UK-CTAP briefing on Vitamin D [originally codified 12 November 2020]

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1. Summary

Rationale and evidence	• Vitamin D has well defined role in regulating inflammatory responses to infection *in vitro* and *in vivo*. • Loss of vitamin D receptor signalling in animal models of LPS induced ARDS is associated with more severe pathology and increased mortality. • Vitamin D suppresses inflammation through direct effects on inflammatory signalling pathways and through modulation of ACE2 and renin-angiotensin axis. • Clinical studies indicate a significant correlation with vitamin D deficiency and increased inflammation • In vitro evidence for anti-viral effects of calcitriol against SARS-CoV-2 with high unphysiological doses • Small interventional, single centre, open-label, randomised, controlled pilot trial in Spain found that Vitamin D therapy (0.532 mg calcifediol loading, 0.266 mg calcifediol maintenance doses on day 3,7 and every week thereafter) in hospitalised patients reduced the need for ICU and mortality. Results need to be interpreted with extreme caution considering many methodological weaknesses of the study and the dosing that is too low according to the modelling included in this document • Observational studies investigating the association of vitamin D levels and COVID-19 outcomes ○ UK-biobank studies suggest that vitamin D deficiency was not associated with infection nor COVID-19 related mortality after adjustment for confounding factors (continuous analysis and categorical analysis ≤25nM/ml).

	○ Meta-analyses suggest that around half of COVID-19 patients have vitamin deficiency and there was an increased risk for individuals with low serum vitamin D (below 20ng/ml) for COVID-19 infection, but not mortality.
Dose, PK, PD	• Vitamin-D advice in UK: 10µg/day (400 IU) supplement (during winter months or at-risk group) • Dietary target in the US: ○ Plasma vitamin D level maintenance of ≥50 nmol/L ○ 1 to 70 years old: 600 IU/d (i.e. 15µg QD) ○ 71 years old and over: 800 IU/d (i.e. 20µg QD) • Food supplements: ○ varying strengths ○ industry standard maximum level 75µg/day (3000IU) • Prescription only medicines containing Vitamin D3 ○ Tablet and liquid forms ○ Strengths: 10µg (400 IU) to 1.25mg (50,000 IU) • Baseline 25(OH)D affects the time it takes to reach optimal 75 nmol/L (300 ng/mL) serum level • 25(OH)D has a 20-day half-life, and it slowly accumulates under 100µg QD vitamin D_3 oral dosing. It may take a month for a severely deficient person to reach the target level at the 100µg QD dose • To rapidly raise 25(OH)D levels and maintain it above 75nmol/L, an oral dosing strategy may involve a high single dose at least 1250 µg (50,000 IU) followed by 100 µg (4000 IU) QD dose starting 30 days after the single dose ○ High loading dose (1250 µg): could be administered by high-dose prescription cholecalciferol, e.g. 1.25mg tablet/liquid. ○ Maintenance dose (100µg): could be covered by lower dosage forms of prescription cholecalciferol.
Safety and interactions	• Contraindication in hypercalcaemia, decreased renal function, metastatic calcification, evidence for vitamin D toxicity • Caution regarding additional vitamin D or calcium supplementation during medicinal intake of high Vitamin D doses • Vitamin D toxicity may occur at levels of 25(OH)D >150 ng/ml (>375 nmol/l). Clinical symptoms include: ○ Acute symptoms: anorexia, headache, vomiting, constipation. ○ Chronic symptoms: dystrophy (weakness, loss of weight), sensory disturbances, possibly fever with thirst, polyuria, dehydration, apathy, arrested growth and urinary tract infections. Hypercalcaemia ensues, with metastatic calcification of the renal cortex, myocardium, lungs and pancreas. • Interactions:

	○ Patients taking cardiac glycosides may be susceptible to hypercalcaemia related to Vitamin D supplementation. This leads to increase in cardiac glycoside related toxicity. ○ Phosphate infusions should not be administered in hypervitaminosis D because of the dangers of metastatic calcification. ○ Concomitant use of glucocorticoids might decrease the effect of vitamin D. (Weak evidence to support this statement)
Clinical trials	• 22 interventional trials with vitamin D (monotherapy) in COVID-19 ○ 6 in prophylaxis setting (WHO scale 0) ○ 16 in treatment setting (Who scale 1-2 n=4, WHO scale 3-4 n=12) • Higher single doses are mostly used in the hospitalised setting (highest 500,000 IU) • Preventative setting: ○ Highest dose regimen (start dose 100,000 IU, maintenance daily 10,000 IU for 16 weeks) (NCT04411446) ○ UK trial organised by Queen Mary University of London in normal risk healthy volunteers, with dosing up to 3200 IU (80 micrograms) for 6 months (NCT04579640)

2. Proposed products

2.1. Vitamin D compounds and metabolism

The term Vitamin D is used for a range of compounds [1]:

- Ergocalciferol (vitamin D2)
- Cholecalciferol (vitamin D3) – most frequently trialled
- Dihydrotachysterol (synthetic vitamin D analogue)
- Alfacalcidol (1α-hydroxycholecalciferol, vitamin D analogue),
- Calcitriol (1,25-dihydroxycholecalciferol, active form of vitamin D).

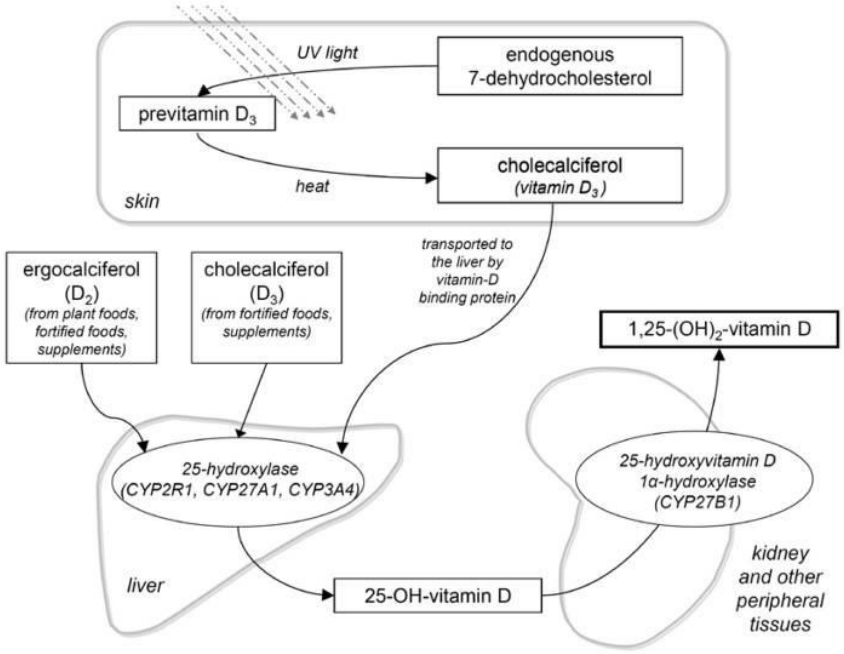

Figure 1: Vitamin D metabolism: supplemental vitamin D is available in two forms, cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). Vitamin D3 is produced endogenously in the skin upon exposure to ultraviolet (UV) radiation and is found in fortified foods and foods of animal origin such as fish, eggs, and liver. Vitamin D2 is only available exogenously, primarily through consumption of plant foods, fortified foods and dietary supplements. The liver is the primary site for the initial hydroxylation reaction that converts both vitamin D2 and D3 to the main circulating form of vitamin D, 25-hydroxycholecalciferol (25(OH)D). This conversion occurs via hepatic 25-hydoxylases, which include the cytochrome P450 (CYP) enzymes 2R1, 3A4, and 27A1. The active steroid hormone form of vitamin D is 1,25-dihydroxycholecalciferol (1,25(OH)2D), which is formed from 25(OH)D at both the local tissue level and in the kidney by an additional hydroxylation of 25(OH)D via 1α-hydroxylase (CYP27B1) 9. Catabolism of vitamin D metabolites occurs via 24-hydroxylase (CYP24A1). (Robyn et al., 2013).

[1] https://bnf.nice.org.uk/treatment-summary/vitamins.html

2.2. Nutritional guidance

Classification of serum 25(OH)D levels

- Target: to maintain plasma vitamin D level ≥50 nmol/L (The Recommended Dietary Allowance (RDA) by US Institute of Medicine (IOM) (Ross et al, 2011)
  - 1 to 70 years old: 600 IU/d (i.e. 15µg QD)
  - 71 years old and over: 800 IU/d (i.e. 20µg QD)
  - Severe deficient: <12 ng/mL < 30 nmol/L (FNB & IOM, 1997)
  - Deficient: 12 – 19 ng/mL 30 – 49 nmol/L (IOM, 2011)
  - Insufficient: 20 – 29 ng/mL 50 – 74 nmol/L (Holick M. F., 2007)
  - Target for prevention: ≥30 ng/mL ≥ 75 nmol/L (Holick M. F., 2007)
  - Danger of toxicity: >100 ng/mL > 250 nmol/L

The Department of Health and Social Care and NHS currently recommend that [2]:

- Children aged 1 to 4 years old should be given a daily supplement containing 10 micrograms of vitamin D.
- Children above 4 years old and adults should consider taking a daily supplement containing 10 micrograms of vitamin D only during the autumn and winter, if there is concern that intake is not enough via food.
- People at risk of vitamin D deficiency are recommended to take a daily supplement containing 10 micrograms of vitamin D throughout the year.
- COVID-19 specific advice: The NHS has made a recommendation to take 10 micrograms (400 IU) of vitamin D a day between October and early March in the context of longer times indoors due to the COVID-19 pandemic. However, there is currently not enough evidence to support taking vitamin D to prevent or treat coronavirus.

However, these recommendations doses are not relevant to achieving target for prevention (75 nmol/L). Further information is provided in the Pharmacology section (Section 4).

2.3. Vitamin D in medicines:

- Simple vitamin D deficiency can be prevented by taking an oral supplement of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) daily. Higher doses may be necessary for severe deficiency.
- Vitamin D deficiency may be caused by intestinal malabsorption or chronic liver disease which usually requires treatment with vitamin D in pharmacological doses.
- Vitamin D requires hydroxylation by the kidney to its active form, therefore the hydroxylated derivatives alfacalcidol or calcitriol should be prescribed if patients with severe renal impairment require vitamin D therapy.
- All of forms are used to treat rickets and osteomalacia.
- Calcitriol is also licensed for the management of postmenopausal osteoporosis.
- Paricalcitol, a synthetic vitamin D analogue, is licensed for the prevention and treatment of secondary hyperparathyroidism associated with chronic kidney disease.

[2] https://www.nhs.uk/conditions/vitamins-and-minerals/vitamin-d/

- A list of all prescribing only vitamin D3 medicines can be found in section 8. It is available in capsule and liquid form containing cholecalciferol in strengths 10µg (400 IU) to 1.25mg (50000 IU).

2.4. Vitamin D in elderly

UK National Diet and Nutrition Survey 2008–20 gives a crude snapshot estimate of the vitamin D status of different populations including the elderly, however they are not sensitive enough to outline prevalence of low vitamin D status in vulnerable groups, and may not reflect important seasonal variations (Table 1; Spiro & Buttriss, 2014).

Aging is a significant cause of vitamin D deficiency due to a reduced renal function, reduced ability to synthesise vitamin D from sunlight and reduced outdoor activity (Gallagher, 2013).

The following data are available highlighting vitamin D deficiency in the elderly (Spiro & Buttriss, 2014):

- Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA) study of older people living independently: 36% of older men and 47% of older women had 25(OH)D serum concentrations below 30 nmol/l
- The Longitudinal Ageing Study Amsterdam (LASA), in community-living older people aged over 65, reported a serum 25(OH)D lower than 25 nmol/l in 8% of men and 14% of women, and lower than 50 nmol/l in 45% of men and 56% of women
- Swiss nursing homes study: 90% of women had levels below 50 nmol/l compared with 57% in non-institutionalised women
- Swedish study in 11 nursing homes: 80% had 25(OH)D levels below 50 nmol/l, and vitamin D deficiency was associated with increased mortality

Age (year)/gender	Mean year-round 25(OH)D nmol/l	% below 25 nmol/l year round	% below 25 nmol/l January–March	% below 25 nmol/l July–September
1.5–3 boys and girls	58.1	7.5	n/a	n/a
4–10 boys	52.3	12.3	31.4	1.7
4–10 girls	48.0	15.6		
11–18 boys	44.9	19.7	40.0	13.4
11–18 girls	41.1	24.4		
19–64 male	43.5	24.0	39.3	8.4
19–64 female	47.3	21.7		
65+ male	47.0	16.9	29.3	3.6
65+ female	42.5	24.1		

Table 1: Year-round mean plasma 25(OH)D and the proportion with 25(OH)D concentration below 25 nmol/l all year round and in summer and winter months in free living children and adults and elderly from UK National Diet and Nutrition Survey 2008–20 (Spiro & Buttriss, 2014)

3. Rationale for development in COVID-19

There is an increasing literature on the potential role of vitamin D deficiency and the development of severe of COVID-19, which may partially explain some of the observed associations with geographical location, age, ethnicity and sex (Rhodes et al., 2020) and (Benskin, 2020).

Vitamin D has long been thought essential for the maintenance of an effective immune response to microbial pathogens. Vitamin D is important for the production of calthicidin (Hewison, 2011) - a cationic antimicrobial peptide in macrophages and epithelial cells, that has antiviral activity against enveloped viruses (Ahmed et al., 2019). Most immune cells express the vitamin D receptor and in monocytes, there are thought to be over 200 Vitamin D responsive genes, however, the major immune modulating effect of vitamin D receptor signalling is through reducing inflammatory responses (Rhodes et al 2020). This is reinforced by a study of inflammation and vitamin D deficiency in the elderly which demonstrated a significant correlation between vitamin D deficiency and increased IL-6 and CRP levels (Laird et al., 2014). It is not well understood how vitamin D reduces inflammatory responses, although studies indicate that vitamin D represses production of inflammatory cytokines such as IL-6 and TNF, by reducing MAPK p38 and NFkB activation (Zhang et al., 2012). Vitamin D also increases the expression of ACE2 and reduces ACE expression. This change in ratio is thought to be protective against the development of pathology that causes ARDS. This has been demonstrated in animal models where vitamin D receptor knock out mice developed more severe lung injury and mortality rates in an LPS sepsis model that was alleviated by ANG-2 antagonists (Kong et al., 2013). In further support of its role in regulating ANG-2 ARDS pathology, vitamin D also suppresses the expression of renin, the rate-limiting enzyme in the renin-angiotensin cascade (Yuan et al., 2007).

Therefore, a beneficial effect of vitamin D could be to reduce inflammatory responses associated with severe COVID-19 particularly viral induced ARDS pathology potentially through modulation of inflammatory cytokines ACE2 and the renin-angiotensin axis.

Observational studies have demonstrated that the prevalence of COVID-19 is negatively associated with estimated 25(OH)D levels in different populations and can be further stratified by latitude, or by race in the US (Figure 2). In the northern part of the US, a vitamin D deficient person at 20 ng/mL 25(OH)D serum level (i.e. 50 nmol/L, marked by the black dashed line) has a 18% SARS-CoV-2 positive rate, compared with a 13.5% rate of a vitamin D sufficient person with 30 ng/mL level (i.e. 75 nmol/L, marked by the black solid line) (Figure 2 upper panel). Strikingly, in terms of race, the curves are steeper for the “Black non-Hispanic” and “Hispanic” groups, compared with the “White non-Hispanic” group.

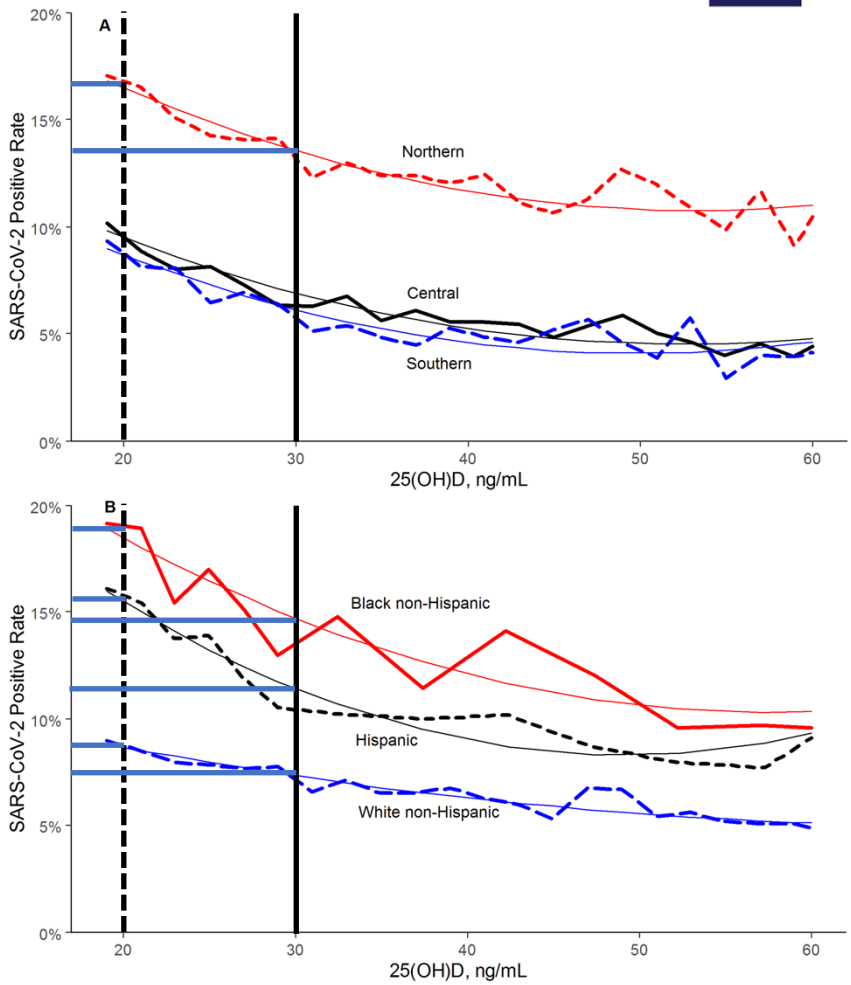

Figure 2. SARS-CoV-2 NAAT Positivity Rates and Circulating 25(OH)D Levels, (A) by Latitude Region and (B) Predominately Black non-Hispanic, Hispanic, and White non-Hispanic Zip Codes. Smooth lines represent the weighted second order polynomial regression fit to the data associating circulating 25(OH)D levels (x) and SARS-CoV-2 positivity rates (y). Black dashed line: marks deficiency. Black solid line: marks insufficiency. Figure is taken from Kaufman et al, 2020.

4. Pharmacology

The PK discussion below relates to vitamin D_3 and its active metabolite 25(OH)D_3, which are referred to as vitamin D and 25(OH)D in this section.

Doses for vitamin D3 (cholecalciferol) and D2 (ergocalciferol) in the BNF are:

- 400 international units for prevention of deficiency and 800 international units for treatment of deficiency. Higher doses can be used for the treatment of severe deficiency following appropriate clinical testing and management.

Individual brands and formulations of vitamin D supplements have different licensed dosing regimens, for example:

- 20 to 40 micrograms (800 to 1,600 international units) of vitamin D3 (cholecalciferol) daily for prevention of deficiency
- 20 to 80 micrograms (800 to 3,200 international units) of vitamin D3 (cholecalciferol) daily for up to 12 weeks for treatment of deficiency (with higher doses used for severe deficiency).

For more detailed dosing information see the summaries of product characteristics for cholecalciferol and ergocalciferol.

Vitamin D has a relatively short half-life of approx. 20h, while 25(OH)D has a much longer half-life of approx. 15 days. Consequently, under vitamin D daily dosing between 10µg QD to 1250µg QD, it would take weeks for 25(OH)D plasma / serum concentration to plateau (Figure 3).

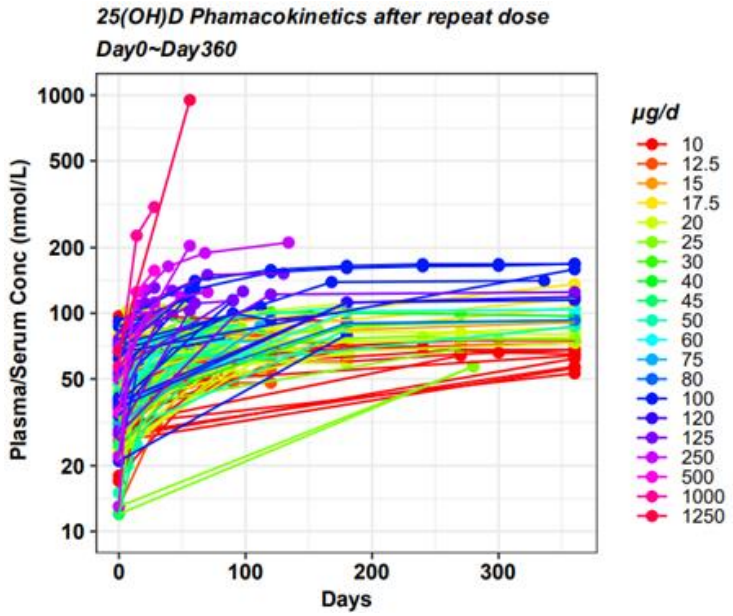

Figure 3. Plasma or serum PK of 25(OH)D after daily dosing of vitamin D across a wide range of doses between 10µg (400IU) QD to 1250µg (10,000IU) QD. Each line represents mean values from a study arm in clinical trials compiled in a meta-analysis plotted in log scale. Figure is taken from (Huang and You, 2020).

Baseline level of 25(OH)D is another factor that affects how quickly serum 25(OH)D may be raised to the target level for prevention i.e. 75 nmol/L or 30 ng/mL. The application submitted to Podio

proposed 50 µg QD and 100 µg QD dosing regimens. Under a 50 µg QD dosing regimen, simulation of a well calibrated and well qualified PBPK model predicted it might take 75 days for severely deficient person (i.e. starting level of 10nmol/L = 4 ng/mL) to reach the target level (Figure 4 A), and would still take 30 days for an insufficient person (i.e. starting level of 50 nmol/L = 20 ng/mL) (Figure 4 C).

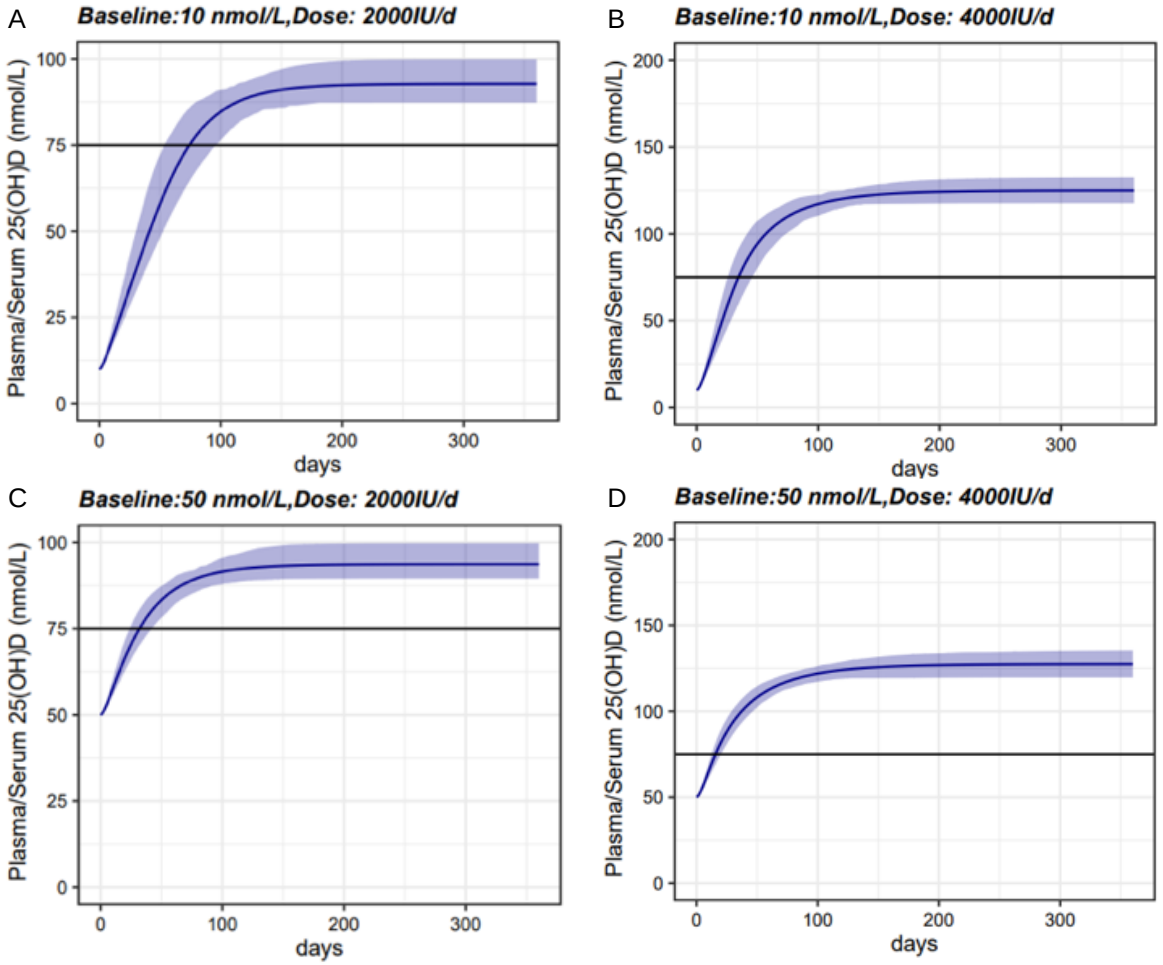

Figure 4. Simulation of 25(OH)D PK in two individuals with 25(OH)D baseline levels at 10 nmol/L (A-B) and 50 nmol/L (C and D). Continuous daily oral dosing at 50 µg (i.e., 2000IU A and C) and 100µg (i.e. 4000IU, B and D) was simulated. Figure is taken from (Huang and You, 2020).

Interestingly, a higher daily dose at 100µg (i.e. 4000IU) is predicted to shorten the duration it takes for these two persons to reach 75 nmol/L target to about 30 days (Figure 4 B) and 20 days (Figure 4 D), respectively. The model structure offers a straightforward explanation for this prediction. It assumes a linear kinetics for vitamin D metabolism (where the production rate of 25(OH)D was assumed to be directly proportional to vitamin D concentration) and saturable kinetics of 25(OH)D clearance (where a Hill-type function was used). Hence, at high doses of vitamin D, 25(OH)D clearance might become saturated, leading to rapid accumulation of 25(OH)D.

Indeed, the PK profile of 25(OH)D under very high doses confirms this prediction. Figure 5 shows these very high oral doses may raise 25(OH)D above target levels within a few days and maintain levels above target levels for weeks. Given that vitamin D has a good tolerability profile (250 nmol/L)

it might be advisable to consider a very high single dose (e.g. 1250 µg ≈) to rapidly achieve target levels, in conjunction with a daily maintenance dose of 100 µg starting at 30 days after the single dose, in the context of prophylaxis or COVID-19-related in-patient treatments.

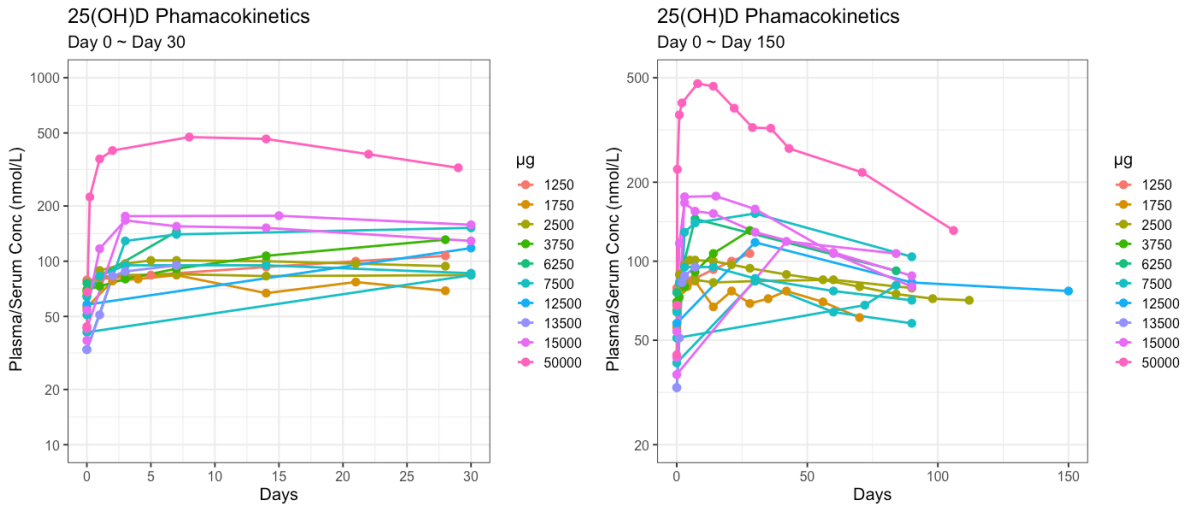

Figure 5. Plasma or serum PK of 25(OH)D after very high single oral dose of vitamin D at doses between 1250 µg (i.e. 50,000 IU) to 50,000 µg (i.e. 2x10^7 IU). A) 0-30 days B) 0-150 days. Figures are adapted from (Huang and You, 2020).

We noticed that the pilot trial for NCT04366908 (COVIDIOL) (Entrenas Castillo et al., 2020) (P14 of this brief) used doses lower than 1250 µg (i.e. 0.532 mg calcifediol on day of admission. 0.266 mg oral calcifediol on day 3 and 7, and then weekly until discharge or ICU admission). To evaluate this, we extracted the 25(OH)D PK data under daily oral doses of 500 µg and 250 µg from Figure 3 and replotted them in Figure 6. Under daily oral dose at 250 µg, it takes approximately 15 days for the severely deficient (below 30 nmol/L in Figure 6) to reach target level of 75nmol/L. Therefore, it would take longer than 15 days for the NCT04366908 dosing regimen to reach target level if it is reached at all. This is also mentioned in P14 where is trial is discussed.

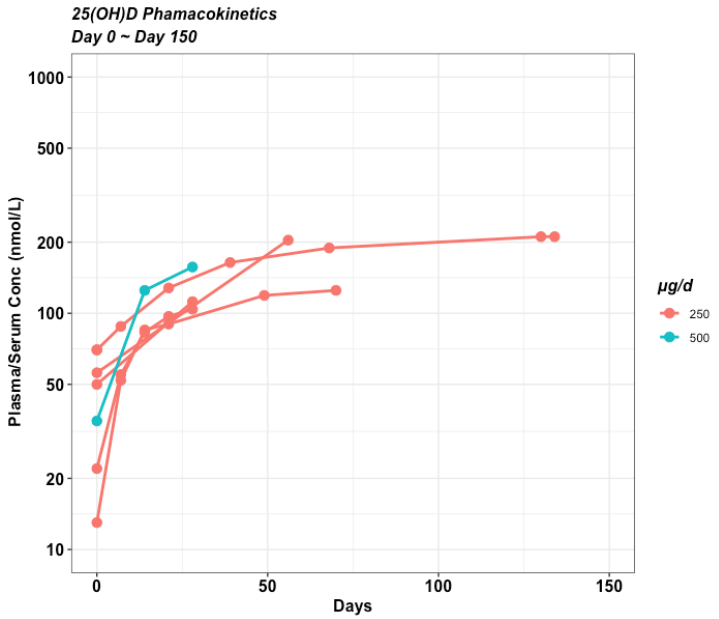

Figure 6. Plasma or serum PK of 25(OH)D after daily dosing of vitamin D at 250µg (10,000IU) QD and 500µg (20,000IU) QD. Each line represents mean values from a study arm in clinical trials compiled in a meta-analysis plotted in log scale. Figure is adapted from (Huang and You, 2020).

Patient compliance is very important for maintaining 25(OH)D above target level. For a severely deficient individual (10 nmol/L 25(OH)D baseline level) who received 50 µg (i.e. 2000IU) QD dosing for 180 days before administration is discontinued, it takes only about 20 days to become insufficient again (Figure 7). Hence, it might be important to consider measures to ensure patient compliance in the settings of prophylaxis and "long COVID-19".

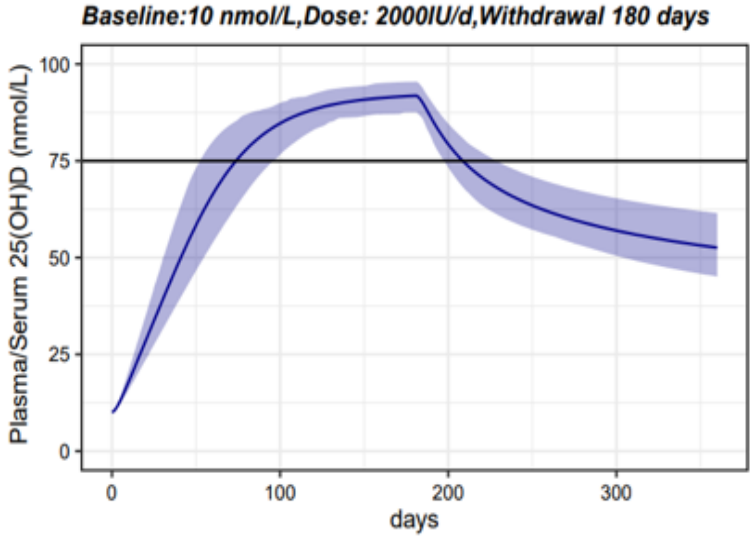

Figure 7. Simulation of 25(OH)D PK in a severely deficient individual (10 nmol/L 25(OH)D baseline level) who received 50 µg (i.e. 2000IU) QD dosing for 180 days before administration is discontinued. Figure is taken from (Huang and You, 2020).

5. Evidence in COVID-19 and related conditions

There are reviews by the NICE reviewing the evidence on vitamin D in COVID. The first full review dates from 29 June 2020 and contains assessment of all observational data (evidence summary ES28)[3]. It was supplemented by a rapid evidence summary on the results of the first interventional clinical trial in Spain [4](see below). There is currently no recommendation for vitamin D therapy in COVID-19 from NICE.

5.1. Non-clinical evidence on anti-viral effects against SARS-CoV-2

Mok et al., 2020 (non peer-reviewed preprint):

- Vero E6 cell line assay, HuH7 cell line assays, human nasal epithelial cells (hNECs):
  - Pre-treatment screen: Compound treatment 2h prior infection
  - Post-treatment screen: Compound treatment 1h post infection
  - SARS-CoV-2 infection at a multiplicity of infection (MOI) of 1 and incubated for 4 days
  - 4 compound libraries were screened:
    - Pre-treatment: 62-compound ACE2-targeted compound library (CADD) (TargetMol), 57-compounds natural product library
    - Post-treatment: 500-compound flavonoids library (TimTec), 1172-compound FDA-approved drug library (Selleckchem)
- Results total:
  - Total of the 121 compounds identified with activity against SARS-CoV-2
  - 7 were shortlisted for validation:
    - Pre-infection: citicoline, pravastatin sodium and tenofovir alafenamide
    - Post-infection: imatinib mesylate, calcitriol, dexlansoprazole, and prochlorperazine dimaleate
- Results for calcitrol (10µM) post-treatment:
  - 10µM represents very high non-physiological concentrations of Vitamin D
  - Vero E6: 1.3 $\log_{10}$ reduction of SARS-CoV-2 titre (Figure 8)
  - HuH7: not recapitulated in HuH7 cells (explained due to CC50 value of 4.7 µM in HuH7 cells) (Figure 9)
  - hNECs: reduction of 0.69 log10 in viral titre (Figure 10)
  - EC50 values in supplement could not be accessed online

[3] https://www.nice.org.uk/advice/es28/evidence/evidence-review-pdf-8777674477
[4] https://www.nice.org.uk/advice/es28/resources/covid19-rapid-evidence-summary-vitamin-d-for-covid19-pdf-1158182526661

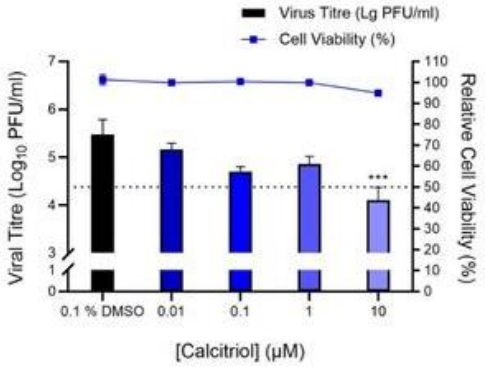

Figure 8: Mok et al 2020 study: Anti-viral effect of calcitrol (10µM) in Vero6 cells (Mok et al., 2020)

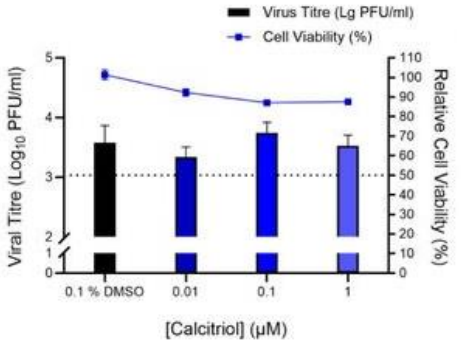

Figure 9: Mok et al 2020 study: Anti-viral effect of calcitrol (10µM) in hNEC cells (Mok et al., 2020)

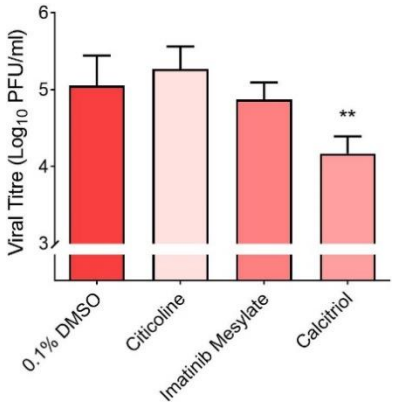

Figure 10: Mok et al 2020 study: Anti-viral effect of calcitrol (10µM) in hNEC cells (Mok et al., 2020)

5.2. Interventional clinical data from COVID-19

There are currently no interventional clinical data available which show that Vitamin D can prevent COVID-19 (pre-/ or post-exposure prophylaxis).

One small pilot study has been conducted in hospitalised patients (see below; Entrenas Castillo et al., 2020). The results should be interpreted with caution due to a lack of blinding and lack of knowledge about best standard of care besides the pharmaceuticals mentioned, due to its small sample size, the observed imbalance regarding patient baseline

characteristics and the potential for confounding, due to the lack of measures on vitamin D levels in order to verify any level of causality between intervention and outcome.

Pilot trial for NCT04366908 (COVIDIOL) (Entrenas Castillo et al., 2020):

- Parallel pilot randomized open label, double-masked clinical trial in single centre (Reina Sofia University Hospital, Córdoba Spain)
- Objective: Evaluated the effect of calcifediol treatment on Intensive Care Unit Admission and Mortality rate among Spanish patients hospitalized for COVID-19.
- Patients:
    - 76 seventy-sixth consecutive patients hospitalized with COVID-19 infection with clinical acute respiratory infection, confirmed by a radiographic pattern of viral pneumonia and by a positive SARS-CoV-2 PCR with CURB65 severity scale (recommending hospital admission in case of total score > 1).
    - Allocation 2:1 into treatment arm
    - Baseline characteristics differed considerably in terms of age (Table 2) and co-morbidities/risk-factors (Table 3). Also, obesity was not considered in co-morbidities/risk-factors. Statistically significant difference was identified for the variable hypertension and close to statistical significance for diabetes 3.
- Intervention (2:1):
    - Intervention: 0.532 mg calcifediol on day of admission. 0.266 mg oral calcifediol on day 3 and 7, and then weekly until discharge or ICU admission.
    - Based on the discussion on daily oral dosing data in P10, the used dosing regimen is not expected to be suitable to reach required 25(OH)D concentrations 3 days
    - Control group and best standard of care (per hospital protocol): combination of hydroxychloroquine (400 mg every 12 h on the first day, and 200 mg every 12 h for the following 5 days), azithromycin (500 mg orally for 5 days) and for patients with pneumonia and NEWS score≥5, a broad spectrum antibiotic (ceftriaxone2 g intravenously every 24 h for 5 days) was added to hydroxychloroquine and azithromycin.
- Analysis:
    - Endpoints: rate of ICU admission and deaths.
    - Exploratory descriptive statistics
    - Due to differences in baseline characteristics a multivariate logistic regression analysis was performed to adjust the model by possible confounding variables such as hypertension and type 2 diabetes mellitus
    - Sample Size Calculation: proportion of a participant treated with Calcifediol could meet the criteria for admission to the Intensive Care Unit which is estimated as 5% (with 90 % confidence intervals) and the proportion of a participant not treated with Calcifediol which could be 10 %.
    - Serum 25OHD concentrations at baseline or during treatment were not measured. The authors claim that adults living in the Córdoba area are relatively vitamin D deficient (16 ng/mL on average) in late winter and early spring.
- Outcomes (Table 4):
    - Calcifediol (n=26): no death, all discharged
    - Control (n=13): two deaths and 11 discharges

	Group receiving Calcifediol (n = 50)	Group without Calcifediol (n = 26)	IC 95 %	P
Age (years)	53.14 +/- 10.77	52.77 +/- 9.35	−0.34 – 9.60	0.07
Males [n (%)]	27 (54 %)	18 (69 %)	−0.38 – 0.07	0.20
Females [n (%)]	23 (46 %)	8 (31 %)	−0.07 – 0.38	0.20
Male's age (years)	56.30 +/ 8.29	52.13 +/- 10.05	−9.67 – 1.41	0.14
Female's age (years)	49.43 +/- 12.28	54.13+/- 7.99	−4.87 – 14.25	0.32

Table 2: Pilot trial for COVIDIOL: baseline characteristic: demography (mean +/- standard deviation) (Entrenas Castillo et al., 2020)

Poor prognosis risk factor	Group receiving Calcifediol (n = 50)	Group without Calcifediol (n = 26)	IC 95 %	P
≥ 60 years	14 (28 %)	5 (19.23 %)	−0.11 – 0.28	0.40
Previous lung disease	4 (8%)	2 (7.69 %)	−0.12 – 0.13	0.96
Previous Chronic kidney disease	0	0	–	–
Previous Diabetes mellitus	3 (6%)	5 (19.23 %)	−0.30 – 0.03	0.08
Previous High blood pressure	11 (24.19 %)	15 (57.69 %)	−0.58 – −0.13	0.002
Previous Cardiovascular disease	2 (4%)	1 (3.85 %)	−0.09 – 0.09	0.97
Immunosuppressed & transplanted	6 (12 %)	1 (3.85 %)	−0.03 – 0.20	0.24
At least one prognostic bad risk factor[a]	24 (48 %)	16 (61.54 %)	−0.37 – 010	0.26
PaO2/FiO2 (mean +/-SD)	346.57 +/- 73.38	334.62 +/- 66.33	−22.29 – 46.19	0.49
C-reactive protein (mg/L) (mean +/-SD)	82.93 +/- 62.74	94.71 +/- 63.64	−42.15 – 18.59	0.44
LDH (U/L)(mean +/-SD)	308.12 +/- 83.83	345.81 +/- 108.57	−82.46 – 7.08	0.10
D-Dimer (ng/mL) (mean +/-SD)	650.92 +/- 405.61	1333.54 +/- 2570.50	−360.29 – 1725.53	0.19
Lymphocytes < 800/ µL	10 (20 %)	6 (23.08 %)	−0.16 – 0.23	0.75
Ferritin (ng/mL) (mean +/-SD)	691.04 +/- 603.54	825.16 +/- 613.95	−166.31 – 434.55	0.36
IL-6 (22/48) (pg/mL) (mean +/-SD)	28.88 +/- 75.05	19.54 +/- 19.45	−41.88 – 23.19	0.41

Table 3: Pilot trial for COVIDIOL: baseline characteristics: risk factors/co-morbidities. SD= standard deviation (Entrenas Castillo et al., 2020)

	Without Calcifediol Treatment (n = 26)	With Calcifediol Treatment (n = 50)	p value (1d712;2) Fischer Test
Need for ICU			<0.001
Not requiring ICU, n (%)	13 (50)	49 (98)	
Requiring ICU, n (%)	13 (50)	1 (2)	

*Table 4: Pilot trial for COVIDIOL: results on ICU admission. *Univariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol: treatment vs Without Calcifediol treatment: 0.02 (95 %CI 0.002- 0.17). ** Multivariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol treatment vs Without Calcifediol treatment ICU (adjusting by Hypertension and T2DM): 0.03 (95 %CI: 0.003-0.25). (Entrenas Castillo et al., 2020)*

5.3. Observational data from COVID-19: UK Biobank studies

There are numerous smaller observational studies and data published that investigated the association between vitamin D-levels and SARS-CoV-2 infection/COVID-19 outcomes (reviewed by Benskin, 2020).

The following section will discuss studies using the UK biobank to understand evidence in the context of the British population. The underlying dataset is the UK biobank (502,624 participants aged 37–73 years between 2006 and 2010) for which Vitamin D levels were measured [25(OH)D]. Data imputation was used when below or above the detection limit (Hastie et al., 2020 and Hastie, Pell, et al., 2020).

Hastie et al, 2020 study:

- Dataset:
  - UK biobank participants who had a confirmed COVID-19 infection
  - 2724 COVID-19 tests conducted on 1474 individuals. Of these, 449 had a positive COVID-19 test.
  -
- Analyses:
  - Univariable logistic regression analysis was performed of the association between 25(OH)D concentration (as a continuous variable) and confirmed COVID-19 infection.
  - Adjustment for sex, month of assessment, Townsend deprivation quintile, household income, self-reported health rating, smoking status, BMI quintile, ethnicity, age at assessment, diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), and long-standing illness, disability or infirmity.
- Results (Table 5):
  - Median 25(OH)D concentration measured at recruitment was lower in patients who subsequently had confirmed COVID-19 infection (28.7 (IQR 10.0–43.8) nmol/L) than other participants (32.7 (IQR 10.0–47.2) nmol/L). Hence, it predicted COVID-19 infection univariably (OR = 0.99, 95% CI 0.99–0.999, p = 0.013).
  - Prediction was not confirmed after adjustment for covariates (OR = 1.00; 95% CI = 0.998–1.01; p = 0.208).
  - Update to results below (Table 6)

	Univariable		Multivariable[a]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Vitamin D (nmol/L)	0.99 (0.99–0.999)	0.013	1.00 (0.998–1.01)	0.208
Vitamin D deficient (<25 nmol/L)	1.37 (1.07–1.76)	0.011	0.92 (0.71–1.21)	0.564
Vitamin D insufficient (<50 nmol/L)	1.19 (0.99–1.44)	0.068	0.88 (0.72–1.08)	0.232

OR odds ratio; CI confidence interval.
[a] Adjusted for ethnicity, sex, month of assessment, Townsend deprivation quintile, household income, self-reported health rating, smoking status, BMI category, age at assessment, diabetes, SBP, DBP, and long-standing illness, disability or infirmity.

Table 5: UK Biobank study: Association between Vitamin D and confirmed COVID-19 infection (Hastie et al, 2020)

Hastie, Pell, et al., 2020 study:

- Dataset:
  - UK biobank participants with COVID-19- related death (Death Register data)
  - In the sample, 203 participants died due to COVID-19 infection (5th of March and 25th of April 2020)
- Analyses:
  - Association between serum 25(OH)D concentration as a continuous measurement, or vitamin D deficiency or insufficiency (defined as serum 25(OH)D < 25 and < 50 nmol/L, respectively), and risk of COVID-19 death using Cox proportional hazards regression analysis.
  - Adjustment for sex, month of assessment, Townsend deprivation quintile, household income, self-reported health rating, smoking status, BMI quintile, ethnicity, age at assessment, diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), and long-standing illness, disability or infirmity.
- Results (Table 6):
  - Lower 25(OH)D concentration and vitamin D deficiency were both associated with higher risk of COVID-19 death univariably
  - This result was not confirmed after adjustment for potential confounders
  - Update to previous study (Hastie et al, 2020): 656 confirmed inpatient COVID-19 cases. 25(OH)D concentration and vitamin D deficiency were associated with COVID-19 infection univariably but not multivariably.

	COVID-19 mortality				Inpatient COVID-19 infection			
	Univariable		Multivariable*		Univariable		Multivariable*	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	IRR (95% CI)	*p* value	IRR (95% CI)	*p* value
25(OH)D (per 10 nmol/L)	0.92 (0.86–0.98)	0.016	0.98 (0.91–1.06)	0.696	0.93 (0.90–0.97)	<0.001	1.00 (0.96–1.05)	0.888
Vitamin D deficient (25(OH) D < 25 nmol/L)	1.61 (1.14–2.27)	0.007	1.21 (0.83–1.76)	0.311	1.56 (1.28–1.90)	<0.001	1.10 (0.88–1.37)	0.404
Vitamin D insufficient (25(OH) D < 50 nmol/L)	1.29 (0.97–1.72)	0.076	1.02 (0.75–1.38)	0.919	1.33 (1.14–1.56)	<0.001	1.06 (0.89–1.26)	0.525

Participants who died of COVID-19 had a median age at death of 76 years (interquartile range 71–78 years)

HR hazard ratio, *CI* confidence interval, *IRR* incidence rate ratio

*Adjusted for age, sex, ethnicity, month of assessment, Townsend deprivation quintile, household income, BMI category, smoking status, diabetes, systolic blood pressure, diastolic blood pressure, self-reported health rating, and long-standing illness, disability or infirmity

Table 6: UK biobank study: Association between baseline serum 25(OH)D and confirmed COVID-19 mortality, and confirmed inpatient COVID-19 infection (Hastie, Pell, et al., 2020)

5.4. Observational data from COVID-19: Meta-analyses

Meta-analysis Ghasemian et al., 2020 (non peer-reviewed preprint):

- Objective: Explore the role of vitamin D in COVID-19
- Sixteen observational studies with a total of 4922 participants were identified by searching PubMed, Scopus, Embase, Cochrane Library, Web of Science, and Google Scholar (intitle) as well as preprint database of medRxiv, bioRxiv, Research Square, preprints.org, and search engine of ScienceDirect up to October 10, 2020.
    - 15 retrospective studies and 1 prospective study
    - Sample size ranged from 10 to 2903
- Methodology and prespecified analyses:
    - The pooling of effect sizes was done with 95% Confident Interval
    - Frequency of Vitamin D status in COVID-19 patients
    - Mean 25(OH)D concentration
    - Association between Vitamin D deficiency and COVID-19
    - Co-morbidity frequency
    - Ethnicity frequency.
- Results:
    - 48% of COVID-19 patients were suffering from vitamin D deficiency (95% CI, 29%-67%) and in 41% of patients, levels of vitamin D were insufficient (95% CI, 10%-82%).
    - Mean 25(OH)D concentration (Figure 11)
        - All patients: 18 ng/ml (95% CI, 13-24)
        - Severe patients: 18.20 ng/mL (95% CI, 1-35)
        - Non-severe cases: 26 ng/mL (95% CI, 23.89-28.70)
    - Co-morbidities: 7.4% cancer, 27.1% chronic kidney disease, 30.4% cardiovascular diseases, 5.1% dementia, 14.5% depression/anxiety, 32.1% diabetes, 47.4% hypertension, 22.0% obesity and 17.5% respiratory diseases.
    - Ethnicity: 1.0% Afro-Caribbean, 10.3% Asian, and 92.1% Caucasian.

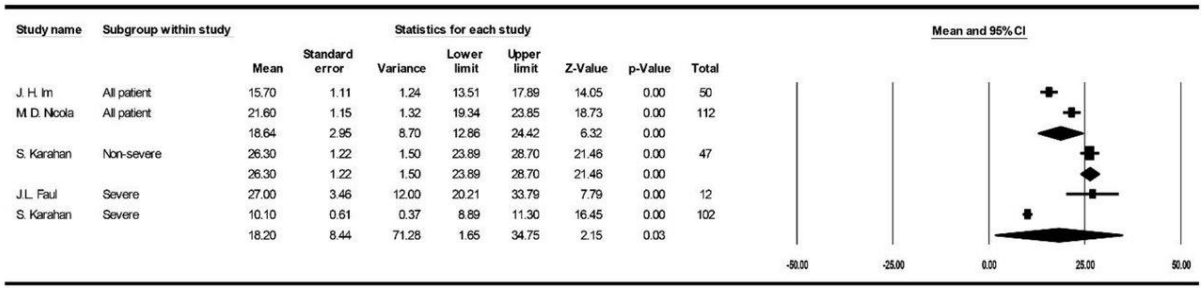

Figure 11: Meta-analysis Ghasemian et al., 2020: outcome on mean 25(OH)D concentration

Meta-analysis Chen et al 2020 (non peer-reviewed preprint):

- Objective: determine whether serum vitamin D is independently associated with COVID-19 infection and outcomes in patients with COVID-19.
- Six observational studies with a total of 377,265 patients were identified by searching the PubMed, Embase, and medRxiv databases from December 2019 to October 1, 2020.

  - Only studies with multivariate adjusted results were included to avoid the impact of potential confounding factors.
  - All studies were deemed as high quality as assessed by Newcastle-Ottawa quality assessment scale
  - 4 studies reported vitamin D levels and COVID-19 positivity
  - 4 reported the association of vitamin D levels and COVID-19 outcomes
  - 3 were cohort studies and 3 were case-control cohort studies.
  - Most of studies (n = 3) were performed in USA, 1 was performed in UK, 1 in Israel and 1 in Germany.
- Methodology and pre-specified analyses
  - Odds ratios (ORs) were pooled using random-effects models
  - Categorical analysis: low serum levels (below 20ng/ml) vs high serum levels (below 30ng/ml)
  - Continuous analysis: study-specific slopes - vitamin D per 5 ng/ml decrement).
- Results:
  - Categorical variable analysis (Figure 12): low serum vitamin D level was associated with an increased risk of COVID-19 infection (OR: 1.47, 95% CI: 1.09-1.97, I2=81%), hospitalization (OR: 1.83, 95% CI: 1.22-2.74, I2=0%), but not in-hospital death (OR: 2.73, 95% CI: 0.27-27.61).
  - Continuous variable analysis (Figure 13): each 5 ng/ml increase in vitamin D level was not associated with any increased risk of COVID-19 infection (OR: 1.04, 95% CI: 0.96-1.12, I2=74%) or in-hospital death (OR: 1.02, 95% CI: 0.93-1.12).

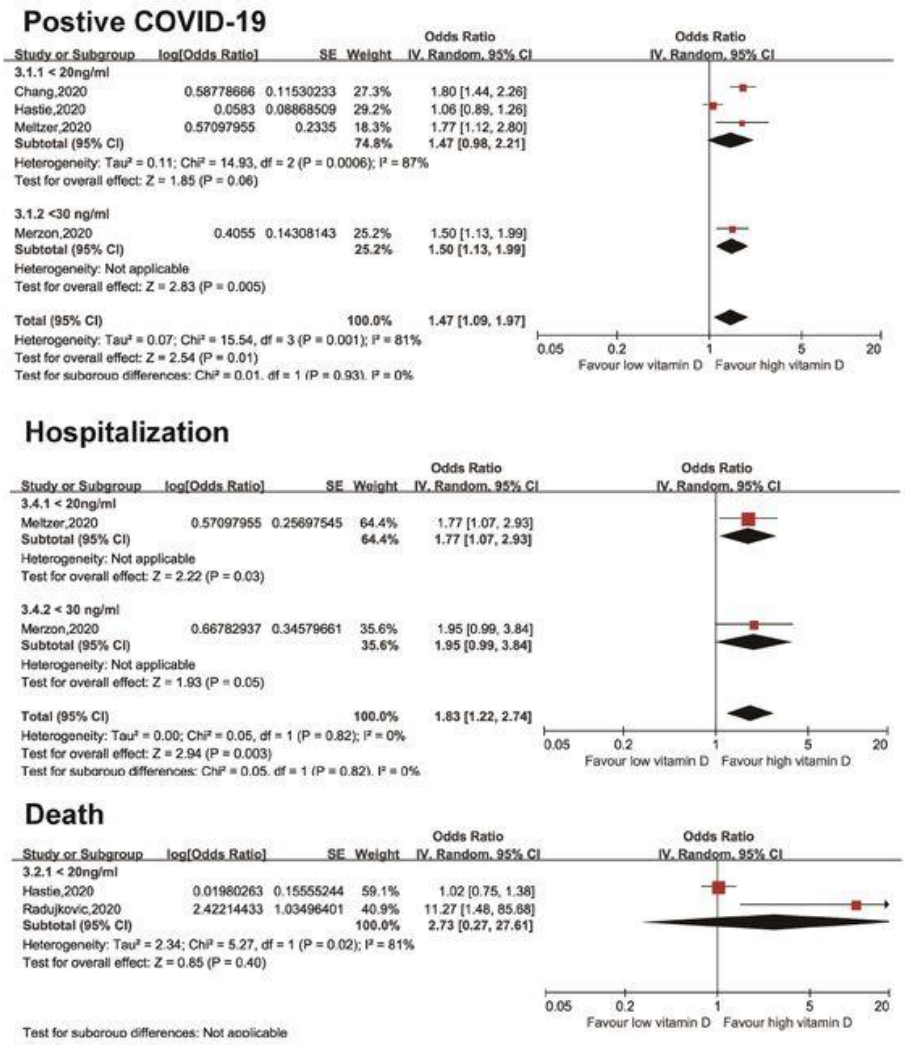

Figure 12: Meta-analysis Chen et al 2020: Outcomes of the categorical analysis on infection, hospitalisation and mortality

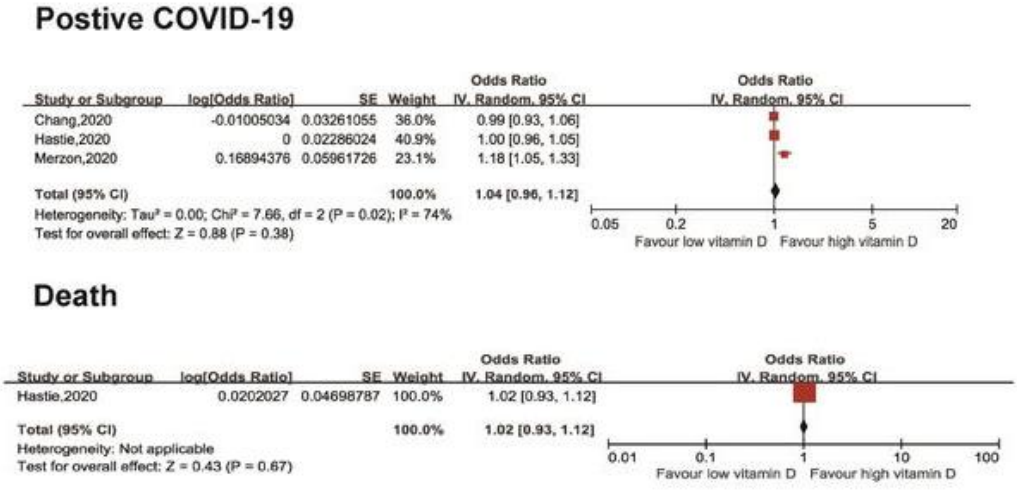

Figure 13: Meta-analysis Chen et al 2020: Outcomes of the continuous analysis on infection and mortality

5.5.

5.6. Interventional data in asthma and acute respiratory infections

Asthma:

- Systematic review and meta-analysis of double-blind, placebo-controlled, randomised controlled trials of vitamin D3 or vitamin D2 supplementation in people with asthma that reported incidence of asthma exacerbation, published between database inception and Oct 26, 2016.
- Primary outcomes: incidence of asthma exacerbation requiring treatment with systemic corticosteroids. Mixed-effects regression models were used to obtain the pooled intervention effect with a 95% CI. Subgroup analyses were done to determine whether effects of vitamin D on risk of asthma exacerbation varied according to baseline 25-hydroxyvitamin D (25[OH]D) concentration, age, ethnic or racial origin, body-mass index, vitamin D dosing regimen, use of inhaled corticosteroids, or end-study 25(OH)D levels; post-hoc subgroup analyses were done according to sex and study duration.
- Studies:
    - 8 eligible randomised controlled trials (total 1078 participants). Individual patient data from 7 studies. Six studies were assessed as being at low risk of bias, and one was assessed as being at unclear risk of bias
    - Vitamin D supplementation reduced the rate of asthma exacerbation requiring treatment with systemic corticosteroids among all participants (adjusted incidence rate ratio [aIRR] 0·74, 95% CI 0·56-0·97; p=0·03; 955 participants in seven studies; high-quality evidence).
- Outcomes:
    - No significant differences between vitamin D and placebo in the proportion of participants with at least one exacerbation or time to first exacerbation.
    - Subgroup analyses of the rate of asthma exacerbations treated with systemic corticosteroids revealed that protective effects were seen in participants with baseline 25(OH)D of less than 25 nmol/L (aIRR 0·33, 0·11-0·98; p=0·046; 92 participants in three studies; moderate-quality evidence) but not in participants with higher baseline 25(OH)D levels (aIRR 0·77, 0·58-1·03; p=0·08; 764 participants in six studies.

Acute respiratory infections:

- Systematic review and meta-analysis of randomised, double-blind, placebo-controlled trials of supplementation with vitamin D3 or vitamin D2 of any duration having incidence of acute respiratory infection as a prespecified efficacy outcome
- Objective: To assess the overall effect of vitamin D supplementation on the risk of acute respiratory infections (ARIs) and to identify factors modifying this effect
- Studies: 25 eligible RCTs (a total of 11,321 participants, aged from 0 to 95 years). IPD were obtained for 10,933 out of 11,321 (96.6%) participants.
- Outcomes:
    - Vitamin D supplementation reduced the risk of ARI among all participants [adjusted odds ratio (aOR) 0.88, 95% confidence interval (CI) 0.81 to 0.96; heterogeneity $p < 0.001$].
    - Subgroup analysis revealed that protective effects were seen in individuals receiving daily or weekly vitamin D without additional bolus doses (aOR 0.81,

95% CI 0.72 to 0.91), but not in those receiving one or more bolus doses (aOR 0.97, 95% CI 0.86 to 1.10; p = 0.05).
- Among those receiving daily or weekly vitamin D, protective effects of vitamin D were stronger in individuals with a baseline 25-hydroxyvitamin D [25(OH)D] concentration of < 25 nmol/l (aOR 0.30, 95% CI 0.17 to 0.53) than in those with a baseline 25(OH)D concentration of ≥ 25 nmol/l (aOR 0.75, 95% CI 0.60 to 0.95; p = 0.006).
- Vitamin D did not influence the proportion of participants experiencing at least one serious adverse event (aOR 0.98, 95% CI 0.80 to 1.20; p = 0.83). The body of evidence contributing to these analyses was assessed as being of high quality.

6. Safety

6.1. Contraindications and cautions (authorised medicines)

Contraindications:
- hypercalcaemia,
- evidence of vitamin D toxicity
- hypervitaminosis D,
- decreased renal function
- metastatic calcification.

Caution is required for additional vitamin D or calcium supplementation.

6.2. Vitamin D toxicity/overdose

Endogenous and exogenous Vitamin D toxicity can be distinguished. In healthy individuals, toxicity is usually caused by exogenous and prolonged intake of high-dose Vitamin D supplements/analogues. Vitamin D toxicity resulting from excessive use of vitamin D is characterized by elevated 25(OH)D >150 ng/ml (>375 nmol/l), and usually normal or slightly increased 1,25(OH)2D concentration. The clinical manifestations of are varied but are related primarily to hypercalcemia, including neuropsychiatric manifestations, such as difficulty in concentration, confusion, apathy, drowsiness, depression, psychosis, and in extreme cases, a stupor and coma. The gastrointestinal symptoms include recurrent vomiting, abdominal pain, polydipsia, anorexia, constipation, peptic ulcers, and pancreatitis. The cardiovascular manifestations include hypertension, shortened QT interval, ST segment elevation, and bradyarrhythmias with first-degree heart block on the electrocardiogram. The renal symptoms include hypercalciuria as the earliest sign, polyuria, polydipsia, dehydration, nephrocalcinosis, and renal failure. Other reported symptoms include band keratopathy, hearing loss, and painful periarticular calcinosis (Marcinowska-Suchowierska et al., 2018).

Overdose of all forms of Vitamin D can lead to toxicity. The toxicity of Vitamin D metabolites is however easier to manage compared to toxicity from vitamin D2 or due to the long half-life in the body (high lipid solubility in the liver, muscles, and fat tissues and the corresponding large storage capacity). Hence, hypercalcemia caused by vitamin D overdose theoretically can last up to 18 months after the administration of vitamin D is discontinued (Marcinowska-Suchowierska et al., 2018).

The SmPC of calcitriol contains information on overdose [5]:

- Acute symptoms of vitamin D intoxication: anorexia, headache, vomiting, constipation.
- Chronic symptoms of vitamin D intoxication: dystrophy (weakness, loss of weight), sensory disturbances, possibly fever with thirst, polyuria, dehydration, apathy, arrested growth and urinary tract infections. Hypercalcaemia ensues, with metastatic calcification of the renal cortex, myocardium, lungs and pancreas.
- Hypercalcaemia at higher levels (>3.2 mmol/L) may lead to renal insufficiency particularly if blood phosphate levels are normal or elevated due to impaired renal function.
- Treatment of accidental overdosage: immediate gastric lavage or induction of vomiting to prevent further absorption. Administration of liquid paraffin to promote faecal excretion. Repeated serum calcium determinations are advisable. If elevated calcium levels persist in the serum, phosphates and corticosteroids may be administered and measures instituted to bring about adequate diuresis.

6.3. Interactions (authorised medicines)

- Concurrent use of cardiac glycosides in the presence of hypercalcaemia due to vitamin D administration increases the potential for cardiac arrhythmias. C
- If patients receive too much cardiac glycoside this leads to toxicity, which can lead to additional heart rate abnormalities, which can be life threatening. With these cardiac glycoside medications toxicity can occur at doses only slightly higher doses than the intended dose. High levels of calcium can increase the likelihood of this toxicity, so calcium levels need to be monitored
- Phosphate infusions should not be administered to lower hypercalcaemia of hypervitaminosis D because of the dangers of metastatic calcification.
- Anti-convulsant e.g. phenytoin, phenobarbital, primidone may diminish the effect due to hepatic enzyme induction.
- Rifampicin may reduce the effectiveness due to hepatic enzyme induction.
- The cytotoxic agent actinomycin and imidazole antifungal agents interfere with vitamin D activity by inhibiting the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D by the kidney enzyme, 25-hydroxyvitamin D-1-hydroxylase.
- Concomitant use of glucocorticoids can decrease the effect of vitamin D. A review on Vitamin D drug interactions reviewed the available literature and concluded that 25(OH)D concentrations are not significantly affected by glucocorticoids (Robien et al., 2013).

[5] https://www.medicines.org.uk/emc/product/11802/smpc#OVERDOSE

7. Manufacturing and availability

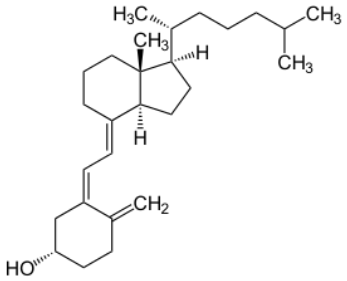

Figure 14: Structure of Vitamin D, IUPAC name: (1S,3Z)-3-[(2E)-2-[(1R,3aS,7aR)-7a-methyl-1-[(2R)-6-methylheptan-2-yl]-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene]ethylidene]-4-methylidenecyclohexan-1-ol

7.1. Synthesis

The four major commercial products of vitamin D are vitamin D3 and D2, 25-hydroxyvitamin D3 and 1α-hydroxyvitamin D3 (Hirsch, 2011).

Vitamin D is manufactured in bulk. The UV irradiation procedure is somewhat specialised but could be accessed using conventional process engineering. Reagents and solvents are commoditised, conventional and freely available (also see below).

7.2. Manufacture

- “Vitamin D3 is available in a variety of forms. Cod liver oil and percomorph liver oil were good sources of vitamin D3 historically but crude cod liver oil processing involves alkali refining, bleaching, winterization, and deodorization. This vigorous treatment of the vitamin containing oil substantially depletes the vitamin activity. Fully cleaned and deodorized cod liver oil is sold with synthetic vitamins added back. Most of the cod liver oils on the market fall into this category. Vitamin D2 as a concentrate or in microcrystalline forms is used in many pharmaceutical preparations, although vitamin D3 is preferred by many manufacturers and consumers because it is the form occurring naturally in animals. Vitamin D2 has been used as a feed supplement for cattle, swine, and dogs, but its use has declined in favour of vitamin D3. As fat as storage and shipping is concerned, Vitamin D is sensitive to air, heat, UV light, and mineral acids. These sensitivities are exaggerated by the presence of heavy-metal ions, such as iron. Therefore, care should be taken to store and ship vitamin D and its various product forms by methods that minimize exposure to these conditions.” (Hirsch, 2011)
- "Vitamin D3 is manufactured from cholesterol which is isolated from wool grease. The cholesterol is converted chemically to 7-dehydrocholesterol which is irradiated with UV light to form pre-vitamin D3 and CIS vitamin D3, the biologically active precursors to the vitamin D3 metabolites. The irradiation and heating processes are carefully controlled to avoid generating the many isomeric inactive forms of the vitamin from being generated. Vitamin D2 is obtained using similar irradiation techniques with ergosterol. Ergosterol is

obtained from yeast fermentation. Most of the manufacturing of cholecalciferol and ergocalciferol is currently being done in China. Worldwide use is 97.3 metric tons. 25-Hydroxyvitamin D3 is predominately made from 25-hydroxy-7-dehydrocholesterol which is produced through a process involving fermentation of modified yeast. 1α-Hydroxyvitamin D3 is made through a chemical process starting with pure vitamin D3." (Hirsch, 2011)

- "There are 2 major forms of vitamin D. Cholecalciferol (vitamin D-3) is produced in the skin after sun exposure. It is produced commercially by extracting 7-dehydrocholesterol from wool fat, followed by UVB irradiation and purification. Ergocalciferol (vitamin D-2) has a different side chain than cholecalciferol (i.e., a C24 methyl group and a double bond between C22 and C23) and is commercially made by irradiating and then purifying the ergosterol extracted from yeast." (Holick, 2005).

7.3. Dependence on special devices for administration

No special devices are needed.

7.4. Supply

Current supply status:
"…there are several manufacturers that provide a vitamin D-2 or cholecalciferol supplement as either 400 or 1000 IU. Thus, diet plus additional vitamin D supplementation can result in attaining the recommended 1000 IU of cholecalciferol." (Holick, 2005).

History of supply:
Vitamin D is widely available as a commodity-scale food supplement.

Geopolitical issues:
No obstacles are apparent. Any interruption from sources such as China could probably be replaced by supplies from Australasia or the Americas and vice versa.

8. Regulatory considerations and clinical trial environment

8.1. Clinical trial environment

There are currently 22 interventional clinical trials that investigate vitamin D (monotherapy) in COVID-19

As discussed in the above PK/PD section, to rapidly raise 25(OH)D levels and maintain it above 75nmol/L, an oral dosing strategy may involve a high single dose at least 1250 µg (50,000 IU) followed by 100 µg QD (4000 IU) dose starting 30 days after the single dose.

Prophylaxis:

- 6 in prophylaxis setting (WHO scale 0)
- Only one trial uses a dosage that would reach the Vitamin D levels that are proposed in the above PK/PD analysis.
- PROTECT (NCT04411446, French-Canada): trial, in healthcare workers at high risk with the following dose regimen: start dose 100,000 IU, maintenance daily 10,000 IU for 16 weeks .
- UK trial organised by Queen Mary University of London in normal risk healthy volunteers, with dosing up to 3200 IU (80 micrograms) for 6 months (NCT04579640)

Treatment:

- 16 in treatment setting (WHO scale 1-2 n=4, WHO scale 3-4 n=12)
- Higher doses are utilised compared to ongoing trials in preventative setting.
- Most trials (n=10) use a one-time administration of high doses.
- 9 trials use a dosage that would reach the Vitamin D levels that are proposed in the above PK/PD analysis.
- The CARED (NCT04411446, Argentina) uses the highest dose with 500,000 IU in recently hospitalised patients (WHO scale 3).

8.2. Legal status: food supplement

- Food supplements are concentrated sources of nutrients (or other substances) with a nutritional or physiological effect. Such food supplements can be marketed in “dose” form, such as pills, tablets, capsules, liquids in measured doses, etc [6].
- The EU Food Supplements Directive 2002/46 came into force on 1 August 2005 and is implemented in the UK by the Food Supplements (England) Regulations 2003 and equivalent regulations in Scotland, Wales and Northern Ireland. The Regulations specify the vitamin and mineral substances permitted for use in food supplements and identify the units of measurement, labelling, presentation and advertising allowed [7].
- The Directive lays down a harmonised list of vitamins and minerals that may be added for nutritional purposes in food supplements (in Annex I to the Directive). Annex II of the Directive contains a list of permitted sources (vitamin and mineral substances) from

[6] https://ec.europa.eu/food/safety/labelling_nutrition/supplements_en
[7] https://www.gov.uk/government/publications/food-supplements-guidance-and-faqs

which those vitamins and minerals may be manufactured. In annex II of Directive 2002/46/EC [8], vitamin D is listed to contain (a) cholecalciferol or (b) ergocalciferol.

- Dietary reference values for vitamins are available in the Department of Health publication: Dietary Reference Values for Food Energy and Nutrients for the United Kingdom: Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. *Report on Health and Social Subjects 41*. London: HMSO, 1991 [9].
- In the UK, the industry standard for maximum dosing of vitamin D supplementation is 75 µg/day [10]

Age	RNI	
0–up to 6 mo	8.5	
6 mo–3 yr	7.0	
4–64 yr	0	provided skin is exposed to sun
65+ yr	10.0	
Pregnant and lactating women	10.0	

Table 7: Dietary Reference Values for Vitamin D 1ug/d, from Dietary Reference Values for Food Energy and Nutrients for the United Kingdom: Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Report on Health and Social Subjects 41. London: HMSO, 1991 [11].

8.3. Authorised medicines for cholecalciferol (vitamin D3)

There are three legal categories: prescription-only medicine (POM), pharmacy medicines (P), and general sales medicines (GSL) [12]. Vitamin D can be found in medicinal products of all three categories (see annex 1).

Based on the above PK/PD modelling on vitamin D3, this section will focus on vitamin D3 (active substance cholecalciferol) in monotherapy.

GSL authorisations:

There are no medicines containing cholecalciferol in monotherapy with general sales licence.

P authorisations:

There are no medicines containing cholecalciferol in monotherapy with pharmacy license. All medicines contain cholecalciferol in combination with calcium. These are authorised for Prevention and treatment of vitamin D and calcium deficiency. Vitamin D and calcium supplement as an adjunct to specific osteoporosis treatment of patients who are at risk of

[8] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32002L0046&from=EN
[9] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/743790/Dietary_Reference_Values_-_A_Guide__1991_.pdf
[10] https://www.pagb.co.uk/latest-news/mpl-vitd-adults/
[11] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/743790/Dietary_Reference_Values_-_A_Guide__1991_.pdf
[12] https://www.gov.uk/guidance/medicines-reclassify-your-product#classifications-of-medicines

vitamin D and calcium deficiency. Many indications are specifically referring to elderly (housebound and institutionalised elderly subjects).

<u>POM authorisations:</u>

- Accord-UK Ltd
  - Plenachol D3 20 000 IU Capsules
  - Plenachol D3 40 000 IU Capsules
- Colonis Pharma Ltd
  - Colecalciferol 1 000 IU Capsules
- Consilient Health Ltd
  - Colecalciferol 800 IU Film-coated Tablets
  - Invita D3 2,400 IU/ml oral drops, solution
  - InVita D3 25,000 IU oral solution
  - InVita D3 25,000 IU soft capsules
  - InVita D3 400 IU soft capsules
  - InVita D3 5,600 IU soft capsules
  - InVita D3 50,000 IU soft capsules
  - InVita D3 800 IU soft capsules
  - invitaD3 50,000 IU oral solution
- Galen Limited
  - THORENS 10 000 I.U. /ml oral drops, solution
  - THORENS 25 000 I.U. /2.5 ml oral solution
- Internis Pharmaceuticals Ltd
  - Fultium-D3 20,000IU capsules
  - Fultium-D3 3,200IU capsules
  - Fultium-D3 800IU capsules
  - Fultium-D3 Drops
- Kyowa Kirin Ltd
  - Stexerol-D3 Tablets
- Mylan
  - Desunin 4000 IU Tablets
  - Desunin 800 IU Tablets
  - Kalcipos-D 500 mg/ 800 IU Chewable Tablets
- Strides Pharma UK Ltd
  - Strivit-D3 20,000 IU Soft Capsules
  - Strivit-D3 3,200 IU Soft Capsules
  - Strivit-D3 800 IU Soft Capsules
- Thame Laboratories
  - Colecalciferol 3000IU/ml Oral Solution
- Tor Generics Limited
  - COLECALCIFEROL 20000-IU SOFTGEL CAPSULES IN 20S

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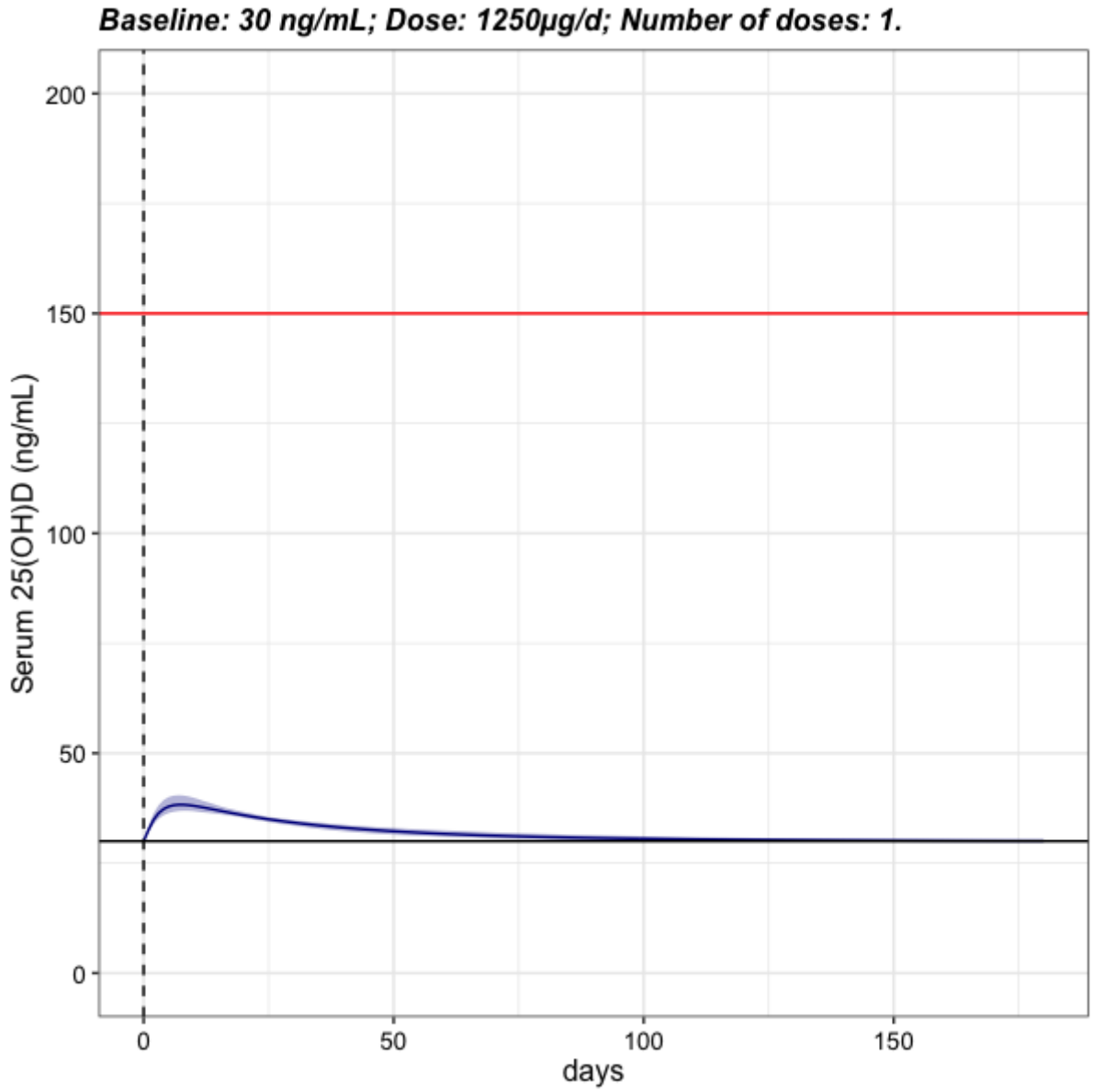
Vitamin D PBPK Simulation

[Prepared by You Tao, Lead PK/PD Modelling & Simulation Scientist]

For data overview, model construction and validation, please refer to the accompanying paper
Huang ZH and You T. (2020) Personalise Dose Regimen of Vitamin D3 Using Physiologically-Based Pharmacokinetic Modelling. Beyond Consulting Technical Report.
PDF at https://www.letsgobeyond.co.uk/vitamin-d

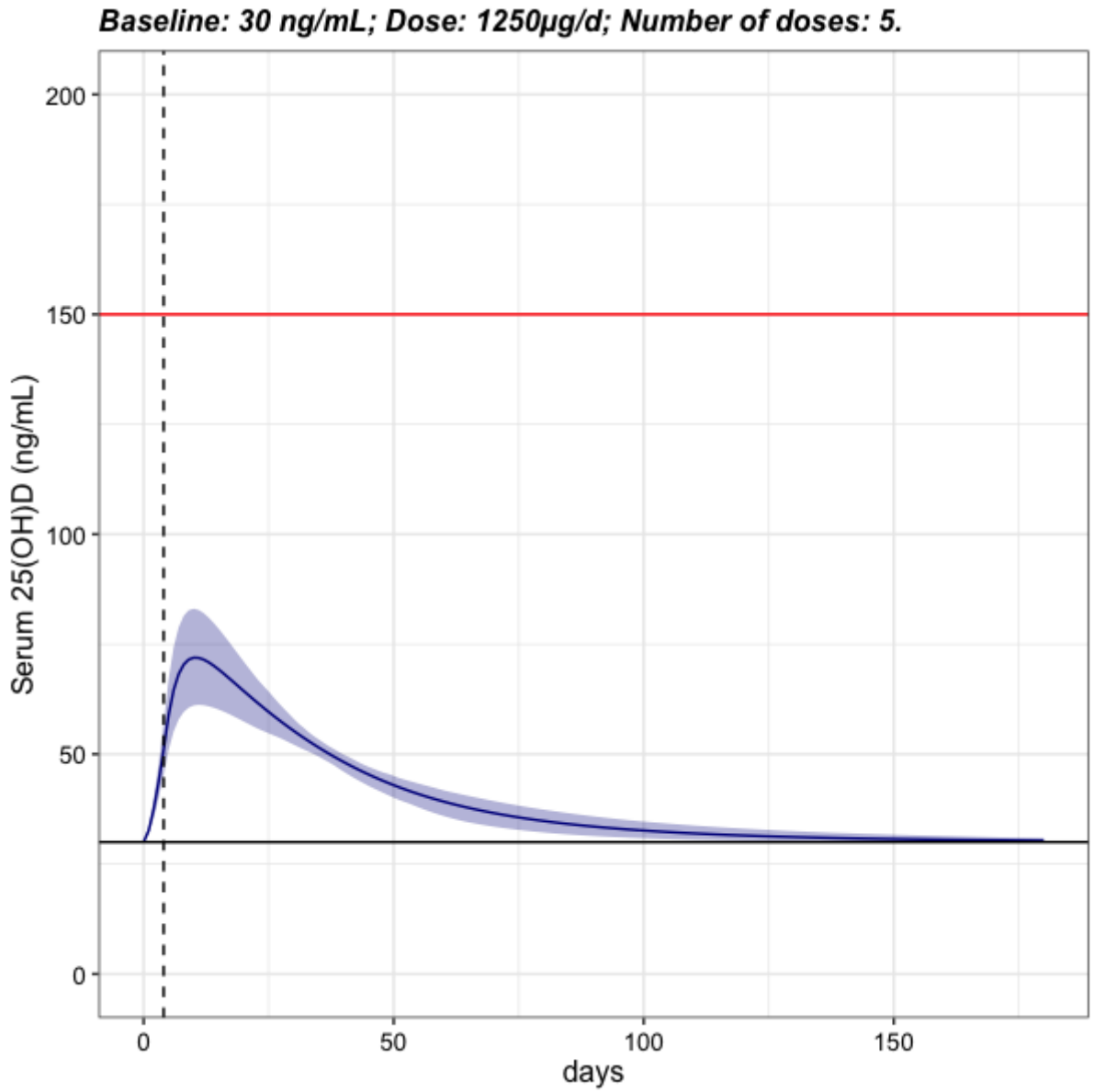


Baseline: 30 ng/mL (i.e. sufficient subject)
Dose: 1250 µg (i.e. 50000 IU)
Single dose (administered at time 0)

Blue line: Expected serum 25(OH)D concentration

Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Interpretation:
For a vitamin D replete subject (baseline at 30 ng/mL), a 1250 µg single dose is safe

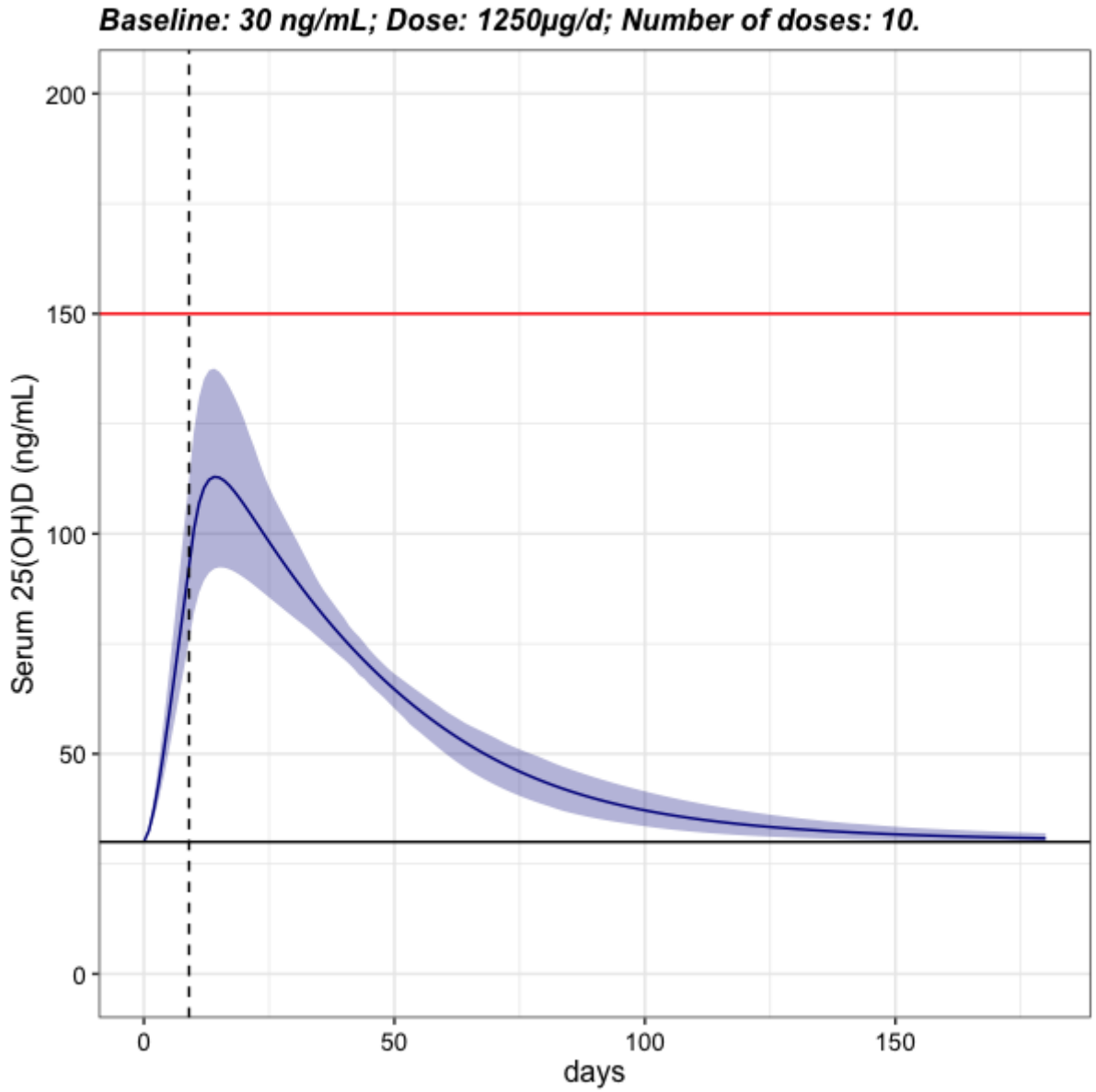


Baseline: 30 ng/mL (i.e. sufficient subject)
Dose: 1250 µg (i.e. 50000 IU)
5-day QD (administration started at time 0)

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)

Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Interpretation:
For a vitamin D replete subject (baseline at 30 ng/mL), 5-day 1250 µg QD PO dosing is safe



Baseline: 30 ng/mL (i.e. sufficient subject)
Dose: 1250 µg (i.e. 50000 IU)
10-day QD (administration started at time 0)

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Interpretation:
For a vitamin D replete subject (baseline at 30 ng/mL), 10-day 1250 µg QD PO dosing is safe

Baseline: 30 ng/mL (i.e. sufficient subject)
Dose: 1250 µg (i.e. 50000 IU)
14-day QD (administration started at time 0)

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Interpretation:
For a vitamin D replete subject (baseline at 30 ng/mL), 14-day 1250 µg QD PO dosing might exceed toxicity threshold

Baseline: 8.6 ng/mL (i.e. severely deficient subject)
Dose: 1250 µg (i.e. 50000 IU) x 7 + 100 µg (i.e. 4000 IU) X 173
administration started at time 0

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Interpretation:
For a vitamin D severely deficient subject (baseline at 8.6 ng/mL), 1250 µg (i.e. 50000 IU) x 7 + 100 µg (i.e. 4000 IU) X 173 might be an effective and safe regimen to rapidly achieve sufficiency

Baseline: 8.6 ng/mL (i.e. 10 nmol/L, severely deficient subject)
Dose: 1500 µg (i.e. 60000 IU)
7-day QD (administration started at time 0 and the last dose was administered at day 6)

Blue line: Expected serum 25(OH)D concentration

Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Rastogi 2020 reported:
Baseline serum 25(OH)D was 8.6 (7.1 to 13.1) in the intervention group. 10 out of 16 patients could achieve 25(OH)D>50 ng/ml by day-7 and another two by day-14 [day-14 25(OH)D levels 51.7 (48.9 to 59.5) ng/ml.

Since our time axis starts at time 0 instead of “day 1”, this statement should be compared with simulation results at the end of day 6 and day 13.

Baseline: 8.6 ng/mL (i.e. 10 nmol/L, severely deficient subject)
Dose: 1500 µg (i.e. 60000 IU)
7-day QD (administration started at time 0 and the last dose was administered at day 6)

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
Black horizontal line: marks target serum 25(OH)D concentration (i.e. 30 ng/mL = 75 nmol/L)
Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)

Baseline: 30 ng/mL (i.e. 75 nmol/L, sufficient subject)
Dose: 1500 µg (i.e. 60000 IU)
7-day QD (administration started at time 0 and the last dose was administered at day 6)

Blue line: Expected serum 25(OH)D concentration
Band: 90% confidence interval (i.e. 5% to 95%) of simulated serum 25(OH)D concentration
Vertical dashed line: marks the day when the last dose was administered
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Red horizontal line: marks upper bound of safe 25(OH)D concentration (i.e. 150 ng/mL = 375 nmol/L)