, C-reactive protein (CRP), albumin and creatinine in all patients completing the Palliative-D study (n=150). Points show mean of unadjusted raw data, + 95% CI.

8.8 **Adverse events**

One patient developed renal failure during the study and the code was broken in this case. Unmasking of treatment allocation revealed that this patient had received placebo. No other serious adverse events (SAE) were recorded. Treatment was well tolerated, with two cases of mild hypercalcemia in the vitamin D group and two in the placebo group (Table 6).

Table 6: Adverse events in the Palliative-D study in all randomised patients throughout the study period of 12 weeks. 244 patients were randomised to vitamin D 4000 IE/day or placebo: 206 patients finished 4 weeks, 169 finished 8 weeks and 150 patients finished 12 weeks; (vitamin D n=67) and placebo (n=83).

	Vitamin D (n=121)	Placebo (n=123)
GI symptoms, mild diarrhoea, nausea and/or stomach pain	2	1
Increased creatinine	1	0
Renal failure	0	1
Breathlessness	0	1
Hypercalcemia (Albumin adjusted S-Ca > 2,60)	2	2

9. DISCUSSION

The results from the Palliative-D show that correction of vitamin D deficiency is safe and may have positive effects on opioid use and fatigue. After 12 weeks, the mean opioid dose (fentanyl) in the vitamin D group was 6.7 µg/h lower than in the placebo group. NNT analysis revealed that 12 patients had to be treated for 12 weeks to obtain a reduction for one patient with the clinically relevant dose 12 µg/h (smallest available fentanyl patch).

Vitamin D supplemented patients exhibited less fatigue compared to the placebo group with a reduction of >1 ESAS point, a change that is assessed as clinically relevant.[32,33] However, vitamin D supplementation had no effect on antibiotic use. No difference in QoL between groups could be observed. As expected, vitamin D treatment significantly increased 25-OHD levels. There was no evidence for that the VDR genotypes Taq1 and Fok1 affected opioid-doses at baseline.

A strength of the "Palliative D" study is the study design. RCTs are difficult to conduct in palliative care[38-42], and thus most data from palliative care facilities is based on observational or case-control studies.[43] Further, data is comprehensive, with very few missing data points in patients completing the study. The mean increase in 25-OHD after 12 weeks of vitamin D treatment (42 nmol/L) was somewhat larger than in the pilot study (33 nmol/L), indicating good compliance. Since we included patients with all types of advanced cancer with varying remaining life span, generalizability of the results may be broad. Significant results regarding opioid dose and fatigue were obtained, although only 150 patients, 40 fewer than planned, completed all 12 weeks. Vitamin D treatment in this group proved safe and well tolerable, and screening for 25-OHD deficiency would be feasible considering analytical costs.

The major limitation of this study was the large drop-out rate with fewer patients completing all twelve weeks in the intervention group, resulting in loss of power and increased risk of both Type I and type II errors. The large drop-out rate highlights the difficulty for even trained palliative care professionals to estimate remaining life span in patients with advanced cancer, often overestimating survival time.[44,45]

There was a larger drop-out rate in the vitamin D arm, especially between 4 and 8 weeks, although overall survival did not differ significantly between the two treatment arms at any time-point. Still, it cannot be entirely ruled out that vitamin D in some way was detrimental, leading to higher drop-out rate in the vitamin D arm, although the mechanistic basis for such an effect remains unclear. However, it should be noted that vitamin D has not been shown to have a negative effect in other cancer-trials.[46,47] In the large VITAL-study on 25 871 healthy subjects ≥50 years old randomized to vitamin D_3 2000 IE/day or placebo with a median follow-up time of 5.3 years, vitamin D actually reduced the incidence of metastatic or fatal cancer.[46] Overall, we believe that vitamin D is safe when given to cancer-patients in the palliative phase, although careful monitoring for potential side-effects is recommended.

The observed effect size of -6.7 µg/h fentanyl per patient after 12 weeks might be of limited clinical importance. However, the results presented here underscore a new conceptual advancement that vitamin D may have direct mechanistic effects on the opioid system in humans. As such, vitamin D supplementation should be explored in cancer pain since it may provide a safe and accessible way to reduce opioid use.

It should also be noted that the study population was predominantly Caucasian. Thus, the results might not be generalizable to more diverse patient populations.

The potential mechanism for a beneficial effect of vitamin D on opioid use remain elusive. Current evidence mainly supports effects of vitamin D on immune response and thus possibly inflammatory pain.[6,8,9] However, in a rat model for hyperalgesia, vitamin D was shown to reduce pain via induction of opioid-associated genes in the cerebrum and spinal cord.[48]

To the best of our knowledge, this is the first large RCT on vitamin D supplementation in advanced cancer patients measuring the effect of vitamin D on pain, infections, fatigue and QoL. Though there is continuous interest in the role of vitamin D in cancer prevention and treatment, [49-53] data on the topic of vitamin D supplementation in symptom management of cancer patients is scarce, and has often been collected from smaller cohorts and/or using case-control design.[18,28]

The results presented here suggest that Vitamin D may improve fatigue, a symptom notoriously difficult to treat pharmacologically.[54] The findings are in accordance with our cross-sectional study of all screened patients at baseline in the 'Palliative-D cohort' (n=530), showing a correlation between low 25-OHD and fatigue severity, especially in men.[37] The decreased need of opioids in the vitamin D group might contribute to decreased fatigue, as might decreased inflammatory immune response and prostaglandin synthesis.[6,8,9] However, a significant effect on fatigue was evident only when assessed with ESAS ($p<0.01$) and not with EORTC QLQ-C15-PAL ($p=0.06$). Thus, this finding should be interpreted with caution.

We did not observe effects of vitamin D supplementation on self-assessed QoL, in line. with a previous study in patients with advanced cancer.[55] Although reduced pain and fatigue may contribute to better QoL, it may still not be enough to affect the overall life-situation of these patients.

10. CONCLUSION

In conclusion, correction of Vitamin D deficiency in cancer patients admitted to palliative care is safe, well-tolerated and may have a positive effect on opioid use and fatigue, but only is those with a survival time more than 12 weeks.

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The study team have no conflict of interest to declare. The funders of this trial took no part in study design, data collection, data interpretation, writing or reviewing of the manuscript.

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14. SIGNED BY THE PRINCIPAL INVESTIGATOR

I, the undersigned, am responsible for the Clinical Study Report of the Palliative D study and herby certifies that all data in the report is correct.

Sponsor & Principal Investigator
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