Feinstein Institutes for Medical Research
Northwell Health

**PROTOCOL: INTERVENTION STUDY (CLINICAL TRIALS)**

**Title:** Impact of Vitamin D Therapy on Thyroid Function and Antibody Levels in Pediatric Graves' Disease: A Pilot Feasibility Trial

**Protocol Number: 25-0285**

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**Investigational Product Name(s): ergocalciferol**

**IND or IDE Number** *(if applicable)*: NA

**Regulatory Sponsor** (*Sponsor-Investigator or IND/IDE holder, if applicable*)**: NA**
**Name**
**Address**
**Phone number**
**Email address**

**Protocol Version and Date: version 1. 6.26.2025**

**Protocol History**

| Protocol Version | Date |
|---|---|
| **Version 1** | **6/26/25** |
| | |
| | |

**Protocol Amendment Summary of Changes**

| Section | Summary of Revisions | Rationale |
|---|---|---|
| | | |
| | | |
| | | |

**Study Team**

| | |
|---|---|
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**Participating Site Teams**

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| **Northwell Health**<br>*CCMC Pediatric Endocrinology*<br>*1991 Marcus Ave, Suite M100*<br>*New Hyde Park, NY 11042* | **PI: Sharon Hyman**<br>**Co-Investigator: Sofya Ilmer**<br>**Study Coordinators: Jillian Charyn, Nafeesa Saadi**<br>**Research Nurses: Joanne Twaits**<br>**Research Pharmacists: Uzma Afzal** |

## CONTENTS

## STATEMENT OF COMPLIANCE

1. The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

   United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, 21 CFR Part 812 and/or other applicable CFRs).

2. Investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of clinical trials have completed the appropriate Human Subjects Protection and ICH GCP Training.

3. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study, except for changes necessary to eliminate an immediate hazard to study participants. In addition, all changes to the consent form will be IRB-approved; a determination will be made if participants will need to be re-consented with the most recent version of the IRB approved consent form.

**ABBREVIATIONS OF TERMS**

*The list below includes abbreviations utilized in this template. Therefore, this list should be customized for each protocol by removing unused abbreviations and adding unlisted abbreviations.*

| | |
|---|---|
| ADE | Adverse Device Effect |
| AE | Adverse Event |
| ANCOVA | Analysis of Covariance |
| CBC | Complete Blood Count |
| CFR | Code of Federal Regulations |
| CLIA | Clinical Laboratory Improvement Amendments |
| CMP | Clinical Monitoring Plan |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRF | Case Report Form |
| DHHS | Department of Health and Human Services |
| DSMB | Data Safety Monitoring Board |
| DRE | Disease-Related Event |
| EC | Ethics Committee |
| eCRF | Electronic Case Report Forms |
| FDA | Food and Drug Administration |
| FDAAA | Food and Drug Administration Amendments Act of 2007 |
| FFR | Federal Financial Report |
| GD | Graves' Disease |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practices |
| GMP | Good Manufacturing Practices |
| GWAS | Genome-Wide Association Studies |
| HIPAA | Health Insurance Portability and Accountability Act |
| IB | Investigator's Brochure |
| ICH | International Conference on Harmonisation |
| ICMJE | International Committee of Medical Journal Editors |
| IDE | Investigational Device Exemption |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |
| ISM | Independent Safety Monitor |
| ISO | International Organization for Standardization |
| ITT | Intention-To-Treat |
| LSMEANS | Least-squares Means |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MOP | Manual of Operating Procedures |
| MSDS | Material Safety Data Sheet |
| NCT | National Clinical Trial |
| NIH | National Institutes of Health |
| OHRP | Office for Human Research Protections |
| PI | Principal Investigator |
| QA | Quality Assurance |
| QC | Quality Control |

| | |
|---|---|
| RCT | Randomized Controlled Trial |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SMC | Safety Monitoring Committee |
| SOA | Schedule of Activities |
| SOP | Standard Operating Procedure |
| TFT | Thyroid Function Test |
| UADE | Unanticipated Adverse Device Effect |
| UP | Unanticipated Problem |
| US | United States |

**PROTOCOL SYNOPSIS**

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|---|---|
| **Protocol Title** | Impact of Vitamin D Therapy on Thyroid Function and Antibody Levels in Pediatric Graves' Disease: A Pilot Feasibility Trial |
| **Study Description** | Our long-term goal is to investigate whether achieving optimal vitamin D levels should be standard of care in pediatric patients diagnosed with Graves' Disease (GD). Our objective is to investigate the feasibility of protocol implementation for a larger clinical trial to help determine whether high dose vitamin D supplementation can promote earlier normalization of thyroid function and earlier reduction in Graves' antibody levels in newly diagnosed pediatric patients with GD compared to monotherapy with methimazole alone. |
| **Funder** | CCMC Pediatric Endocrinology – Departmental Funding |
| **Clinical Phase** | Pilot Feasibility Study |
| **Study Objective(s)** | • Primary: To evaluate the feasibility and refine procedures for a future, adequately powered randomized controlled trial of high-dose vitamin D supplementation in pediatric GD<br>• Secondary (exploratory): To explore the trends in thyroid function (TSH, T3, FT4, and T4) in pediatric GD patients receiving either methimazole alone or methimazole plus high-dose vitamin D supplementation.<br>• Tertiary (exploratory): To evaluate the impact of high-dose vitamin D supplementation on TSH receptor antibody (TRAb) and Thyroid Stimulating immunoglobulin (TSI) levels in children with newly diagnosed GD. |
| **Interventions or Test Article(s)** | The investigational product is vitamin D ergocalciferol 50,000 IU, administered orally. In this study, participants will be randomized to either the experimental (vitamin D + methimazole) or control (methimazole only) group at the time of the initial visit. The starting dose of methimazole and dose adjustments will be made by the treating endocrinologist based on established clinical practice guidelines. The control arm will receive no additional intervention other than methimazole (standard of care). While the control group will not receive the high-dose vitamin D intervention, they will be allowed to take up to 1000 IU of vitamin D daily. This approach balances the need for a comparison group against the ethical responsibility of not withholding a potentially beneficial supplement for those with low levels. |
| **Study Design** | This is an open label randomized clinical pilot feasibility trial |
| **Subject Population** | Specify the sample size, gender, age, demographic group, general health status, and geographic location. |
| **Criteria for Inclusion and Exclusion:** | Inclusion Criteria:<br>○ All new pediatric<br><br>Exclusion Criteria:<br>○ Initial hydroxy vitamin D levels |

| | | |
|---|---|---|
| | participants aged 9-17 years with a new diagnosis of GD who will be started on methimazole, will be offered to participate at the time of diagnosis.<br>○ Biochemical features include:<br>○ Suppressed TSH <0.1.<br>○ Elevated T3<br>○ Elevated Free T4<br>○ Elevated T4<br>○ **Positive TSI or TRAb.** The presence of antibodies is diagnostic.<br>○ Our study will offer enrollment to non-English speaking participants | >80 ng/mL<br>○ **Hypocalcemia, corrected calcium based on albumin <8.4 mg/dL**<br>○ **Hypercalcemia, corrected calcium based on albumin >10.5 mg/dL**<br>○ Conditions that affect vitamin D metabolism such as: malabsorption, chronic kidney or liver disease, nephrocalcinosis, hyperparathyroidism<br>○ Current use of medications which are known to affect thyroid function or vitamin D metabolism such as thyroid hormone replacement, corticosteroids, anticonvulsants<br>○ Allergy to vitamin D or methimazole<br>○ Diagnosis of Hashitoxicosis or thyrotoxicosis (both TSH receptor antibody (TRAb) and thyroid-stimulating immunoglobulin (TSI) levels are negative)<br>○ Participants under the age of 9 years at the time of diagnosis<br>○ Pregnant participants<br>○ Active or uncontrolled infections, other significant medical conditions deemed by the investigator to interfere with study participation or pose undue risk to the participant. |
| **Target Enrollment** | 30 participants are to be enrolled at CCMC Pediatric Endocrinology clinical practice. | |
| **Study Duration:** | August 1, 2025 – August 31, 2026. | |
| **Participant Duration:** | 24 weeks from initial enrollment in the study | |
| **Data and Safety Monitoring Plan** | DSMP officer and PI | |

**TABLE 1: SCHEDULE OF STUDY PROCEDURES**

| ***Study Phase*** | ***Screening/ Baseline*** | ***Treatment/Intervention*** | | |
|---|---|---|---|---|
| ***Study Procedure*** | ***D-0 (±1 d)*** | ***VISIT 1 (6-week Phone call (±1 d)*** | ***VISIT 2 (12 week/3-month visit (±1 d)*** | ***VISIT 3 - FINAL (24 week/6-month visit) (±1 d)*** |
| *Informed Consent/Assent* | *x* | | | |
| *Inclusion/Exclusion Criteria* | *x* | | | |
| *Demographics* | *x* | | | |
| *Medical History- Past and current medical conditions* | *x* | | *x* | *x* |
| *Physical Examination* | *x* | | *x* | *x* |
| *Vital Signs: BP, HR, RR* | *x* | | *x* | *x* |
| *Height and Weight* | *x* | | *x* | *x* |
| *Pregnancy Test (PRN)* | | | | |
| *Prior/Concomitant Medications* | *x* | *x* | *x* | *x* |
| *Clinical Laboratory Evaluation* | *x* | *x* | *x* | *x* |
| *Randomization* | *x* | | | |
| *Dispense Investigational Product* | *x* | | | |
| *IP Accountability/Return* | | | | *x* |
| *Drug Compliance* | | *x* | *x* | *x* |
| *Adverse Event Assessment* | | *x* | *x* | *x* |

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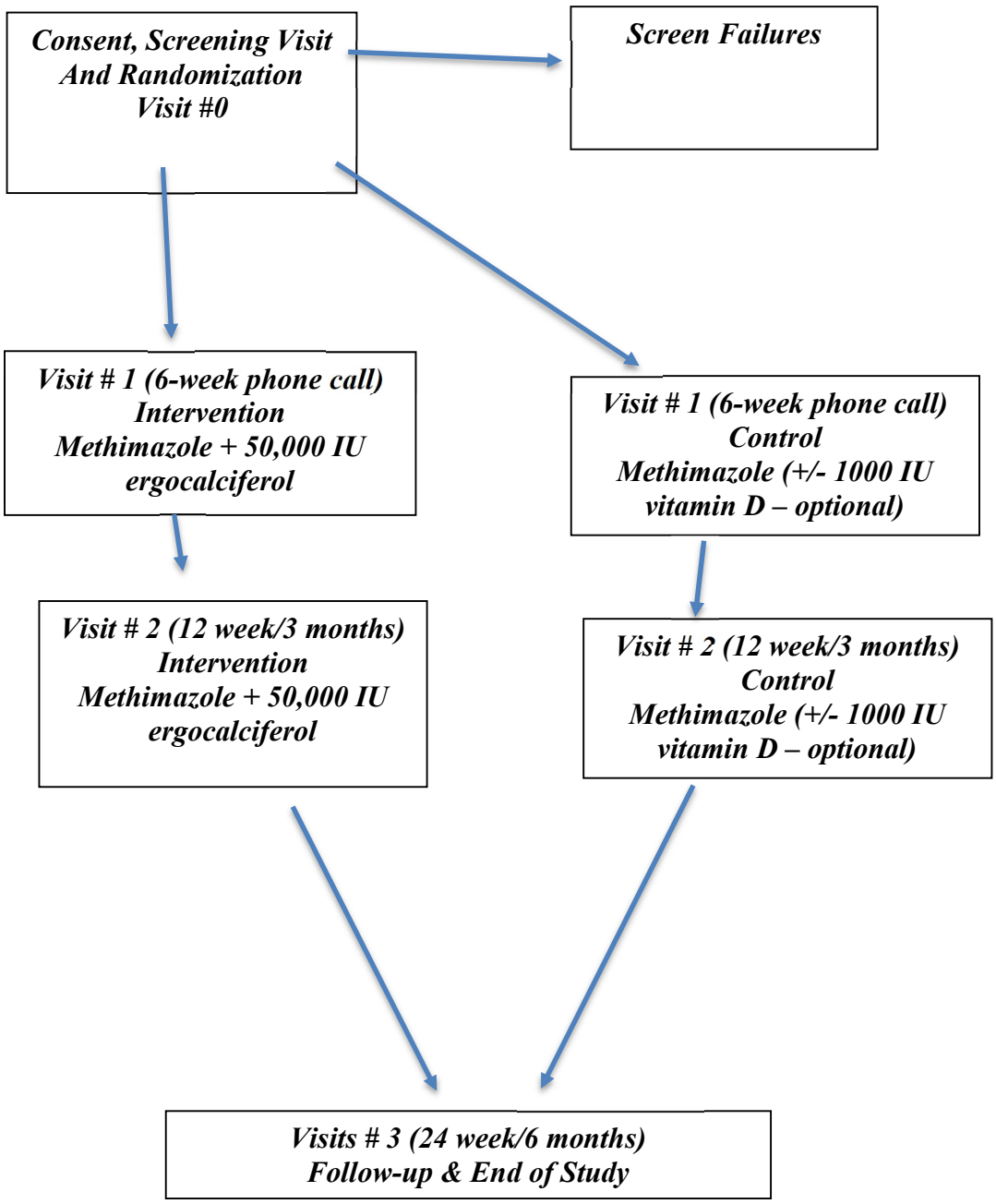
**FIGURE 1: STUDY DIAGRAM**



# 1. INTRODUCTION

## 1.1 Background Information and Rationale

Graves' disease (GD) is an autoimmune disorder causing hyperthyroidism, with remission (sustained euthyroidism off medication) being the ultimate treatment goal. Clinical features of GD include thyroid enlargement or goiter; palpitations, diarrhea, weight loss, polyphagia, ophthalmopathy, poor sleep, hyperactivity, mood swings, anxiety, increased risk of osteoporosis and fractures from higher bone turnover, proximal muscle weakness, hyperhidrosis, heat intolerance, fatigue, growth acceleration and advanced bone maturation.[1] Most pediatric endocrinologists start treatment with methimazole and titrate the dose to maintain normal thyroid hormone levels. The goal of treatment in GD is to ultimately achieve remission. Remission is defined as remaining euthyroid for at least 12 months after discontinuation of anti-thyroid therapy. In children, the predictive factors for earlier remission are: lower baseline severity of disease at diagnosis as measured by free thyroxine (FT4), total thyroxine (T4), total triiodothyronine (T3) levels, Thyroid stimulating immunoglobulin (TSI), TSH Receptor Antibody (TRAb) levels; older age, smaller thyroid size at diagnosis, higher Body Mass Index (BMI), lower heart rate at diagnosis, rapid normalization of thyroid hormone levels after starting treatment, rate of decline of TRAb after 6 months of treatment.[2-4]

While predictive factors for remission in both adults and children include lower baseline disease severity (as measured by FT4, T3, TSI, and TRAb levels) and smaller thyroid size, TRAb and TSI levels are key factors influencing treatment decisions. Persistently positive antibody levels often necessitate continued anti-thyroid drug (ATD) therapy, as they indicate a high likelihood of relapse. Monitoring the rate of TRAb decline, particularly at 6 months and upon ATD withdrawal, has been shown to predict remission in adults[5]. Furthermore, emerging evidence suggests a strong association between vitamin D and autoimmune regulation. Studies have linked low vitamin D levels to an increased risk of GD in both adults and children, with some research indicating a dose-response relationship between vitamin D levels and GD risk[6-8]. While one small pediatric trial (n=25) found that vitamin D supplementation alongside standard GD therapy improved TSH levels faster[9], data on the impact of vitamin D supplementation on treatment outcomes in children with GD remains extremely limited.

This study addresses the critical gap in research regarding the effect of vitamin D supplementation on antibody and thyroid hormone trends in children with GD. Current literature demonstrates a correlation between vitamin D deficiency and GD, but the impact of supplementation on disease course, particularly antibody levels which are crucial for treatment decisions, remains unexplored in pediatric populations. This study will investigate the feasibility of protocol implementation for a larger clinical trial. The secondary goals will be to investigate whether high-dose vitamin D supplementation, in conjunction with standard methimazole therapy, influences the trajectory of TRAb and TSI levels, as well as thyroid hormone levels, in children newly diagnosed with GD. By examining these trends, the study will contribute valuable scientific knowledge regarding the potential role of vitamin D in modulating the autoimmune response in pediatric GD. **Positive findings could significantly impact clinical practice by providing evidence to support the use of vitamin D supplementation as an adjunctive therapy, potentially improving remission rates and reducing the duration of ATD treatment in children with this condition.**

## 1.2 Investigational Product

**Study Intervention:** High-dose Vitamin D (Ergocalciferol) Supplementation
**Description of Investigational Agent**: The intervention in this study is ergocalciferol (vitamin D2), a fat-soluble vitamin crucial for calcium absorption, bone health, and immune system regulation. Ergocalciferol is a white to yellowish-white crystalline powder practically insoluble in water but soluble in organic solvents like ethanol and ether. It is commercially available as oral capsules and is not an investigational drug.
**Market Status:** FDA-approved (Over the counter and prescription)

**Dosage Form:** Oral capsules containing 50,000 International Units (IU) of ergocalciferol will be used.
**Justification for Route, Dosage, Regimen, and Duration:**
Oral administration is the standard and most convenient route for vitamin D supplementation. Participants in the intervention arm will receive 50,000 IU of ergocalciferol weekly for the first 8 weeks, followed by 50,000 IU every two weeks for another 4 months of the 6-month study period. This high-dose regimen is designed to rapidly replete vitamin D stores and achieve optimal serum levels. This regimen is supported by a previous study by Nwosu et al. [10] which used the same high-dose vitamin D supplementation protocol in patients with new-onset type 1 diabetes without significant adverse effects. The 6-month intervention period is chosen to align with the typical timeframe for initial treatment response assessment in pediatric Graves' disease. This duration allows sufficient time to evaluate the effects of vitamin D supplementation on thyroid hormone and antibody levels. Furthermore, it is a reasonable timeframe for a pilot study to assess feasibility before embarking on a larger, longer-term trial.
**Justification for Off-Label Use:** While high-dose vitamin D is sometimes used clinically, the specific regimen in this study (50,000 IU weekly followed by every two weeks) might be considered off-label in some contexts. This is justified by:

- **Safety:** The chosen dosage is well below the tolerable upper intake level for vitamin D in children older than 9 years, minimizing the risk of toxicity [10]. Careful monitoring of 25-hydroxyvitamin D levels and calcium will be implemented throughout the study to ensure participant safety. Any participant with a vitamin D level > 80 ng/mL will have their dosage reduced to 1000 IU daily to mitigate potential toxicity.
- **Rationale:** The dosing regimen is modeled after a successful and safe protocol used in a prior study in a similar patient population (type 1 diabetes)[10]. This provides evidence to support the safety and potential efficacy of this approach.

**Pilot Study Design:** This is a pilot feasibility study. The primary objective is to determine the feasibility of the intervention and its impact on antibodies and thyroid hormones, rather than claiming any therapeutic benefit. The data generated by this study can inform optimal dosing regimens in subsequent trials.
**Safety Monitoring:** Close monitoring of 25-hydroxyvitamin D levels at regular intervals will help mitigate any risks associated with high-dose vitamin D. Participants will be contacted regularly to reinforce adherence and ensure they are undergoing the required monitoring.

### 1.3 Findings from Previous Studies

Graves' disease (GD) is an autoimmune process which is caused by autoantibodies that bind to the thyrotropin receptor, stimulating growth of the thyroid and overproduction of thyroid hormone. GD in children presents significant treatment challenges. The only available antithyroid drug (ATD) therapy for children, methimazole, has low remission rates (<25%), often necessitating more aggressive interventions like radioactive iodine or surgery, each with inherent risks. The risk of severe adverse reactions to ATDs, such as hepatic failure and bone marrow suppression, further complicates management and contributes to the overall morbidity thus necessitating need for alternative treatments.
Historically, vitamin D has been associated with the regulation of bone metabolism. Recent evidence suggests a strong association between vitamin D and autoimmune regulation in diseases such as Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS) and Type 1 Diabetes. Due to its unique capability to bind to vitamin D receptor and serve as a transcriptional factor, vitamin D can regulate gene expression and further exert its immunomodulatory effects on immune cells. It has been shown to inhibit Th17 cytokine production, enhance Treg activity, induce NKT cell functions, suppress Th1, and promote Th2 cytokine production.[11] Because of the high prevalence of vitamin D insufficiency and deficiency in patients with MS, T1DM, and SLE, vitamin D supplementation has been considered a potential candidate for the treatment of autoimmune diseases with some studies showing promising results in these autoimmune diseases. The relationship between vitamin D and GD has not been studied as extensively. Monitoring and replacing low vitamin D levels is not a standard of care in patients with GD. While some adult studies suggest that vitamin D supplementation improves GD treatment outcomes, there is extremely limited data in children.

### 1.4 Benefit and Risk Assessment

#### 1.4.1 Known Potential Risks

This study involves the use of high-dose vitamin D (ergocalciferol) supplementation in conjunction with standard methimazole therapy for pediatric Graves' disease. The following potential risks have been identified:

**Risks Associated with High-Dose Vitamin D Supplementation:**

- **Hypercalcemia:** The primary risk of high-dose vitamin D is hypercalcemia (elevated blood calcium levels). Symptoms can include nausea, vomiting, constipation, abdominal pain, muscle weakness, confusion, and in severe cases, cardiac arrhythmias. Regular monitoring of calcium levels will be performed to mitigate this risk. If hypercalcemia occurs, the vitamin D dose will be reduced to 1000 IU daily or discontinued, and appropriate medical management will be implemented.
- **Hypercalciuria:** Increased urinary calcium excretion can occur with high-dose vitamin D, potentially leading to kidney stones in susceptible individuals. Adequate hydration will be encouraged, and urine calcium levels may be monitored if clinically indicated.
- **Other potential side effects:** Less common side effects of vitamin D supplementation can include nausea, vomiting, constipation, loss of appetite, weakness, and weight loss. These are usually mild and transient.

**Risks Associated with Methimazole Therapy:**

- **Minor side effects:** Common side effects of methimazole include rash, itching, nausea, vomiting, joint pains and altered taste sensation. These are usually mild and self-limiting.
- **Serious adverse events:** Rare but serious adverse events associated with methimazole include agranulocytosis (severe decrease in white blood cells), hepatotoxicity (liver damage), and vasculitis (inflammation of blood vessels). Regular monitoring of blood counts and liver function tests will be conducted to detect these potential complications early. Participants will be educated about the signs and symptoms of these adverse events and instructed to report them immediately.
- **Allergic reactions:** Allergic reactions to methimazole, ranging from mild skin rashes to severe anaphylaxis, can occur. Participants will be closely monitored for signs of allergy.

**Risks Related to Study Procedures:**

- **Phlebotomy:** The study involves multiple blood draws for laboratory testing. Risks associated with phlebotomy include bruising, pain, infection, and rarely, nerve damage or fainting. Standard phlebotomy procedures will be followed by trained personnel to minimize these risks.
- **Discomfort or anxiety:** Some participants may experience discomfort or anxiety related to study procedures, such as blood draws or clinic visits. The study team will strive to create a supportive and comfortable environment for all participants.

**Immediate, Short-Term, and Long-Term Risks:**

- **Immediate risks:** The most immediate risks are related to phlebotomy (bruising, pain, infection) and potential allergic reactions to methimazole.
- **Short-term risks:** Short-term risks include the potential side effects of vitamin D and methimazole, primarily hypercalcemia, hypercalciuria, gastrointestinal symptoms, rash, joint pains and minor allergic reactions.
- **Long-term risks:** The long-term risks of this study are minimal. The duration of exposure to high-dose vitamin D is limited to 24 weeks. Long-term monitoring of thyroid function and antibody levels will be performed as part of standard clinical care for Graves' disease.

**Alternative Procedures Considered:**

- **Lower dose vitamin D:** A lower dose of vitamin D supplementation was considered. However, the chosen high-dose regimen is supported by prior research[10] and is more likely to achieve optimal serum vitamin D levels within the study timeframe.

- **Placebo control:** A placebo control group was considered. However, due to the ethical implications of potentially withholding vitamin D in participants with low levels, a standard of care control group (methimazole alone with the option of taking up to 1000 IU of vitamin D daily) was chosen. This allows all participants to receive appropriate management of their vitamin D levels while still permitting comparison between the intervention and control groups. **Should the patients in the control group have vitamin D levels <20 ng/mL, patients will be strongly advised to take the 1000 IU of vitamin D daily for repletion.**

**Other Risks:**

- **Social, legal, and economic risks:** There are no anticipated social, legal, or economic risks associated with study participation. Participation is voluntary, and participants can withdraw at any time without penalty. Confidentiality will be maintained according to HIPAA regulations.
- **Psychological risks:** Participation in the study is not expected to pose significant psychological risks. However, the study team will be sensitive to any emotional distress that may arise and will provide appropriate support and referrals as needed.

### 1.4.2 Known Potential Benefits

**Direct Benefits to Study Participants:**
Potential Improvement in Graves' Disease Symptoms: While not a guaranteed outcome of this pilot study, some participants may experience improvements in their GD symptoms (e.g., reduced fatigue, palpitations, anxiety, improved sleep) due to either the vitamin D supplementation or the optimized management of their GD as part of the study protocol. It is crucial to emphasize that this is not the primary aim of the study and cannot be guaranteed.
Close Monitoring and Medical Care: Participants will receive close monitoring of their thyroid function, antibody levels, vitamin D levels, and overall health throughout the study. This enhanced medical attention could lead to earlier detection and management of any potential complications related to GD or vitamin D supplementation.
Increased Knowledge and Understanding: Participants will gain a better understanding of GD, its management, and the potential role of vitamin D in autoimmune diseases. They will also learn about the importance of research and contribute to scientific knowledge.
**Potential Earlier Identification of Vitamin D Deficiency:** Participants will have their vitamin D levels measured, which may identify a deficiency that could be addressed, even in the control group (who can take up to 1000 IU/day). Correcting vitamin D deficiency can have general health benefits beyond GD, such as improved bone health and immune function.
**Indirect Benefits (to Individuals or Society):**
Contribution to Scientific Knowledge: The study will generate valuable data on the feasibility of conducting larger trials on vitamin D supplementation in pediatric GD. This will pave the way for future research that could ultimately lead to improved treatments and better outcomes for children with GD.
**Potential for Improved Treatment Strategies**: If the pilot study findings are positive and a subsequent larger trial demonstrates the efficacy of vitamin D supplementation, it could lead to changes in standard clinical practice, potentially offering a safe and effective adjunctive therapy for pediatric GD. This could improve remission rates, reduce the need for more aggressive treatments, and minimize long-term complications.
**Enhanced Understanding of Vitamin D's Role in Autoimmune Diseases:** The study may contribute to a broader understanding of vitamin D's role in autoimmune diseases, potentially informing research and treatment strategies for other conditions beyond GD.
**Immediate, Short-Term, and Long-Term Potential Benefits:**

**Immediate Benefits:** Primarily include the close monitoring and enhanced medical care received throughout the study, as well as the increased knowledge about GD and vitamin D. Some participants might also experience an early improvement in symptoms, though this is not guaranteed.
**Short-Term Benefits:** Similar to immediate benefits but may also include improved control of GD symptoms and earlier identification and management of potential complications.

**Long-Term Benefits:** Primarily relate to the potential for improved treatment strategies and a better understanding of vitamin D's role in autoimmune diseases, benefiting future generations of children with GD and potentially other autoimmune conditions.

### 1.4.3 Assessment of Potential Risks and Benefits

The risks associated with this pilot study are generally low and manageable with the implemented mitigation strategies. The potential benefits, while not guaranteed for individual participants, are substantial in terms of the potential for future advancements in pediatric GD treatment. The knowledge gained from this feasibility study, including data on recruitment, adherence, and data collection, is crucial for designing a future, adequately powered RCT that could ultimately lead to improved outcomes for children with this condition. Therefore, the potential benefits of conducting this pilot study, in terms of informing future research and potentially improving patient care, outweigh the relatively low and manageable risks.

### 1.5 IND or IDE Applicability

This research study does not involve an investigational new drug (IND) or an investigational device (IDE). The intervention, vitamin D (ergocalciferol), is an FDA-approved product available both over the counter and by prescription. While the specific high-dose regimen used in this study may be considered off-label use, it does not meet the criteria for an IND application as defined in 21 CFR 312. Therefore, no IND is required for this study. Similarly, no devices are being investigated, so an IDE application under 21 CFR 812 is not applicable.

# 2 STUDY OBJECTIVES AND ENDPOINTS

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| **Primary** | | |
| To evaluate the feasibility and refine procedures for a future, adequately powered randomized controlled trial of high-dose vitamin D supplementation in pediatric GD. | This pilot will assess: (a) recruitment rates and strategies; (b) adherence to the high-dose vitamin D protocols, including evaluating different adherence monitoring strategies (e.g., pill counts, medication logs, phone calls); and (c) the completeness and variability of collected data for primary and secondary outcome measures; (d) identify barriers to large-scale implementation. | These data will direct the design and sample size calculation of a subsequent definitive large-scale trial. |
| **Secondary/Exploratory** | | |
| To explore the trends in thyroid function (TSH, T3, FT4, and T4) in pediatric GD patients receiving either methimazole alone or methimazole plus high-dose vitamin D supplementation. | These preliminary data will be used to estimate effect sizes and inform sample size calculations for a future, fully powered trial investigating the impact of vitamin D on thyroid hormone normalization. We hypothesize that patients receiving high-dose vitamin D will achieve normal TSH, T3, Free T4 and T4 levels significantly faster than those receiving methimazole alone. Thyroid function tests will be measured at baseline, 6, 12 and 24 | Faster normalization can improve patient well-being, reduce methimazole dosage requirements and potentially reduce long-term complications. |

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| | weeks, and time to normalization will be compared between groups. | |
| **Tertiary/Exploratory** | | |
| To evaluate the impact of high-dose vitamin D supplementation on TSH receptor antibody (TRAb) and Thyroid Stimulating immunoglobulin (TSI) levels in children with newly diagnosed GD | We hypothesize that patients receiving high-dose vitamin D will experience a significantly greater percentage reduction in TSH R-Ab and TSI levels at 24 weeks compared to those receiving methimazole alone. Antibody levels will be measured at baseline and 24 weeks, and the percentage change from baseline will be compared between groups. | A greater reduction in antibody levels may indicate a more effective treatment response and potentially predict a higher likelihood of remission. |

# 3 INVESTIGATIONAL PLAN

## 3.1 Overall Study Design and Plan

A. Rationale: This pilot study represents a critical first step towards evaluating the therapeutic potential of high-dose vitamin D supplementation in pediatric GD. By focusing on feasibility aspects such as recruitment, adherence, and data collection, this pilot will lay the foundation for a future definitive trial designed to assess the efficacy and safety of this promising adjunctive therapy.

B. Study Overview: This study aims to determine the feasibility of protocol implementation for investigating the potential benefits of adding high dose vitamin D to standard therapy for pediatric Graves' Disease. To efficiently accomplish the goals of this study, we will recruit pediatric patients aged 9-17 years that are newly diagnosed with Graves' disease in our division of pediatric endocrinology at Cohen Children's Medical Center.

C. Study Design and Methods:

This is an open label randomized clinical pilot feasibility trial. In this study, participants will be randomized to either the experimental (vitamin D + methimazole) or control (methimazole only) group at the time of the initial visit. The starting dose of methimazole and dose adjustments will be made by the treating endocrinologist based on established clinical practice guidelines. The control arm will receive no additional intervention other than methimazole (standard of care). While the control group will not receive the high-dose vitamin D intervention, they will be allowed to take up to 1000 IU of vitamin D daily. This approach balances the need for a comparison group against the ethical responsibility of not withholding a potentially beneficial supplement for those with low levels. The experimental arm will be given vitamin D 50000 IU weekly for 8 weeks followed by 50000 IU every 2 weeks for another 16 weeks in addition to standard of care (methimazole). This dose is below the standard tolerable upper intake level for ergocalciferol for people older than 9 years and thus unlikely to lead to ergocalciferol toxicity. This dosing regimen was modeled after a randomized controlled trial for vitamin D supplementation in type 1 diabetes mellitus by Nwosu et al and there were no toxicity issues during the course of that study.[10]

Participants enrolled in the study will obtain bloodwork for 25-hydroxy vitamin D levels at baseline, 12 and 24 weeks in addition to thyroid function tests to monitor for toxicity. They will also obtain thyroid function levels at 6 weeks which is standard of care. CMP for calcium levels will be drawn at baseline, 6, 12 and 24 weeks to minimize risk of hypercalcemia from vitamin D supplementation and for monitoring of the liver function tests while on methimazole which is standard of care.

D. Key Variables: Demographic data (Date of diagnosis, Age, Sex, BMI z-scores, Race, Ethnicity, Weight), Hyperthyroidism Disease severity (based on Free thyroxine levels). We will document whether the thyroid hormone levels have normalized at each specific time point. We will also document whether there was at least a 50% decrease in the TSH Receptor Antibody level or Thyroid stimulating immunoglobin at 24 weeks. Thyroid levels at diagnosis, 6, 12 and 24 weeks will be recorded as well as 25 hydroxy vitamin D levels at diagnosis, 12 and 24 weeks.

E. Exposure & Outcome Measures: We will compare changes and trends in thyroid (T4, FT4, T3, TSH) in the treatment group (vitamin D + methimazole) compared to controls (methimazole only) at 6, 12 and 24 weeks. We will compare changes in Graves' antibody levels (TRAb, TSI) at baseline and 24 weeks compared to levels at diagnosis to see if there was a >50% decrease in the antibodies in the treatment group compared to controls at 24 weeks. We will also evaluate the trends in thyroid function and vitamin D at 12 weeks and 24 weeks and changes or trends in Graves' antibodies at 24 weeks compared to levels at diagnosis in both groups.

G. Statistical Plan: Data will be analyzed based on intention to treat. Descriptive statistics will be computed for all aims (frequencies and proportions for categorical data, means ± standard deviation (SD) or medians and Q1-Q3 for continuous data), as appropriate. For each thyroid hormone level and for TSH, logistic regression will be performed using a generalized linear mixed model (GLMM) to simultaneously assess the odds of normalization at 6, 12 and 24 weeks. GLMMs can account for correlations of measurements at different timepoints that are from the same patient. Logistic regression will be utilized to assess the odds of >50% decrease in antibody levels at 24 weeks from diagnosis. If the sample size is sufficient, we will adjust for disease severity at diagnosis in each model. If GLMMs are not feasible, analysis will be performed separately at each timepoint. A linear mixed model (appropriate data transformations may be applied) with an interaction term of group by time will be utilized to compare absolute and percentage changes in thyroid function (T4, FT4, T3) at 6, 12 and 24 weeks, vitamin D levels at 12 & 24 weeks, and Graves' antibody levels (TRAb, TSI) at 0 & 24 weeks within participants in each group (treatment and control) from time of diagnosis and between groups at each time point. Separate models will be built for each thyroid function hormone (T4, FT4, T3), for vitamin D, and for each Graves' antibody (TRAb, TSI) for absolute and for percentage change. As with GLMMs, linear mixed models can account for correlations of measurements at different timepoints that are from the same patient. For all analyses, results yielding p-values <0.05 will be considered statistically significant. All analyses will be conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

### 3.2 Participating Sites and Enrollment

This pilot study will be conducted at a single site: Cohen Children's Medical Center (CCMC), within the Division of Pediatric Endocrinology, Northwell Health. We aim to enroll a total of 30 participants. Recruitment will cease once 30 participants have consented and been enrolled in the study. We anticipate that all enrolled participants will be evaluable, assuming adherence to study procedures and complete data collection. Therefore, the target number of evaluable participants is also 30. While unforeseen circumstances may lead to some participants becoming ineligible for analysis, our recruitment target accounts for a reasonable level of attrition.

### 3.3 Study Population

Our target population consists of pediatric patients aged 9-17 with newly diagnosed GD. We will recruit participants from Northwell Pediatric Endocrinology practice, where approximately 20-30 patients meeting the inclusion criteria are seen annually, indicating a sufficient pool of potential participants. Recruitment will occur over 11 months (Dec 2024 - Oct 2025), with a target enrollment of up to 30 participants. Physicians at our practice will be trained to identify and refer eligible patients using provided study criteria. Potential participants will be informed about the study during their initial clinic visit. If enrollment is slower

than anticipated, we will explore expanding recruitment efforts to another site and/or extending the recruitment period.

This study focuses on a pediatric population under the care of our pediatric endocrinology practice aged 9-17 years old. Graves' disease can significantly impact growth and development in children, and there is a lack of data on the efficacy of adjunctive vitamin D therapy in this age group. This research has the potential to improve treatment outcomes and quality of life for children with Graves' disease. The study is designed to minimize risks through rigorous safety monitoring, including regular blood tests and dose adjustments as needed. Informed consent will be obtained from parents/guardians, and assent will be obtained from children. Participation is voluntary, and withdrawal is permitted at any time. The potential benefits of this research outweigh the minimal risks, and the study will be overseen by the IRB to ensure participant safety and ethical conduct If applicable and available, describe high-level strategies and methods for recruiting participants.
No ads will be used.

### 3.4 Subject Eligibility

#### 3.4.1 Inclusion Criteria

To be eligible for participation in this study, individuals must meet all the following inclusion criteria:

1. Provision of signed and dated informed consent form by the participant or by the participant's parent/legal guardian (if the participant is aged 9-17 years). Assent will be obtained from participants aged 9-17 years.
2. A new diagnosis of Graves' disease (GD), defined as:
    - Suppressed TSH level
    - Elevated free T4 (FT4) and/or total T4 (T4) and/or total T3 (T3) level
    - Positive TSH receptor antibody (TRAb) or thyroid-stimulating immunoglobulin (TSI) level – note patient may sign the consent form prior to these results coming back. If both are negative the patient will be excluded from the study and considered a screen failure.
3. Age 9-17 years, inclusive.
4. Initiation of methimazole therapy as part of standard GD treatment.
5. Willingness and ability to comply with all study procedures, including attending scheduled study visits, taking study medications as prescribed, and undergoing required laboratory testing.
6. Ability to take oral medications.
7. For females of reproductive potential:
    - Not currently pregnant or breastfeeding
8. Willingness to have their 25-hydroxyvitamin D levels measured and ability to take vitamin D supplementation if initial vitamin D level is <80ng/mL.
9. Our study will offer enrollment to non-English speaking participants

#### 3.4.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Initial hydroxy vitamin D levels >80 ng/mL (which may not be available at the time of initial screening)
- Hypocalcemia, corrected calcium based on albumin <8.4 mg/dL
- Hypercalcemia, corrected calcium based on albumin >10.5 mg/dL

- Conditions that affect vitamin D metabolism such as: malabsorption, chronic kidney or liver disease, nephrocalcinosis, hyperparathyroidism
- Current use of medications which are known to affect thyroid function or vitamin D metabolism such as thyroid hormone replacement, corticosteroids, anticonvulsants
- Allergy to vitamin D or methimazole
- Diagnosis of Hashitoxicosis or thyrotoxicosis (both TSH receptor antibody (TRAb) and thyroid-stimulating immunoglobulin (TSI) levels are negative)
- Participants under the age of 9 years at the time of diagnosis
- Pregnant participants
- Active or uncontrolled infections, other significant medical conditions (e.g., uncontrolled diabetes mellitus, active malignancy, severe liver or kidney disease), or any condition deemed by the investigator to interfere with study participation or pose undue risk to the participant.

### 3.4.3 Screen Failures

Screen failures will be notified of their ineligibility and thanked for their interest in the study. Appropriate medical care and referrals will be provided as needed, regardless of eligibility status. Data collected during screening for screen failures will be documented (e.g., screening log) and retained per study protocol and institutional requirements but will not be included in the final study analysis.

- **Vitamin D Level:** Participants with a 25-hydroxyvitamin D level > 80 ng/mL at screening will not be eligible for re-screening. This is because high vitamin D levels at baseline could confound the study results and potentially increase the risk of hypercalcemia with supplementation. These participants will be counseled on the importance of appropriate vitamin D intake and may be referred for additional clinical care as needed.
- **Negative TRAb and TSI:** Participants who are screen failures due to negative TRAb and TSI antibodies, confirming the absence of Graves' disease, will not be eligible for re-screening. This is because the study is specifically designed to evaluate the effect of vitamin D supplementation in newly diagnosed pediatric Graves' disease.

# 4 EVALUATIONS BY VISIT

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## 4.1 Screening Visit

Baseline/Screening Visit (Day 0):

- **Combined Visit Rationale**: To minimize participant burden and streamline the study process, the screening and baseline visits will be conducted on the same day. This approach allows for immediate enrollment of eligible participants without requiring a separate screening visit.
- **Procedures:**
    1. Eligibility Assessment and Informed Consent/Assent: The visit will begin with a comprehensive eligibility assessment, including:
        - Review of inclusion/exclusion criteria based on medical history, current medications, and any relevant pre-existing conditions.
        - Physical examination, including height, weight, and vital signs.

- If eligible, the informed consent process will begin. This includes explaining the study's purpose, procedures, risks, benefits, and the participant's rights, as well as obtaining written informed consent from the parent/legal guardian. Assent will also be obtained from the participants aged 9-17 years.

2. Laboratory Testing (Contingent on Consent): Following informed consent/assent, initial laboratory tests will be performed, including:
    - Thyroid function tests (TSH, free T4, total T4, total T3)
    - TSH receptor antibodies (TRAb) and Thyroid Stimulating Immunoglobulin (TSI)
    - 25-hydroxyvitamin D level
    - Complete blood count (CBC)
    - Comprehensive metabolic panel (CMP) to assess calcium, renal and liver function
    - Except for vitamin D level all the above would be considered standard of care.
3. Enrollment Confirmation (Contingent on Eligibility): If the patient qualifies for the study based on initial laboratory testing from the referral (suppressed TSH and elevated T4 or Free T4), they would be offered to participate. If any eligibility criteria are not met based on the laboratory results, the participant will be classified as a screen failure and appropriate procedures for screen failures will be followed.
4. Study Randomization (For Enrolled Participants): Enrolled participants will be randomized to either the intervention arm (methimazole + high-dose vitamin D) or the control arm (methimazole alone, with the option of taking up to 1000 IU of vitamin D2 daily) at the time of the initial visit following the consent.
5. Medication Dispensing and Instructions (For Enrolled Participants):
    - Intervention Arm: Dispense the initial dose of vitamin D2 (50,000 IU ergocalciferol) and provide detailed instructions on the dosing schedule (weekly for 8 weeks, then every two weeks). Provide a study medication diary/log for participants to track their doses.
    - Control Arm: Reinforce standard methimazole dosing instructions and provide education on the optional use of up to 1000 IU/day of vitamin D2 if levels are below recommendations.
6. Review of Potential Adverse Events (For Enrolled Participants): Educate participants/families on how to recognize and report any potential adverse events related to study medications or any changes from baseline.

### 4.2 Visit 1 (Phone call follow-up, 6 weeks from initiation)

**Purpose:** This phone call serves as a mid-point check-in to assess medication adherence, reinforce study procedures, and remind participants about their upcoming Week 6 laboratory testing.

**Procedures:**

1. Contact the Participant/Parent/Guardian: Attempt to contact the participant or their parent/guardian by phone. Document the date and time of the call, as well as the outcome (successful contact, left voicemail, no answer). If unable to reach the participant after multiple attempts (at least 3 attempts should be made on different dates during different times), document this and consider alternative contact methods.

2. Medication Adherence Assessment: If contact is made, inquire about the participant's adherence to both methimazole and (if applicable) vitamin D supplementation. Utilize open-ended questions to assess adherence (e.g., "Can you tell me how you've been taking your medications over the past few weeks?"). Review the medication logs/diaries and reconcile pill counts (if feasible). Address any reported adherence issues and provide appropriate guidance and support. Refer to study clinicians if clinically significant deviations from the protocol are found. For patients in the control arm, ask the parent or participant whether they are taking any supplemental vitamin D and how often.
3. Laboratory Test Reminder: Remind the participant/parent/guardian about the importance of completing the required Week 6 laboratory tests:
   - Thyroid function tests (TSH, free T4, total T4, total T3)
   - Complete blood count (CBC)
   - Comprehensive metabolic panel (CMP) with calcium
     Provide clear instructions on how to schedule the blood draw and where to submit the samples (Northwell labs). Answer any questions the participant/parent/guardian may have about the laboratory procedures.
4. Adverse Event Inquiry: Inquire about any potential adverse events experienced since the Baseline Visit. Document any reported adverse events using standardized terminology and follow appropriate reporting procedures as outlined in the study protocol. If there are clinically significant reports, contact the study physician.
5. Answer Questions and Provide Support: Address any questions or concerns the participant/parent/guardian may have about the study. Provide ongoing support and encouragement.
6. Confirm Next Contact: Confirm the date and time of the next scheduled in person follow-up visit (Week 12).

### 4.3 Visit 2 (Week 12/3month study visit)

**Purpose:** This visit allows for a comprehensive assessment of the participant's progress, including medication adherence, thyroid function, vitamin D levels, safety labs, and any potential adverse events. The visit will be combined with a formal outpatient visit that is normally scheduled at this timeframe.

**Window:** The Week 12 visit should ideally occur within a +/- 1-week window (i.e., between Week 11 and Week 13) to allow for flexibility in scheduling. Document the actual visit date.

**Procedures:**

1. **Medication Review and Adherence Assessment:**
   1. Review the participant's medication adherence using pill counts, medication logs, and participant/parent reports.
   2. Address any reported adherence issues and provide appropriate guidance and support.
   3. Refer to study clinicians for clinically significant deviations from the protocol or any other issues/concerns requiring physician input.
2. **Physical Examination:**
   Documented height, weight, and vital signs (blood pressure, heart rate).
   Document any relevant physical findings.
3. **Laboratory Testing:** Obtain the following laboratory tests:

- Thyroid function tests (TSH, free T4, total T4, and total T3)
- 25-hydroxyvitamin D level
- Complete blood count (CBC)
- Comprehensive metabolic panel (CMP) including calcium

4. **Adverse Event Assessment:**
    - Systematically inquire about any adverse events experienced since the last visit (Week 6 phone call).
    - Document the onset, duration, severity, and relationship to study medications for each reported adverse event.
    - Follow appropriate reporting procedures as outlined in the study protocol for all adverse events and contact study clinicians as needed.
5. **Concomitant Medication Review:** Inquire about any new medications or changes to existing medications since the last visit. Document all current medications.
6. **Answer Questions and Provide Support:** Address any questions or concerns the participant/parent/guardian may have about the study. Provide ongoing support and encouragement.
7. **Schedule Next Visit/Contact:** Schedule the next follow-up visit (24 weeks / 6 months) and remind participants about the procedures to be conducted at that visit. Provide contact information for the study team in case of any questions or concerns.

**4.4 Visit 3 (24 weeks/ 6 months: Final study visit)**

1. **Purpose**: This visit marks the conclusion of the 6-month study period. The purpose is to collect final data on thyroid function, antibody levels, vitamin D status, safety labs, and any potential adverse events.

1.3 **Visit Window**: The 24 weeks / 6-month visit should ideally occur within a +/- 2-week window (between Week 22 and Week 26) to allow for flexibility in scheduling. Document the actual visit date.

2. Procedures:
    1. **Medication Review and Adherence Assessment:**
        - Conduct a final review of medication adherence for both methimazole and vitamin D (if applicable): Utilize pill counts, medication logs, and participant/parent reports.
        - Collect any remaining unused study medication (vitamin D)
    2. **Physical Examination:**
        - Measure height, weight, and vital signs (blood pressure, heart rate, respiratory rate).
        - Document any relevant physical findings.
    3. **Laboratory Testing: Obtain the following laboratory tests:**
        - Thyroid function tests (TSH, free T4, total T4, and total T3)
        - TSH receptor antibodies (TRAb) and Thyroid Stimulating Immunoglobulin (TSI)
        - 25-hydroxyvitamin D level
        - Complete blood count (CBC)
        - Comprehensive metabolic panel (CMP)
    4. **Adverse Event Assessment:**
        - Conduct a thorough assessment of any adverse events experienced since the last visit (Week 12 visit).
        - Document all adverse events, including their onset, duration, severity, and relationship to study medications using standardized terminology.
        - Follow the study's adverse event reporting procedures.
    5. **Concomitant Medication Review:** Inquire about any changes to concomitant medications since the last visit. Document all current medications.
    6. **Study Completion Procedures:**
        - Thank the participant and their family for their participation in the study.
        - Provide contact information for the study team in case of any future questions or concerns.

**4.5 Visit 4**

N/A

**4.6 Unscheduled Visits**

**Handling of Unscheduled Visits:**

1. **Contacting the Study Team:** Participants will be instructed to contact the study team or their primary endocrinologist immediately if they experience any significant adverse events, worsening of their Graves' disease symptoms, or have any other concerns that necessitate an unscheduled visit. Clear contact information (phone number, email address) for the study team will be provided to all participants at the Baseline/Screening visit.
2. **Triage and Assessment:** Upon contact, the study team will triage the situation to determine the urgency and nature of the visit. This may involve phone consultation with the study physician to determine the appropriate course of action. Depending on the reason for the

unscheduled visit, the study team will guide the participant and may recommend seeking immediate medical attention if the situation warrants it.

3. **Documentation:** All unscheduled visits will be documented in the participant's study record, including the date and time of the visit, the reason for the visit, any assessments performed, and any actions taken.

**Assessments During Unscheduled Visits:**

The specific assessments performed during an unscheduled visit will depend on the reason for the visit. Possible assessments include:

- **Physical Examination:** If the participant presents for an in-person unscheduled visit, a physical examination may be conducted, focusing on relevant systems based on the participant's reported symptoms or concerns.
- **Laboratory Testing:** Laboratory tests (e.g., thyroid function tests, vitamin D levels, CBC, CMP including calcium) may be ordered as needed to evaluate the participant's condition and assess for potential adverse events.
- **Adverse Event Assessment:** A thorough assessment of any reported adverse events will be conducted, including their onset, duration, severity, and relationship to study medications. Appropriate reporting procedures will be followed for all adverse events.
- **Medication Review:** The participant's current medications, including study medications and any concomitant medications, will be reviewed.
- **Other Assessments:** Other assessments may be performed as deemed clinically necessary by the study physician.

**Study Discontinuation Due to Unscheduled Visits:**

In some cases, an unscheduled visit may result in study discontinuation. This could occur if:

- The participant experiences a serious adverse event that necessitates discontinuation of study medication.
- The participant's condition worsens significantly, requiring alternative treatment strategies.
- The participant is non-compliant with study procedures.
- The participant withdraws consent.

### 4.7 Early Termination Study Visit

Participants may withdraw from the study at any time, for any reason, without penalty. Every effort will be made to understand the reason for withdrawal, but the participant is not obligated to provide a reason.

**Procedures for Early Termination:**

Ideally, participants who withdraw from the study will complete an Early Termination Visit. The procedures conducted during this visit will be adapted based on the timing of withdrawal and the participant's willingness to undergo further assessments. The following procedures will be attempted, whenever feasible:

1. **Contact Information and Reason for Withdrawal:** Document the date of withdrawal and attempt to contact the participant/parent/guardian to confirm the withdrawal and, if possible, ascertain the reason for withdrawal (e.g., adverse events, lack of efficacy, personal reasons). Respect the participant's decision if they decline to provide a reason.
2. **Medication Reconciliation:** Inquire about any remaining study medication (vitamin D) and advise on appropriate disposal. This can be evaluated via phone.
3. **Final Laboratory Testing (Ideal, but not required):** If the participant is willing, the study team will request that they undergo final laboratory testing, mirroring the tests performed at the 24 weeks / 6-month visit (thyroid function tests, TRAb/TSI, 25-hydroxyvitamin D, CBC,

CMP including calcium. This is to capture the participant's status at the time of withdrawal. Explain that their primary endocrinologist would be drawing these labs at that time frame except for vitamin D. While the vitamin D level will not be required, it will be encouraged.

4. **Adverse Event Assessment:** Inquire about any adverse events experienced since the last study visit, even if these events are not believed to be related to the study. Document all reported adverse events using standardized terminology. It is especially important to capture AEs in early withdrawal visits in case they provide insights as to why the patient withdrew.
5. **Final Interview (Optional):** A brief interview may be conducted (with appropriate consent) to gather feedback on the participant's experience in the study. This information can be valuable for improving future research protocols. This could be done via phone.
6. **Referral and Follow-up Care:** Ensure that the participant has appropriate follow-up care for their Graves' disease with their primary endocrinologist. Remind the participant of the importance of continued monitoring and treatment. Document these referrals and recommendations in the participant's study file.

**If a participant withdraws and declines an Early Termination Visit:**
If a participant withdraws from the study and declines any further contact or assessments, document the date of withdrawal and the reason provided (if any). No further study-related procedures will be conducted.

### 4.8 Subject Discontinuation and Withdrawal

**Discontinuation of Study Intervention:**
Participants may be discontinued from the study intervention (vitamin D supplementation) for the following reasons:

- **Hypervitaminosis D:**
  - **Monitoring Test:** Vitamin D levels measured at study visits (baseline, 12 and 24 week) and at any unscheduled visits.
  - **Clinical Decision Point:** If a participant's serum 25-OH vitamin D level >80 ng/mL at any study visit, Vitamin D (ergocalciferol) will be reduced to 1000 IU daily or temporarily discontinued and 25 hydroxy vitamin D level rechecked in 1 month. If unchanged or higher at that time, subject will be taken off therapy and followed until end of study period.
  - **Restarting Study Intervention:** After normalization of serum vitamin D to <60 ng/mL, vitamin D supplementation may be restarted per original protocol. The decision to restart and the adjusted dose will be made in consultation with the study physician.
- **Hypercalcemia:**
  - Monitoring Test: Serum calcium levels measured at each study visit (baseline, weeks 6, 12, 24 weeks) and at any unscheduled visits.
  - Clinical Decision Point: If a participant's serum calcium level exceeds the upper limit of normal (>10.5 mg/dL corrected for albumin levels), additional screening will be done for symptoms of hypercalcemia. If the participant complains of symptoms concerning for hypercalcemia: hypotonia, poor feeding, vomiting, constipation, severe abdominal pain, lethargy, polyuria, dehydration, failure to thrive; Vitamin D will be held until the serum calcium level returns to within the normal range. If no additional symptoms are experienced and the calcium levels are <11.0 mg/dL, therapy may be continued with repeat calcium levels repeated every 1-2 weeks until normalization under 10.6 mg/dL

    - Restarting Study Intervention: After normalization of serum calcium, vitamin D supplementation may be restarted. The decision to restart and the adjusted dose will be made in consultation with the study physician. If hypercalcemia recurs, the vitamin D supplementation will be permanently discontinued.
- **Other Adverse Events:** Vitamin D supplementation may be temporarily or permanently discontinued if the participant experiences other adverse events deemed related to the study medication and clinically significant by the study physician. Please see appendix for list of adverse events.
- **Non-compliance:** If a participant consistently fails to adhere to the prescribed vitamin D regimen, they may be discontinued from the study intervention. For details see section 6.3.5.
- **Participant Request:** Participants may choose to discontinue the vitamin D supplementation at any time for any reason.

**Data Collected at the Time of Discontinuation:**

At the time of discontinuation of the study intervention, the following data will be collected:

- Date of discontinuation
- Reason for discontinuation
- Current medications
- Recent laboratory results (including serum calcium, 25-hydroxyvitamin D, CBC, and CMP)
- Assessment of any adverse events
- Concomitant medications and medical conditions
- Participant-reported outcome measures (as applicable)

**Withdrawal of Subjects:**

Participants may withdraw from the study entirely at any time, for any reason, without penalty or prejudice to their future medical care. The procedures for early termination (described in Section 4.7) will be followed.

**Provision of Care After Withdrawal:**

Following withdrawal from the study or discontinuation of the study intervention, participants will be continuing care with their primary endocrinologist for their Graves' disease. The study team will provide any necessary information or documentation for the participant's medical records. Participants will continue to receive standard medical care for their condition, regardless of their study participation status.

### 4.9 Lost to Follow Up

A participant will be considered lost to follow-up if they meet all the following criteria:

1. **Missed Visit:** The participant fails to attend a scheduled study visit (Week 12 or week 24) or complete a required phone call (Week 6) and does not contact the study team to reschedule within a defined timeframe. Rescheduling and completing the required visit within a week will be appropriate for study purposes.
2. **Unreachable After Multiple Attempts:** The study team has made multiple documented attempts to contact the participant using various methods. These attempts should include:
    - **Phone calls:** At least three phone calls at different times of the day and on different days of the week to the participant's primary contact number and any alternative contact numbers provided. Document the date, time, and outcome of each call (e.g., no answer, left voicemail, spoke with participant).
    - **Other contact methods:** Consider other contact methods such as sending a certified letter to the participant's last known mailing address. Document any such attempts.
3. **No Response:** The participant does not respond to any of the study team's contact attempts within a reasonable timeframe (within 1 week of the initial missed visit/call).

**Actions to be Taken for Missed Visits/Calls:**

- **Prompt Contact:** The study team will attempt to contact the participant as soon as possible after a missed visit/call to reschedule and determine the reason for the missed appointment.
- **Counseling:** The study team will counsel the participant/parent/guardian on the importance of adhering to the study visit schedule and the potential impact of missed visits on the study results.
- **Assessment of Continued Participation:** The study team will assess whether the participant still wishes to and/or should continue in the study. If the participant expresses a desire to withdraw, the procedures for early termination (Section 4.7) will be followed.
- **Documentation:** All contact attempts and their outcomes will be carefully documented in the participant's study record. Also, any actions taken (e.g. rescheduling a visit) should be clearly documented.

**Consequences of Being Lost to Follow-Up:**

Once a participant is deemed lost to follow-up, they will be considered withdrawn from the study. Their data will be handled according to the study's data analysis plan. Every effort will be made to prevent participants from becoming lost to follow-up.

# 5 STUDY PROCEDURES AND EVALUATIONS

### 5.1 Eligibility Assessments

The screening process will follow these steps:

1. **Initial Contact and Information Provision:** Potential participants will be identified through clinic referrals. Study staff will provide information about the study and answer any initial questions at the time of their initial visit in the clinic.
2. **Screening Consent:** If the participant is interested, they will be asked to sign a screening consent form.
3. **Medical History Review:** The investigator or co-investigator will review the participant's medical history to assess for any pre-existing conditions or medications that may meet exclusion criteria.

4. **Physical Examination:** A brief physical exam will be conducted by a qualified medical professional (physician, nurse practitioner, or physician assistant) to assess overall health and measure height, weight, and vital signs.
5. **Laboratory Screening:** If the participant meets initial eligibility criteria based on medical history and physical exam, laboratory tests will be ordered (thyroid function tests, TRAb/TSI antibodies, 25-hydroxyvitamin D, CBC, CMP including calcium).
6. **Eligibility Determination:** The study physician will review all screening data (medical history, physical exam, and laboratory results) to make a final determination of eligibility. Participants meeting all inclusion criteria and none of the exclusion criteria will be deemed eligible.
7. **Informed Consent/Assent:** Eligible participants will be asked to provide written informed assent (if 9-17 years old), along with parental/guardian consent. The study coordinator will review the consent form in detail with the participant/parent/guardian, answer questions, and ensure understanding of the study procedures, risks, and benefits

**5.2 Efficacy and Safety Assessments**

- **Study Intervention Administration:** Vitamin D supplementation (50,000 IU ergocalciferol weekly for 8 weeks, then bi-weekly for another 16 weeks), and Methimazole as prescribed per current standard of care.
- **Follow-Up Procedures:**
    - **Radiographic or other imaging assessments: N/A**
    - **Physical Examinations:** Conducted by a qualified medical professional (physician, nurse practitioner, or physician assistant) at baseline and all subsequent in-person visits (Week 12 and 24 weeks). Physical examinations will include assessment of height, weight, vital signs, and overall health. Any new or worsening physical findings will be documented and evaluated by the study physician.
    - **Laboratory Evaluations:** Blood will be collected at baseline and at weeks 6, 12, and 24, and at any unscheduled visits as clinically indicated. All laboratory testing will be performed at a CLIA-certified laboratory (Northwell Lab). The study physician will review all labs. Except vitamin D levels, all other laboratory testing is considered standard of care for treatment and monitoring of Graves' Disease response to methimazole.
        - **Clinical Laboratory Tests:**
            - Hematology: CBC with differential: frequency of monitoring determined by the patient's primary endocrinologist
            - Complete Metabolic Panel: at baseline, 6, 12 and 24 weeks
            - Thyroid Function Tests: TSH, free T4, total T4, total T3 at baseline, 6, 12 and 24 weeks
            - TSH Receptor Antibodies (TRAb) and Thyroid Stimulating Immunoglobulin (TSI) at baseline and 24 weeks.
            - 25-hydroxyvitamin D Level at baseline, 12 and 24 weeks
        - **Research Laboratory Tests: None**
        - **Genetic Testing.** None
        - **Pregnancy Testing**: Only performed if applicable
        - **Specimen Handling:** Specific instructions for specimen collection, handling, storage, and shipment will be detailed in the study's Manual of Operating Procedures (MOP).
    - **Medication Adherence:** Assessed at each visit (baseline, 6, 12 and 24 weeks) through pill counts, medication logs, and participant/parent self-report.

- **Adverse Event Assessment:** Assessed at every study visit and phone calls. Participants will also be instructed to report any adverse events to the study team between visits.
- **Medical Record Review:** Relevant medical history, including the history of Graves' disease, concomitant conditions, and medication history, will be abstracted from the participant's electronic medical record.
- **Questionnaires/Patient-Reported Outcomes:** medication and supplementation adherence logs
- **Patients will be provided with their test results.**

### 5.3 Assessment of Adverse Events.

Adverse events will be assessed at every study visit (baseline, 6, 12, and 24 weeks) and during any unscheduled visits or phone calls. Details regarding adverse event documentation, grading, and reporting will be provided in Section 8.

### 5.4 Other Assessments

N/A

# 6 STUDY INTERVENTION

### 6.1 Treatment Regimen

**(1) Vitamin D Supplementation (Intervention Arm):**

- Drug: Ergocalciferol (Vitamin D2)
- Dose: 50,000 IU
- Route of Administration: Oral capsules
- Treatment Duration: 24 weeks total
- Loading Dose: 50,000 IU weekly for the first 8 weeks
- Maintenance Dose: 50,000 IU every two weeks for the remaining 16 weeks.
- Dose Modification Criteria: See section 4.8 for details on managing hypervitaminosis D or hypercalcemia, which may necessitate dose adjustments or discontinuation of vitamin D.

**(2) Methimazole (Both Arms):**

- Drug: Methimazole
- Dose: Standard dosing per established pediatric guidelines for Graves' disease (typically weight-based). Initial and follow-up doses are determined by the treating physician.
- Route of Administration: Oral
- Treatment Duration: Ongoing, as determined by the treating physician, as part of standard Graves' disease management. Methimazole use during the 24-week study period will be recorded.
- Dose Modification Criteria: Dose adjustments are per standard clinical practice and not study directed. All adjustments will be recorded in the medical record.

**(3) Vitamin D Supplementation (Optional, Control Arm):**

- Drug: Cholecalciferol or Ergocalciferol
- Dose: Up to 1000 IU daily

- Route of Administration: Oral (tablets, capsules, or other commercially available forms)
- Source: Over the counter (OTC), obtained independently by participants
- Study Team Involvement: The study team will monitor vitamin D use via participant self-report in the medication adherence log. Data on the type, dose, and frequency of OTC vitamin D used will be collected. However, the study team will not provide vitamin D or manage any related adverse events.
- Multivitamins: If participants take a multivitamin containing vitamin D, the combined total daily intake from the multivitamin and any additional vitamin D supplements should not exceed 1000 IU.

### 6.2 Randomization and Blinding

- **Randomization:**
  1. **Allocation:** Participants will be randomized 1:1 to either the intervention arm (methimazole + high dose vitamin D) or the control arm (methimazole alone, with the option of taking up to 1000 IU/day of vitamin D2).
  2. **Method:** Randomization will be performed using the RedCap randomization tool.
  3. **Stratification:** Stratification will not be performed during the randomization process for this pilot study. However, data collected on baseline disease severity (based on Free thyroxine levels) and vitamin D deficiency status (deficient <20 ng/mL, insufficient 20-30 ng/mL, sufficient ≥30 ng/mL) will be carefully documented and used for stratification purposes in the design of a subsequent, larger clinical trial. This pilot study will inform the development of the stratification strategy for the larger trial, allowing for better balance between treatment groups and potentially increasing the power of the larger study to detect treatment effects. The information gathered regarding the distribution and impact of these variables will be crucial in optimizing the design of the future trial. While these factors won't be used for stratification in the current study, they will be considered during data analysis to assess their potential influence on the outcomes and to inform the stratification plan for the larger trial.
  4. **Implementation:** After providing informed consent/assent and completing the baseline assessment, the designated study staff member will access the randomization system to obtain the participant's assigned treatment group.
- **Blinding:**
  - Due to the nature of the intervention (vitamin D supplementation), complete blinding of participants and study staff is not feasible. Participants and clinicians will be aware of the treatment assignment due to the difference in supplementation regimens.
  - Blinding is deemed unethical in this study, as it would necessitate withholding vitamin D supplementation from participants with low levels, a practice considered unethical given the established standard of care and potential health benefits of vitamin D. Participants in the control group will have the option of taking low-dose (up to 1000 IU daily) vitamin D supplementation and even encouraged to, if they are severely vitamin D deficient.

### 6.2.1 Unblinding Procedures

Due to the nature of the intervention (vitamin D supplementation), complete blinding of participants and study staff is not feasible. Participants and clinicians will be aware of the treatment assignment due to the difference in supplementation regimens.

## 6.3 Procedures for Study Product

### 6.3.1 Intervention Description

This study involves two medications: vitamin D (ergocalciferol) and methimazole.

**(1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Market Status:** Commercially available.
- **Manufacturer:** Avet Pharmaceuticals. Please note the manufacturer may change depending on availability of the product.
- **Formulation:** Oral capsules. 50000 IU ergocalciferol
- **Packaging:** The study pharmacist will repackage the capsules into smaller bottles of 16 capsules each
- **Labeling:** Study-specific labels will be affixed to each dispensed bottle/package and will include:
    - Study name and number
    - Participant ID (to be filled by the study coordinator)
    - Drug name (Ergocalciferol)
    - Dosage and administration instructions (50,000 IU weekly for 8 weeks, then 50,000 IU every two weeks for 4 months)
    - Storage instructions
    - Dispensing date (to be filled by the study coordinator)
    - Expiration date
    - Study contact information
- **Manufacturing:** The commercially available vitamin D will be purchased from a reputable supplier and is manufactured in compliance with Good Manufacturing Practices (GMP).

**(2) Methimazole (Both Arms):**

- **Methimazole** will be prescribed by the participant's treating physician and dispensed by the participant's usual pharmacy as part of their standard medical care. The study will not supply methimazole directly. Methimazole prescribing and management will be per standard of care and usual clinical practice and not dictated by the study protocol.

**(3) Vitamin D2 (Optional, Control Arm):**

- Participants in the control arm will be informed that they may take standard, OTC vitamin D2 supplements at a dose of up to 1000 IU per day, if desired. This is optional and will not be supplied by the study. Participants will be instructed to follow the manufacturer's instructions on the OTC product label. If vitamin D levels are below recommendations, vitamin D2 at 1000 IU daily may be recommended, but this is not mandated.

***Devices:***

- *N/A*

### 6.3.2 Procurement

**1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Provider:** The study's CCMC Pediatric Research Pharmacy will procure the commercially available vitamin D (ergocalciferol) from a reputable, licensed wholesale distributor/supplier.
- **Requesting Product:** The Pediatric Research Pharmacy will maintain an adequate supply of vitamin D and will manage inventory. Study staff will submit requests to the pharmacy through secure Northwell e-mail and order forms.
- **Recipient:** The Pediatric Research Pharmacy will receive the bulk supply of vitamin D. The pharmacy will then dispense the vitamin D to individual participants according to study protocol and maintain appropriate records.

**(2) Methimazole (Both Arms):**

- **Procurement and Dispensing:** Methimazole is standard of care and will not be procured or dispensed by the research pharmacy. Participants will obtain methimazole through their usual pharmacy, as part of their routine medical care. The study team will not be involved in the procurement or dispensing of methimazole. This is important to emphasize for the IRB.
- **Study Responsibilities:** The treating physician will document the dose, frequency, and any changes to the methimazole regimen during the study period, as this is important clinical information.

**(3) Vitamin D2 (Optional, Control Arm):**

- **Procurement:** Vitamin D2 (up to 1000 IU daily) is optional for the control arm, available OTC, and will be obtained by participants independently, if desired and if recommended by their physician for low vitamin D levels. The study is not involved in the procurement, dispensing, or management of vitamin D2 in the control arm.

### 6.3.3 Storage

**1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Storage Location:** The Pediatric Research Pharmacy will store the bulk supply of vitamin D in a dedicated, secure, and temperature-controlled area within the pharmacy.
- **Temperature Requirements:** The vitamin D will be stored according to the manufacturer's labeled storage recommendations: 25°C (77° F), excursions permitted between 15°-30° C
- **Security:** The vitamin D storage area within the pharmacy will be secure and locked to prevent unauthorized access. Access will be limited to authorized pharmacy personnel. Once dispensed to the study personnel, the vitamin D will be stored in a secured cabinet under key.

**(2-3) Methimazole (Both Arms) and Vitamin D2 (Optional, Control Arm):**

- As previously stated, these are not handled by the study team or research pharmacy, and therefore no study-specific storage procedures apply. Participants are responsible for storing their own medications according to the manufacturer's instructions.

### 6.3.4 Preparation and Administration of IP

**(1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Preparation:**
  - **Location:** Vitamin D will be dispensed to the study staff by the Pediatric Research Pharmacy. No further preparation (e.g., mixing, reconstitution) is required for the commercially available oral capsules.
  - **Documentation:** The pharmacy will maintain records of all dispensing activities, including date dispensed, lot number, and quantity dispensed. The study staff will provide logs to the research pharmacy regarding participant ID, date dispensed, lot number, and quantity dispensed.
  - Per the Northwell policy and federal regulations, the investigational product label must display the below language:
    - "Caution: investigational use only"

- **Administration:**
  - **Route:** Oral
  - **Schedule:**
    - **Initial Phase:** 50,000 IU once weekly for 8 weeks.
    - **Maintenance Phase:** 50,000 IU every two weeks for the remaining 4 months.
  - **Timing:** Participants will be instructed to take their vitamin D dose on the same day each week, at a time convenient for them. No specific time of day is required.
  - **Instructions to Participants:** Participants will receive clear written and verbal instructions on how and when to take their vitamin D. They will be encouraged to establish a routine to aid adherence. Written instructions will be provided as well.
  - **Safety Precautions:** Participants will be instructed to contact the study team immediately if they experience any adverse events, particularly symptoms of hypercalcemia (nausea, vomiting, constipation, increased thirst, increased urination, muscle weakness, confusion).
  - **Expiration:** Participants will be instructed to check the expiration date on the vitamin D bottle/package and to notify the study team if the medication is expired. The Pediatric Research Pharmacy will ensure that dispensed vitamin D has a sufficient remaining shelf life.

**(2) Methimazole (Both Arms):**

- **Preparation and Administration:** Methimazole preparation and administration will be directed as part of standard medical care by the treating physician and patient. The study will not dictate these procedures. The dose, frequency, and any adjustments to the methimazole regimen will be documented by the study team, but the study does not provide specific instructions regarding methimazole.

**(3) Vitamin D2 (Optional, Control Arm):**

- The study team will not provide any instructions on taking vitamin D2. Participants may choose to take commercially available vitamin D2 and will be instructed to follow the manufacturer's instructions.

### 6.3.5 Subject Compliance Monitoring

**(1) Vitamin D (Ergocalciferol): Intervention Arm**

The following methods will be used to assess and track participant compliance with the vitamin D supplementation regimen:

- **Pill Counts:** At each study visit (baseline, 12 and 24 week) study staff will count the remaining vitamin D capsules to estimate the number of doses taken.
- **Medication Logs:** Participants/parents will be provided with medication and adherence logs to record each dose of vitamin D taken. They will be instructed to bring the completed log to each study visit.
- **Self-Report:** At each study visit and during the Week 6 phone call, study staff will ask participants/parents about their vitamin D intake to confirm adherence.

**Procedures for Non-Compliance:**

- **Definition of Significant Non-Compliance:** Significant non-compliance will be defined as missing more than two doses of vitamin D during the study period.
- **Actions for Significant Non-Compliance:**
    1. **Contact and Counseling:** If significant non-compliance is identified, the study coordinator will contact the participant/parent to discuss the reasons for non-compliance and reinforce the importance of adherence to the study protocol.
    2. **Problem-Solving:** The study coordinator will work with the participant/parent to identify and address any barriers to adherence (e.g., difficulty remembering to take the medication, side effects, concerns about the medication). Strategies may include providing medication reminders, adjusting the dosing schedule (if feasible and with physician approval), or addressing any misconceptions about the study medication.
    3. **Documentation:** All instances of non-compliance, discussions with the participant, and actions taken will be documented in the participant's study record.
    4. **Discontinuation from Study Intervention (if necessary):** If non-compliance persists despite interventions, the participant may be discontinued from the vitamin D supplementation portion of the study but should remain in follow-up for the remainder of the study duration to allow for data collection for the primary study outcome. The rationale for this approach should be clearly explained to the IRB, e.g., to preserve the integrity of the primary outcome analysis, to maintain monitoring of thyroid function, and to assess potential long-term impacts of the study. This is a nuanced point that the IRB is likely to question.
- **Withdrawal from Study**: If the participant chooses to discontinue from the study completely due to non-compliance issues, they will be able to do so without penalty. The early termination visit procedures outlined in section 4.7 will be followed.

**Mandatory Documents and Source Documents for Compliance Calculation:**

- **Mandatory Documents:**
    - Medication logs completed by participants/parents

- **Source Documents:**
    - Pill count records
    - Medication and Adherence logs
    - Notes from discussions with participants/parents regarding adherence

The primary source document for calculating study intervention compliance will be the pill count records, supported by medication logs and participant self-report.

### 6.3.6 Accountability

**(1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Responsibilities of Investigators and Designees:** The following personnel will have designated responsibilities for drug accountability:
    - **Principal Investigator (PI) or co-investigators:** Overall responsibility for ensuring compliance with the drug accountability procedures outlined in the protocol. Responsible for dispensing vitamin D to participants at the study visits. To facilitate participant convenience, given the study site's location separate from the hospital, the PI or the co-investigator will dispense study medication directly to participants at the clinic. This reduces participant burden by eliminating the need for an additional trip to the hospital pharmacy.
    - **Research Pharmacist:** Responsible for receiving, storing, dispensing, and maintaining inventory records of the vitamin D.
    - **Study Coordinator:** Collecting returned unused medication, documenting administration, and maintaining participant-specific drug accountability records.
- **Drug Life-Cycle Accountability:**
    - **Delivery to Site:** Upon delivery to the Pediatric Research Pharmacy, the pharmacist will verify the shipment, document the receipt (date, quantity, lot number, expiration date), and store the vitamin D according to the designated storage procedures.
    - **Dispensing to Participants:** While the main supply of vitamin D will be stored and managed by the Pediatric Research Pharmacy, a limited supply of study drug will be kept on hand at the study site for dispensing to participants to facilitate study visits. The study site will maintain a detailed inventory log of all vitamin D received from the pharmacy and dispensed to participants. This log will include the participant ID, date dispensed, quantity dispensed, lot number, and the initials of the study staff member dispensing the medication. The study site's inventory will be reconciled with the pharmacy's inventory monthly to ensure accountability. The study coordinator will be responsible for requesting additional vitamin D from the pharmacy when needed.
    - **Participant Use and Documentation:**
        - Participants will be instructed to record each dose of vitamin D taken in a participant drug log/diary (Medication Adherence log). The log should include date and time of dose, quantity taken, and any missed doses or other relevant information (e.g., adverse events).

        - Study coordinators will review participant drug logs at each study visit to monitor compliance and address any questions or concerns.
        - At each visit, pill counts will be performed to verify adherence and reconcile with the participant's drug log.
    - **Return of Unused Product:** Participants will be instructed to return any unused vitamin D to the study team at the end of their participation or at any time they discontinue the study intervention. Study staff will count and document the returned medication and its disposition.
    - **Final Disposition:** All unused or expired vitamin D will be returned to the Pediatric Research Pharmacy for disposal according to institutional policies and applicable regulations. The pharmacy will maintain records of drug destruction.
- **Documentation:**
    - **Pharmacy Inventory Logs:** The pharmacy will maintain detailed logs of all vitamin D received, dispensed, returned, and destroyed.
    - **Participant Drug Logs/Diaries:** Participants will document each dose taken in a provided Medication Adherence and Supplementation logs
    - **Dispensing Records:** The pharmacy will maintain dispensing records for each participant, including the date, quantity, and lot number dispensed.

**(2) Methimazole (Both Arms) and Vitamin D2 (Optional, Control Arm):**

- As previously stated, these medications are not part of the study's drug accountability procedures. Participants will obtain methimazole through their usual prescriptions, and vitamin D2 is an optional OTC supplement. The study team will document the methimazole dose and any adjustments during the study, but no formal accountability procedures apply. This is important to state clearly for the IRB.

### 6.3.7 Return or Destruction of IP

1) Vitamin D (Ergocalciferol): Intervention Arm

- **Final Reconciliation:** At the conclusion of the study (after the final participant's last visit/follow-up), a final reconciliation of vitamin D will be performed. This will involve:
    1. **Study Site Reconciliation:** The study team will account for all vitamin D dispensed to participants. This includes vitamin D dispensed directly at the study site and any remaining study drug returned by participants.
    2. **Pharmacy Reconciliation:** The Pediatric Research Pharmacy will perform a final inventory reconciliation, accounting for all vitamin D received from the supplier, dispensed to the study site or directly to participants, and returned from the study site or participants.
    3. **Comparison and Discrepancy Resolution:** The study site's and pharmacy's records will be compared to ensure agreement. Any discrepancies will be thoroughly investigated and documented. The PI or co-investigator, research pharmacist, and study coordinator will collaborate to resolve any discrepancies before the final disposition of the study drug. A written report of the discrepancy and resolution should be filed with the study documentation.
- **Return or Destruction:**

- **Expired/Damaged Vitamin D:** Any expired or damaged vitamin D will be returned to the Pediatric Research Pharmacy for destruction.
- **Unused Vitamin D:** At the study's conclusion, all unused vitamin D at both the study site and the research pharmacy will be destroyed according to the research pharmacy's procedures.

- **Documentation:**
  - **Drug Accountability Logs:** All transactions (receipt, dispensing, return, destruction) will be documented in the designated drug accountability logs maintained by both the study site and the research pharmacy. These logs must include dates, quantities, lot numbers, participant IDs (for dispensed medication), and the initials of the personnel involved in each transaction.
  - **Destruction Records:** If vitamin D is destroyed, the research pharmacy will maintain detailed destruction records, including the date of destruction, method of destruction, quantity destroyed, lot numbers, and the witness signature(s) of authorized personnel. Provide the template or specify the location where this record can be found in study materials for IRB review.
  - **Final Reconciliation Report:** A final reconciliation report will be generated, summarizing the overall drug accountability for the study. This report should document the initial quantity of vitamin D received, the quantity dispensed, the quantity returned, the quantity destroyed, and the final disposition of the study drug. The report must be signed and dated by the PI and research pharmacist.

**(2) Methimazole (Both Arms) and Vitamin D2 (Optional, Control Arm):**

These medications are not subject to the study-specific return/destruction procedures. Methimazole is handled as part of routine patient care, and optional vitamin D2 is an OTC product obtained independently by participants. The study team has no responsibility for the disposition of these medications. It's important to clearly reiterate this distinction for the IRB.

### 6.4 Prior and Concomitant Therapy

This section outlines permitted and prohibited concomitant medications/therapies during the study. It aligns with the medication restrictions specified in the inclusion/exclusion criteria.

- **Data Collection Time Points:** Information on prior and concomitant medications will be collected at the following time points:
  - Screening Visit: A comprehensive medication history, including all current and past medications (prescription, over the counter, and supplements), will be obtained.
  - All Subsequent Study Visits (baseline, 6, 12, and 24 week) and at any unscheduled visits: Participants/parents will be asked about any changes to their medication regimen, including new medications, discontinued

medications, and changes in doses. This includes recording standard of care methimazole regimens and noting any dose adjustments/changes.

- **Prior and Concomitant Medical Therapy to be Collected:** As this study focuses on Graves' disease in the pediatric population and vitamin D, all prior and concomitant therapies will be recorded, but the study team will focus on medications/supplements that might affect vitamin D or calcium levels, interact with vitamin D or methimazole, or affect thyroid function. *The study team will pay particular attention to calcium, vitamin D (including OTC supplements), medications that affect bone metabolism, and other thyroid-related medications.*
- **Permitted Concomitant Medications/Therapies:**
    - **Medications for Co-existing Conditions:** Participants are permitted to take medications for pre-existing medical conditions (e.g., asthma, allergies) if these medications are deemed safe by the study physician and do not meet the exclusion criteria. For any permitted medication, the rationale will be clearly documented.
    - **Over-the-Counter Medications (OTC):** Occasional use of OTC medications (e.g. analgesics, antipyretics, antihistamines, antacids) is permitted, provided they are used according to standard dosing guidelines and do not meet the exclusion criteria. Participants should report their use of OTC medications to the study team at each visit. The control group may take OTC vitamin D2
    - 1000 IU/day if desired. Total amount of OTC vitamin D that can be taken is not to exceed 1000 IU/day which may include multivitamin supplements.
- **Prohibited Concomitant Medications/Therapies.**
    - **Vitamin D Supplementation (Intervention Arm):** Participants in the intervention arm are prohibited from taking any other vitamin D supplements, including OTC vitamin D, other than the study-provided ergocalciferol.
    - **Medications Affecting Calcium or Bone Metabolism:** Medications known to significantly affect calcium or bone metabolism (e.g., high-dose calcium supplements, bisphosphonates, thiazide diuretics) are prohibited unless deemed medically necessary by the study physician.
    - **Other Thyroid Medications:** Any medications, other than methimazole, used to treat thyroid disorders (e.g., levothyroxine, propylthiouracil) are prohibited, as they could confound the study results.
    - **Other Excluded Medications:** Current use of medications known to significantly affect thyroid function will be prohibited (e.g. corticosteroids, amiodarone) or vitamin D metabolism (e.g., anticonvulsants, rifampin). These medications will also be prohibited during the study period.

- **Potential Effects of Permitted Concomitant Therapy and Assessment of Independent Effects: Drug-Drug Interactions:** The study physician will review all concomitant medications for potential drug-drug interactions with vitamin D or methimazole. If any potentially significant interactions are identified, the study physician will assess the risk/benefit of continued study participation and may recommend alternative therapies or dose adjustments.
- While detailed concomitant medication data will not be systematically collected in REDCap for this pilot study, information on key medications/supplements known to potentially affect vitamin D, calcium levels, or thyroid function (e.g., calcium

supplements, vitamin D supplements, medications affecting bone metabolism, other thyroid medications) will be documented in the medical record and summarized in the study record. This limited information will be used to describe potential confounding medications and to inform the development of a more comprehensive medication assessment strategy for the subsequent, larger clinical trial. Given the limitations of the medication data in this pilot, a full disentanglement of the independent effects of vitamin D from other medications may not be feasible. However, descriptive analyses of the use of these key medications/supplements will be performed and considered when interpreting the study results and planning the larger trial. The statistical analysis plan for the larger trial will include robust methods to address potential confounding from concomitant medications, based on the learnings from this pilot study.

### 6.5 Rescue Medication Administration

**1) Vitamin D (Ergocalciferol): Intervention Arm**

- **Hypervitaminosis D or hypercalcemia: Please see section 4.8 for dose adjustments in those clinical scenarios.**
- **Other Adverse Events:** If a participant experiences other adverse events deemed related to vitamin D and requiring intervention, the study physician will determine the appropriate course of action, which may include symptomatic treatment or discontinuation of vitamin D supplementation.

**(2) Methimazole (Both Arms)**

- Management of adverse events or inadequate response to methimazole is part of standard medical practice for Graves' disease and will be handled by the participant's treating physician, not the study. The study team will document any changes in methimazole regimen (dose, frequency), but these decisions will be made by the treating physician based on the participant's clinical condition and standard of care guidelines.

**(3) Vitamin D2 (Optional - Control Arm)**

- Participants in the control arm may choose to take OTC vitamin D2 supplements. The study team will not provide any specific recommendations or guidance for the management of adverse events potentially related to OTC vitamin D2 use. It is recommended that they follow the manufacturer's instructions or consult with their physician or pharmacist.

# 7 STATISTICAL CONSIDERATIONS

### 7.1 Brief Description of Study Design

This is an open label randomized clinical pilot feasibility trial. In this study, participants will be randomized to either the experimental (vitamin D + methimazole) or control (methimazole only) group at the time of the initial visit. The starting dose of methimazole and dose adjustments will be made by the treating endocrinologist based on established clinical practice guidelines. The control arm will receive no additional intervention other than methimazole (standard of care). While the control group will not receive the high-dose vitamin D intervention, they will be allowed to take up to 1000 IU of vitamin D daily. This approach balances the need for a

comparison group against the ethical responsibility of not withholding a potentially beneficial supplement for those with low levels.

### 7.2 Primary Endpoint(s)

This pilot study's primary objective is to assess the feasibility of conducting a larger randomized controlled trial (RCT) of high-dose vitamin D in pediatric Graves' disease (GD). Therefore, the primary "endpoints" of this pilot are feasibility metrics, not clinical outcomes. These metrics will inform the design and sample size calculation of the future, larger RCT. Specifically, the primary feasibility endpoints are:

- **(a) Recruitment Rate:** The proportion of eligible participants who consent/assent to participate in the study within the designated recruitment period March 2025-October 2025. This will be calculated as the number of enrolled participants divided by the number of eligible participants approached.
- **(b) Adherence Rate:** The proportion of prescribed vitamin D doses taken by participants in the intervention arm, as measured by pill counts at each visit, corroborated by medication logs and patient/parent self-report. This will help to determine the feasibility of achieving adequate adherence in a larger trial and help in evaluating the various adherence monitoring strategies employed.
- **(c) Data Completeness:** The proportion of completed data fields for pre-specified primary and secondary outcome measures in the final dataset. This will be assessed for all enrolled participants and will help identify potential challenges in data collection for a larger RCT. Data variability will also be evaluated as descriptive statistics to understand potential dispersion of these values.
- **(d) Barriers to Implementation:** A qualitative assessment of any challenges or obstacles encountered during the pilot study that could hinder the successful implementation of a larger-scale RCT. This assessment will be based on the experiences of the study team, feedback from participants/families, and any unforeseen logistical or operational issues encountered.

**Sample Size Justification:**

The sample size of 30 participants is considered adequate for this pilot study based on feasibility considerations and recommendations for pilot studies. This sample size is likely not powered to detect statistically significant differences in clinical outcomes, as the primary aim is to assess feasibility for a larger RCT. It is expected that this sample size will provide sufficient data to evaluate recruitment rates, adherence to the intervention, data completeness, and potential barriers to implementation, thus informing the design and sample size calculation of a subsequent, adequately powered RCT.

### 7.3 Secondary Endpoint(s)

**Exploratory Outcomes**

This pilot study will explore the following outcomes to generate preliminary data and inform the design of a future, larger randomized controlled trial. These are not formal secondary endpoints, as this pilot is likely not powered to detect statistically significant differences in these outcomes. The purpose of collecting this data is to observe trends, estimate effect sizes and their variability, and assess the feasibility of collecting these data in a larger trial.

- **Thyroid Function Normalization:** Time to normalization of thyroid function tests (T3, FT4, and T4) will be explored. Normalization will be defined as achieving age-appropriate normal ranges for T3, FT4 and T4 tests simultaneously. Time to normalization of TSH will be explored separately. Time to normalization will be calculated as the time from baseline to the first visit at which T3, FT4 and T4 tests are within normal limits and a separate TSH time

to normalization will be documented. The proportion of participants achieving normalization in each arm (methimazole alone vs. methimazole + vitamin D) will also be described. These exploratory analyses will inform the sample size calculation for a future, adequately powered trial investigating the impact of vitamin D on thyroid hormone normalization.

- **TSH Receptor Antibody (TRAb) and Thyroid Stimulating Immunoglobulin (TSI) Levels:** The percentage change from baseline in TRAb and TSI levels at 24 weeks will be explored. The percentage change will be calculated as [(24-week value - Baseline value) / Baseline value] * 100. The difference in percentage change between the two treatment arms (methimazole alone vs. methimazole + vitamin D) will be described. This information will inform the design of a future trial examining the impact of vitamin D on these antibody levels.

### 7.4 Safety Endpoints

Safety and tolerability of high-dose vitamin D are important considerations in this pilot study. All participants receiving at least one dose of vitamin D or methimazole will be included in the safety analysis. Adverse events (AEs) will be collected, coded, and analyzed as follows:

- **AE Definition:** An AE is any untoward medical occurrence temporally associated with the use of a medicinal product, whether or not related to that product. This includes abnormal laboratory findings, symptoms, and disease. AEs related to both vitamin D (study intervention) and methimazole (standard of care) will be documented. Methimazole AEs, while not study-related, will be monitored for participant safety and to distinguish them from potential vitamin D effects in this open-label study.
- **AE Coding:** AEs will be coded using MedDRA terminology (System Organ Class and PT).
- **AE Calculation and Presentation:** Each distinct AE will be counted once per participant. AE data will be presented by SOC and PT, including start/stop dates, severity (using CTCAE v [5.0]), relationship to vitamin D or methimazole (causality for methimazole AEs assessed per routine clinical practice), expectedness, outcome, duration, and any action taken regarding vitamin D dosing (with rationale).
- **AEs Leading to Discontinuation:** All AEs leading to vitamin D discontinuation will be reported. Discontinuation of vitamin D only, with continued study follow-up, will be distinguished from complete study withdrawal. Details, rationale, and outcome of AEs leading to vitamin D discontinuation will be documented.
- **Serious Adverse Events (SAEs):** All SAEs will be reported. An SAE is any untoward medical occurrence that: results in death; is life-threatening; requires or prolongs hospitalization; results in persistent disability/incapacity; is a congenital anomaly/birth defect; or is deemed serious by the study physician.
- **Treatment-Emergent AEs:** AEs occurring or worsening after the first dose of vitamin D will be analyzed.
- **Safety and Tolerability Assessment:** The safety and tolerability of high-dose vitamin D will be evaluated by monitoring all reported AEs, with particular attention to those potentially related to vitamin D or hypercalcemia (e.g., nausea, vomiting, constipation, polyuria, polydipsia).

### 7.5 Intention – To-Treat (ITT) and Per Protocol (PP) Analysis Sets

Data from this pilot study will be analyzed using an intention-to-treat (ITT) approach. The ITT analysis set will include all participants who are randomized to either the intervention arm

(vitamin D + methimazole) or the control arm (methimazole alone), regardless of whether they receive any study intervention (vitamin D) or complete all follow-up visits. This approach is consistent with the intention-to-treat principle, which aims to minimize bias by analyzing participants based on their assigned treatment group, rather than their actual treatment received. For this pilot, descriptive statistics for key feasibility and exploratory outcomes will be calculated for the entire ITT population. While a modified intention-to-treat (mITT) analysis set may also be used for certain exploratory outcomes requiring a minimum amount of follow-up data for meaningful interpretation (e.g. thyroid function or antibody levels), the primary analysis set for this pilot feasibility study is the ITT population. Any deviations from the ITT principle for individual exploratory analyses (e.g. use of mITT), will be pre-specified prior to data analysis and the rationale clearly documented in the statistical analysis plan accompanying study publication.

## 7.6 Statistical Methods

The primary objective of this pilot study is to assess the feasibility of conducting a larger RCT, not to test hypotheses about treatment efficacy. Therefore, the statistical analyses will primarily be descriptive. Inferential statistics will be used for exploratory analyses of clinical outcomes, but these analyses will focus on estimating effect sizes and variability to inform the design of the future RCT, rather than on drawing definitive conclusions about treatment effects.

### 7.7.1 Analysis of the Primary Endpoint

The primary "endpoints" of this pilot study are feasibility metrics. These will be analyzed as follows:

- **Recruitment Rate:** Calculated as the number of enrolled participants divided by the number of eligible participants approached. Presented as a percentage with 95% confidence intervals.
- **Adherence Rate:** Calculated as the percentage of prescribed vitamin D doses taken, based on pill counts corroborated by medication logs and self-report. Presented as a percentage with 95% confidence intervals. Different adherence monitoring strategies (pill counts, logs, phone calls) will be compared descriptively.
- **Data Completeness:** Calculated as the proportion of completed data fields for pre-specified outcome measures. Presented as a percentage. Variability in collected data will be described using descriptive statistics (means, standard deviations, interquartile ranges).
- **Barriers to Implementation:** Qualitative data on barriers will be summarized thematically.

**Missing data** for feasibility outcomes will be minimal as they are primarily process related. Any missing data will be documented and described.

### 7.7.2 Analysis of Secondary Endpoint(s)

Exploratory outcomes will be analyzed descriptively to understand trends and estimate effect sizes for the future RCT.

- **Thyroid Function Normalization:** For each thyroid hormone level and for TSH, logistic regression will be performed using a generalized linear mixed model

(GLMM) to simultaneously assess the odds of normalization at 6 weeks, 12 week, 24 weeks. GLMMs can account for correlations of measurements at different timepoints that are from the same patient. Logistic regression will be utilized to assess the odds of >50% decrease in antibody levels at 24 weeks from diagnosis. If the sample size is sufficient, we will adjust for disease severity at diagnosis in each model. If GLMMs are not feasible, analysis will be performed separately at each timepoint.

- **TRAb and TSI Levels:** A linear mixed model (appropriate data transformations may be applied) with an interaction term of group by time will be utilized to compare absolute and percentage changes in thyroid function (T4, FT4, T3) at 6, 12 and 24 weeks, vitamin D levels at 12 and 24 weeks months, and Graves' antibody levels (TSH-R Ab, TSI) at baseline and 24 weeks within participants in each group (treatment and control) from time of diagnosis and between groups at each time point. Separate models will be built for each thyroid function hormone (T4, FT4, T3), for vitamin D, and for each Graves' antibody (TSH-R Ab, TSI) for absolute and for percentage change. As with GLMMs, linear mixed models can account for correlations of measurements at different timepoints that are from the same patient.
- **Missing Data:** Missing data for exploratory outcomes will be addressed using simple imputation methods (e.g., last observation carried forward, mean imputation) for exploratory purposes only. The limitations of these methods and the potential impact of missing data will be discussed.

### 7.7.3 Analysis of Safety Endpoints (if applicable)

Safety endpoints will be analyzed descriptively. The incidence and severity of adverse events (AEs) will be summarized by treatment arm, system organ class, and preferred term. The relationship of AEs to study interventions will be assessed and described. Serious adverse events (SAEs) will be reported individually.

### 7.7.4 Pre-specified Subgroup Analyses

Subgroup analyses are not planned for this pilot study due to the small sample size and feasibility focus. The feasibility of conducting subgroup analyses in the larger RCT will be assessed based on the variability observed in this pilot.

### 7.7.5 Interim Analysis and Early Stopping Rules (if applicable)

No interim analyses or early stopping rules are planned for this pilot feasibility study.

### 7.7.6 Sample Size Justification

The sample size of 30 is based on feasibility considerations, anticipated recruitment rates, and recommendations for pilot studies. It is not based on a formal power calculation for clinical outcomes. This sample size is expected to be sufficient to assess feasibility, explore trends in the exploratory outcomes, and inform the design of a future, adequately powered RCT. The sample size is justified by the available patient volume and ensures adequate data can be collected to estimate effect sizes and their variability for the future RCT sample size calculation.

# 8 ADVERSE EVENT, SERIOUS ADVERSE EVENT AND UNANTICIPATED PROBLEM

## 8.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a participant temporally associated with the use of a medicinal product, whether or not related to that product. This includes abnormal laboratory findings, symptoms, intercurrent illnesses, and injuries. Abnormal diagnostic results are AEs if they result in study withdrawal, are associated with an SAE or clinical signs/symptoms, lead to additional treatment/tests, or are considered clinically significant by the investigator.

- **Specific Considerations:** In this pediatric study, special attention will be paid to age-appropriate AE manifestations and potential long-term effects. Parents/guardians will be actively involved in AE reporting. AEs associated with vitamin D (hypervitaminosis D, hypercalcemia) and methimazole (rash, itching, nausea, vomiting, joint pains, altered LFTs; see package inserts for expected AEs) will be monitored. AEs related to study procedures will also be documented.

## 8.2 Serious Adverse Events (SAE)

An SAE is any untoward medical occurrence that: results in death; is life-threatening (at the time of the event); requires or prolongs hospitalization; results in persistent disability/incapacity; or is deemed a serious important medical event.

- **Specific Considerations:** SAEs will be evaluated with attention to developmental stage and potential long-term impacts. Methimazole-related SAEs (e.g., agranulocytosis, hepatotoxicity, vasculitis), though related to standard of care, will be documented and reported.

## 8.3 Unanticipated Problems (UP)/ Unanticipated Device Effect (UADE)

A UP is any incident, experience, or outcome that is unexpected (given the research procedures and participant characteristics), related or possibly related to research participation, and suggests increased risk of harm. UADE is not applicable (no device).

- **Specific Considerations:** The threshold for reporting UPs may be lower in children. Unexpected AEs related to vitamin D or methimazole, breaches of confidentiality, and significant protocol deviations (e.g., delayed or missed doses of vitamin D, incorrect doses such as doses significantly over or under recommended, deviations from dose modification schedule listed in 4.8) will be evaluated as potential UPs. Serious non-compliance leading to increased risk (e.g., disallowed concomitant medications) may be reported as a UP.

## 8.4 Recording

AEs will be elicited at each visit/contact using open-ended and targeted questions, physical exams, and lab tests (including serum calcium). Documentation in source documents (medical record) will include date of onset, description, severity, relationship to interventions/procedures (methimazole causality assessed by treating physician), expectedness, outcome, and action taken/rationale. AE data will be entered into REDCap. All AEs will be assessed using CTCAE v5.0. The relationship of AEs to vitamin D will be categorized as Definitely, Probably, Possibly, Unlikely, or Not Related (see 8.5.2 for details).

### 8.5 Assessment

All AEs must be assessed by the study clinician using a protocol-defined grading system.

#### 8.5.1 Severity of Event

The CTCAE grading scale defines the following severity grades for AEs:

- **Grade 1 (Mild):** Mild adverse event; intervention not indicated.
- **Grade 2 (Moderate):** Moderate adverse event; limiting age-appropriate instrumental activities of daily living (IADL); intervention indicated.
- **Grade 3 (Severe):** Severe adverse event; limiting self-care ADL; hospitalization indicated.
- **Grade 4 (Life-Threatening):** Life-threatening consequences; urgent intervention indicated.
- **Grade 5 (Death related to AE):** Death related to adverse event.

#### 8.5.2 Relationship to Study Intervention

The relationship of an AE to a study intervention (vitamin D) will be assessed by the clinician who examines and evaluates the participant. This assessment will consider the following factors:

- Temporal Relationship: The time between the start of the study intervention (vitamin D administration) and the onset of the AE.
- Known Pharmacology/Toxicology: The known pharmacology and toxicology of vitamin D, including expected adverse effects and the biological plausibility of a causal relationship. The package insert will serve as a reference.
- Underlying Medical Conditions: The participant's underlying medical conditions and their potential contribution to the AE.
- Concomitant Medications: Concomitant medications, both prescription and over the counter, and their potential for interaction with vitamin D or for causing the AE independently.
- Dechallenge/Rechallenge (if applicable): The response of the AE to discontinuation (dechallenge) and reintroduction (rechallenge) of vitamin D, if ethically and clinically appropriate. This may not always be feasible or ethical.
- Other Potential Causes: Other potential causes of the AE, including intercurrent illnesses, environmental factors, or other study procedures.

  The relationship of the AE to the study intervention (vitamin D) will be categorized using the following terms:

- Definitely Related: Clear evidence suggests a causal relationship between vitamin D and the AE, and other contributing factors can be ruled out with reasonable certainty. The event occurs in a plausible time relationship to vitamin D administration and cannot be readily explained by other factors.
- Probably Related: Evidence suggests a causal relationship, and the influence of other factors is unlikely. The event occurs within a reasonable time after vitamin D administration and is unlikely to be attributed to other causes.
- Possibly Related: There is some evidence to suggest a causal relationship (e.g., a known association, a plausible temporal relationship). However, other factors may have also contributed.

- Unlikely Related: The temporal relationship and/or other factors make a causal relationship between vitamin D and the AE improbable. Other explanations are more likely.
- Not Related: The AE is clearly independent of vitamin D administration, and evidence supports an alternative etiology.

Relationship to Methimazole (Standard of Care):

For AEs potentially related to methimazole, the treating physician (not the study team) will determine the relationship to methimazole based on their clinical judgment and standard of care practice. This assessment will be documented in the participant's medical record and transcribed into the study database.

If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

### 8.5.3 Expectedness

Expectedness will be determined by the study physician based on package inserts and literature. Unexpected AEs are those not listed, more severe/frequent than listed, or different in nature than listed.

### 8.5.4 Time Period and Frequency for Event Assessment and Follow-Up

AEs/SAEs will be followed until resolution, stabilization, or non-related determination. Mild AEs: follow-up until resolution or next visit. Moderate AEs: weekly assessments until resolution/stabilization. Severe AEs/SAEs: frequent assessments, continued follow-up after study intervention discontinuation. Documentation in source documents and REDCap. Long-term follow-up ensured for unresolved events.

## 8.6 Reporting

### 8.6.1 Adverse Event (AE) Reporting

All AEs, regardless of causality or severity, will be documented as described in Section 8.5. While all AEs will be recorded, routine, expected AEs associated with Graves' disease itself, such as those related to fluctuating thyroid hormone levels (e.g., palpitations, anxiety, fatigue, heat intolerance) or the known effects of methimazole (e.g., mild rash, nausea) will be managed as part of routine clinical care by the study physicians and not reported to the sponsor or IRB as protocol deviations or UPs, unless they meet the criteria for an SAE or UP (unexpected, related or possibly related to research participation, and suggesting increased risk of harm). All such events will be carefully documented in the source documents and the study database (REDCap) for comprehensive safety monitoring. The distinction between routine disease management and reportable AEs/UPs will be clearly explained to study staff during training. For methimazole AEs, causality will be assessed by the treating physician per routine clinical practice.

### 8.6.2 Serious Adverse Event (SAE) Reporting

All SAEs, regardless of causality or expectedness, will be reported to the Principal Investigator (PI) within 24 hours of the study team becoming aware of the event. The PI

will then report the SAE to the IRB within [Specify timeframe, e.g., 7 calendar days] of becoming aware of the event, and other regulatory authorities, including the FDA (if applicable), using the appropriate reporting forms and procedures, following institutional and federal guidelines. For the purposes of SAE reporting in this open-label pilot study, where methimazole and vitamin D are used concomitantly, an SAE occurring after the first dose of either methimazole or vitamin D will be considered a treatment-emergent SAE and should be reported to the PI.

### 8.6.3 Unanticipated Problem/ Unanticipated Device Effect Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) according to the institution's policies and procedures. This study does *not* involve a device; therefore, Unanticipated Adverse Device Effects (UADEs) are not applicable. This should be explicitly stated in the protocol for the IRB.
The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number
- A detailed description of the event, incident, experience, or outcome
- An explanation of the basis for determining that the event represents a UP, specifically addressing the three criteria of unexpectedness, relatedness, and increased risk of harm.
- An assessment of the event's impact on the risks and benefits of study participation
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP

**Timeframe for Reporting UPs:**

- UPs that are also SAEs will be reported to the IRB within 7 calendar days of the investigator becoming aware of the event.
- All other UPs will be reported to the IRB within 14 calendar days of the investigator becoming aware of the problem.
- The PI will ensure that all UPs are reported to appropriate institutional officials (as required by the institution's written reporting procedures), the appropriate regulatory authorities, and the Office for Human Research Protections (OHRP), if applicable, within the required timeframes per institutional policy.

### 8.6.4 Reporting to Participants (If Applicable)

- **Individual AE/SAE Reporting:** Participants (or their parents/guardians) will be informed of any AEs or SAEs considered related or possibly related to the study intervention (vitamin D) or study procedures. This will be done in a timely manner, using age-appropriate language, during study visits or via phone or other secure communication methods. The information shared will include the nature of the event, its severity, the suspected relationship to the study, and any actions taken or recommended (e.g., dose modification, additional monitoring). Participants will be encouraged to report any new or worsening symptoms to the study team promptly.
- **UP Reporting to Participants:** If a UP occurs that might affect the safety or well-being of participants, those affected (or their parents/guardians) will be informed promptly. The information provided will include the nature of the UP, its potential impact, and any actions taken to mitigate risks.

- **Study Results related to Vitamin D Levels:** As Vitamin D levels are collected for study purposes, clinically relevant results (e.g., significant deficiency, toxicity) will be communicated to participants (and their parents/guardians) and their primary care physician for appropriate management and referral if necessary. This will occur regardless of randomization arm.
- **Incidental Findings:** Thyroid function tests (TFTs) and other tests such as CMP and CBC obtained as part of standard care will be reviewed by the participant's primary endocrinologist. Any abnormal TFT, CBC and CMP results will be managed according to usual clinical practice, including communication of results and any necessary follow-up with the participant and their parent/guardian.

# 9 REGULATORY, ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

This section details the ethical, regulatory, and oversight procedures for this pilot study, guided by the principles outlined in the Statement of Compliance.

## 9.1 Informed Consent/Assent and HIPAA Authorization

Written informed consent will be obtained from parents/guardians, and assent from child participants (9-17 years). The process will occur in a private setting, allowing adequate time for decision-making (at least 24 hours) and ensuring comprehension through age-appropriate language. Participation is voluntary, with no coercion or impact on standard care. Signed consent/assent forms and HIPAA Authorization will be documented and securely stored, with copies provided to participants. Participants reaching age 18 during the study will be reconsented as adults. While we do not *anticipate* enrolling non-English speaking participants in this study, we will not exclude them from participation. In the event that a non-English speaking individual expresses interest in enrolling, we will utilize a short form consent and HIPAA Authorization in accordance with IRB policy. A qualified interpreter will be provided through Language Line Solutions (either via video or phone) to facilitate a line-by-line interpretation of the full English consent form and any other study-related documents. This interpretation will be documented on the short form consent, noting the interpreter's name, language, and identification number. An impartial witness, who is present with the participant and understands the language of the consent, will also sign the short form consent, attesting that the information was reviewed with the participant in their preferred language with the assistance of the interpreter.

## 9.2 Safety Oversight

Data and Safety Monitoring Plan **(DSMP) and Clinical Monitoring Plan (CMP)**

To ensure participant safety and data integrity, this pilot study will implement a combined DSMP and CMP, recognizing the smaller scale and minimal risk.

- **DSMP:** The PI will oversee study safety, including review of all AEs and SAEs, recruitment/adherence rates, and data completeness. An independent Safety Officer, with expertise in pediatric endocrinology and free from conflicts of interest, will conduct formal safety reviews every 6 months and provide ad hoc consultations. DSMB meeting will take place every 6 months to review study safety data. The safety report for these reviews will include the number of AEs/SAEs, types of AEs/SAEs by system organ class, severity of AEs (using CTCAE v5.0), relationship of AEs to the intervention, and any actions taken. SAEs will be immediately reported to the PI, co-investigators, Safety Officer, Northwell IRB, and other care providers as appropriate. The study will adhere to OHRP/FDA/NIH guidelines and reporting requirements. Hypervitaminosis D and

hypercalcemia will be closely monitored through 25-hydroxyvitamin D and CMPs, with specific dose modification protocols in Section 4.8.

- CMP: The PI and co-investigator will monitor study conduct, including:

    - 100% Source Data Verification (SDV) for primary feasibility outcomes: recruitment rate, adherence (verified against pill counts, logs, self-report), and data completeness.
    - Targeted SDV for secondary and safety outcomes: This targeted source data verification will include thyroid function tests, antibody levels (TRAb and TSI), CMP including calcium, vitamin D levels, and adverse events.
    - Review of informed consent procedures.
    - Vitamin D drug accountability and adherence monitoring (procedures in Section 6.3).
    - Review of AE/SAE reporting for timeliness and completeness.
    - Assessment of protocol compliance and data quality (through REDCap range checks, validation rules, and data review meetings).
    - Regular communication/meetings with study staff.

    Monitoring Frequency: Monitoring visits will occur weekly for the first month and then monthly thereafter documented in monitoring visit reports. Summaries will be provided to the IRB for review.

### 9.2.1 Medical Monitoring

The study PI will provide medical monitoring for the study, ensuring appropriate assessment and reporting of adverse events, implementation of the data and safety monitoring plan, and regular assessment of the number and type of SAEs.

### 9.2.2 Independent Data and Safety Monitoring Board

Please see section 9.2 and 9.2.1

### 9.2.3 Stopping Rules

The study may be stopped prematurely for the following reasons:

- Evidence of significant or unexpected risks to participants related to vitamin D supplementation or study procedures.
- Serious or persistent non-compliance with the study protocol.
- Inability to recruit an adequate number of participants to assess feasibility.
- PI or sponsor decision based on logistical or administrative reasons.
- If the study is stopped, the PI or the co-investigator will promptly inform all participants, the IRB, and any other relevant stakeholders. The reasons for stopping will be documented.

## 9.3 Data Handling and Record Keeping

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP and regulatory and institutional requirements for the protection of

confidentiality of participants. The following subsections should include a description of the data handling and record keeping for the conduct of the trial.

### 9.3.1 Data Collection and Management

Data will be collected electronically using REDCap, a secure, web-based data capture system hosted at Northwell Health. Source documents will be stored in the participant's medical records. Data from source documents will be entered into REDCap by trained study staff. Data entry will be ongoing throughout the study. CRFs will be submitted electronically within 1 week of each study visit. Data quality will be ensured through range checks, data validation rules, and regular monitoring by the study team.

- **Access Control and User Roles:** Access to the REDCap database will be strictly controlled through a role-based access system. Each study team member will be assigned a unique username and password and granted access only to the data necessary for their role. The following roles and permissions will be implemented:
    - **Principal Investigator (PI) and Co-investigators:** Full access to all study data.
    - **Study Coordinator:** Access to all de-identified study data. Ability to enter and modify data.
    - **Research Staff:** Limited access to data based on their specific responsibilities (e.g., data entry personnel will only have data entry permissions; those performing data analysis may have read-only access to de-identified data).
    - **Safety Officer:** Access to de-identified safety data (AEs, SAEs).
    - **Clinical Monitor:** Access to de-identified data for monitoring purposes.
    - **Statistician**: Access to de-identified data for analysis purposes. Depending on the analysis plan, the statistician may have read-only access or permission to generate derived variables or perform data transformations within REDCap. If interim analyses are planned, access to blinded/unblinded data will be managed as per those procedures.
- **Password Management:** Strong password policies will be enforced, requiring a minimum length, complexity (combination of uppercase/lowercase letters, numbers, and symbols), and regular password changes. Passwords will never be shared. Two-factor authentication will be implemented if available.
- **Data Security Training:** All study personnel with REDCap access receive annual training on data security procedures and HIPAA compliance per institutional policy.
- **Confidentiality:** All data will be stored securely within the REDCap database. Participant identifiers will be replaced with study-specific numeric codes to protect confidentiality. The code linking participant identifiers to study codes will be stored separately in a secure, locked location accessible only to the PI and authorized study coordinator. This key will be destroyed after the study and all required audits are complete.
- **Security:** REDCap has robust security measures to prevent unauthorized access, including intrusion detection systems and regular security audits.
- **De-identification:** Prior to any publication or sharing of study data, all identifying information will be removed and replaced with unique study identifiers.

### 9.3.2 Confidentiality and Privacy

All study data will be treated as confidential. Participant privacy will be protected in accordance with HIPAA regulations. Access to study data will be restricted to authorized study personnel. Identifiers will be removed from any published or shared data. No personal identifying information will be released to third parties without explicit consent. Study staff will be trained on HIPAA regulations and data security procedures. The PI, co-investigators, study coordinator, and designated research staff will have access to study records and data. The clinical monitor, IRB representatives, and regulatory agencies may also have access to de-identified data for monitoring and audit purposes.

### 9.3.3 Source Documents

Source documents will consist of the participant's medical records, where all clinical information, including AEs, will be initially documented.

### 9.3.4 Case Report Forms

Data will be entered in REDCap electronic CRFs. The CRFs will collect data on demographics, medical history, vitamin D levels, thyroid function tests, antibody levels, adherence data, and AEs. The case report form will not serve as the sole record of study participation. Study enrollment will also be documented in the participant's medical record to ensure all healthcare providers are aware of the participant's study involvement.

### 9.3.5 Availability and Retention of Records

Study records will be retained for at least 7 years after study completion, consistent with institutional policy and any applicable regulations. All essential documents will be accessible to the IRB, sponsor, and regulatory authorities upon request.

### 9.3.6 Future Use of Stored Specimens and Data

No biological specimens will be stored for future research in this study. Genetic testing will not be performed.

## 9.4 Study Oversight

### 9.4.1 Protocol Deviations

- Protocol deviations will be documented and reviewed by the PI.
- **Major Protocol Deviation:** A deviation that affects participant safety, rights, welfare, or data integrity (e.g., administering the wrong dose of vitamin D, failing to report an SAE, falsification of data). Major deviations will be reported to the IRB within 10 working days of discovery.
- **Minor Protocol Deviation:** A deviation that does not significantly impact participant safety, rights, or data integrity (e.g., minor scheduling changes, missed phone call follow-up if appropriately rescheduled). Minor deviations will be tracked in a log and reported to the IRB at the time of continuing review or annual check-in.
- All deviations and corrective actions will be documented.

#### 9.4.2 Study Status

The study may be terminated or suspended for reasons outlined in section 9.2.4. The PI will promptly inform study participants and the IRB of any termination or suspension, providing written notification with reasons.

#### 9.4.3 Conflict of Interest

All investigators involved in this study will disclose any potential conflicts of interest to the Northwell Health Compliance Department for review and management, as per institutional policy.

#### 9.4.4 Institutional Review Board/Independent Ethics Committee Review and Approval

This study will be conducted in accordance with all applicable regulations and ethical guidelines, including the Declaration of Helsinki, CIOMS guidelines, and ICH GCP. The protocol, informed consent/assent documents, and all study materials will be submitted to the IRB for review and approval before study initiation. Annual progress reports and reports of UPs and SAEs will be submitted to the IRB as required. No other regulatory agencies (IBC, RDRC) require review for this study.

#### 9.4.5 Publication

Aggregate study findings, without any individually identifiable information, will be submitted for publication in a peer-reviewed journal. Authorship will be determined per ICMJE guidelines.

### 9.5 Quality Control and Quality Assurance

#### 9.5.1 Clinical Monitoring

Please see section 9.2 and 9.2.1 for details

#### 9.5.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, any sponsor, regulatory bodies, and institutional compliance offices, providing access to all study documents and facilities.

## 10 REFERENCES


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## APPENDIX

### Anticipated AEs with Severity and Actions

*The CTCAE grading scale (v5.0) defines the following severity grades for AEs:*

- ***Grade 1 (Mild):*** *Mild adverse event; intervention not indicated.*
- ***Grade 2 (Moderate):*** *Moderate adverse event; limiting age-appropriate instrumental activities of daily living (IADL); intervention indicated.*
- ***Grade 3 (Severe):*** *Severe adverse event; limiting self-care ADL; hospitalization indicated.*
- ***Grade 4 (Life-Threatening):*** *Life-threatening consequences; urgent intervention indicated.*
- ***Grade 5 (Death related to AE):*** *Death related to adverse event.*

| System Organ Class | Preferred Term | Severity (CTCAE v5.0) | Related to Vitamin D | Related to Methimazole | Actions |
|---|---|---|---|---|---|
| Gastrointestinal | Nausea | Grade 1-2 | Possibly/Probably | Possibly/Probably | Evaluate clinically and consider repeating thyroid function, calcium and vitamin D levels sooner. Adjust vitamin D dose accordingly. If calcium and vitamin D are normal and nausea persists, consider methimazole adjustment based on thyroid function levels. |
| Gastrointestinal | Vomiting | Grade 1-2 | Possibly/Probably | Possibly/Probably | Similar to nausea |
| Gastrointestinal | Constipation | Grade 1-2 | Possibly/Probably | Unlikely | If persistent or severe (Grade 2), manage per standard clinical practice, which may include stool softeners or laxatives. Consider dose reduction of vitamin D if suspected. |

| System Organ Class | Preferred Term | Severity (CTCAE v5.0) | Related to Vitamin D | Related to Methimazole | Actions |
|---|---|---|---|---|---|
| Gastrointestinal | Abdominal Pain | Grade 1-2 | Possibly | Unlikely | Evaluate for other causes. If severe or persistent, manage per clinical practice. Consider dose reduction or discontinuation of either medication if thought to be contributing based on clinical judgment. |
| Metabolism | Hypercalcemia | Grade 2-4 | Probably/Definitely | Not Related | If Grade 2, reduce vitamin D dose to 1000 IU daily and monitor calcium levels closely (e.g., weekly). If Grade 3 or persistent Grade 2, discontinue vitamin D and initiate appropriate medical management per standard of care. Grade 4 is an SAE requiring immediate medical intervention. |
| Metabolism | Hypercalciuria | Grade 1-2 | Possibly/Probably | Not Related | Encourage increased fluid intake. Monitor calcium levels closely. If severe or persistent, consider vitamin D dose reduction or discontinuation. |
| Renal and Urinary | Kidney Stones (Nephrolithiasis) | Grade 2-4 | Possibly (long-term) | Not Related | This is a potential long-term consequence of hypercalciuria. Manage as clinically indicated. |
| Skin | Rash | Grade 1-2 | Possibly/Probably | Possibly/Probably | If mild, monitor. If moderate (Grade 2), or if concerning to the participant, consider antihistamines. If severe or persistent, consider discontinuation of vitamin D or methimazole and evaluate for other causes. |

| System Organ Class | Preferred Term | Severity (CTCAE v5.0) | Related to Vitamin D | Related to Methimazole | Actions |
|---|---|---|---|---|---|
| Skin | Pruritus (Itching) | Grade 1-2 | Possibly | Possibly/Probably | Similar management to rash |
| Hepatobiliary | Elevated Liver Enzymes (ALT/AST) | Grade 1-3 | No | Possibly/Probably | Monitor liver function tests (LFTs). If the AST/ALT are >5 times the upper limit of normal, consult with hepatology. |
| Hematologic | Leukopenia (Decreased White Blood Cell Count) | Grade 1-4 | No | Possibly (rare) | Monitor CBC. If Grade 3-4 (severe or life-threatening), or if concerning trends are observed, consult with hematology and consider discontinuation of methimazole. Grades 3 or 4 are SAEs. |
| Hematologic | Agranulocytosis | Grade 4 | No | Possibly (rare but serious) | This is an SAE requiring immediate discontinuation of methimazole and medical intervention. |
| Musculoskeletal | Joint Pains | Grade 1-2 | Unlikely | Possibly/Probably | Manage per clinical practice (e.g., analgesics). Evaluate for other causes. |
| Musculoskeletal | Bone pain | Grade 1-2 | Possibly/Probably | Unlikely | If Grade 2 and possibly vitamin D-related, temporarily hold supplement and re-evaluate after resolution or reduce to 1000 IU daily. |
| General | Headache | Grade 1-2 | Unlikely | Possibly | Manage per clinical practice (e.g., analgesics). Evaluate for other causes. |

| System Organ Class | Preferred Term | Severity (CTCAE v5.0) | Related to Vitamin D | Related to Methimazole | Actions |
|---|---|---|---|---|---|
| General | Fatigue/Malaise | Grade 1-2 | Possibly | Possibly | Evaluate clinically and consider repeating thyroid function, calcium and vitamin D levels sooner. Adjust vitamin D dose accordingly. If calcium and vitamin D are normal and fatigue persists, consider methimazole adjustment based on thyroid function levels. |