Department of Pediatrics
University of Minnesota

Prevention of Bone Loss After Pediatric Hematopoietic Cell Transplantation

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Study Product: Pamidronate Disodium

University of Minnesota
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Confidential

Revision History

revision #	version date	summary of revisions	consent form changed
	10/13/2013	Original IRB approval	n/a

1	10/20/2014	prior to enrollment start –formatting change to match the Cancer Center's template (see details in the last bullet) and the following revisions:
		• Title page delete Lynda Polgreen as a co-investigator as she has left the institution
		• Minor clarifications to the inclusion/exclusion criteria
		• Section 8 – Tests and Procedures: move research related tests and procedures from Study Entry to during BMT work-up period
		• Section 8 - Research specimens will be batched tested in UMN Cytokine Lab (previously using Advanced Research and Diagnostic Laboratory)
		• Section 8.1.3 – clarified subject reimbursement and compensation
		• Section 12.5 – early stopping rule for excessive toxicity
		• Replaced Amplatz with Masonic throughout document when referring to UMN Children's Hospital
		• Updated protocol to Cancer Center template:
		  • Update document cover page to list affiliate institution
		  • Add Sponsor and site PI contact information to page 2
		  • Added revision history page and schema
		  • Section 6 - Added subject registration and randomization in OnCore
		  • Section 9 - Replaced previous AE reporting section with new Event Monitoring, Documentation and Reporting section which includes a new targeted toxicity form (appendix IV), affiliate institution reporting requirements and updated IRB reporting requirements
		  • Section 10 – New section covering study status update, data management, compliance with the Cancer Center's Data and Safety Monitoring Plan, affiliate monitoring and record retention
		  • Section 13 – New section addressing ethical and regulatory considerations
		  • Move Vitamin D/calcium supplementation and questionnaires to appendix II - IV, add eligibility checklist as appendix I
			yes

2	12/03/2014	• Throughout protocol: Revise Day 100 to Day 90 and if appropriate generalize the time point as 3 months • Throughout protocol: Delete references to study entry as "day of admission for transplant" • Throughout the protocol refer to affiliate institution as Fred Hutchinson Cancer Research Center as this is where the PI and IRB are located, although the study will continue to be conducted at Seattle Children's Hospital • Section 6.2, *Randomization Day 90 Post-HCT Visit:* Add statements that we will request to collect data from routine tests (DXA scans done at 1 year post-transplant and other relevant data) from subjects who are not eligible for or refuse randomization at Day 90 • Section 7.2, *Calcium*: Clarify how calcium level will be calculated if	yes

revision #	version date	summary of revisions	consent form changed
		subject's total calcium level is below the normal range. • Section 7.6 and 7.7, *Duration of Study Treatment and Participation* Update and clarify • Section 8, *Study Related Tests and Procedures*: Update • Section 8.1.3 *Subject Compensation*: Update • Section 8.2.5 *Diet and Activity Questionnaire*: Update to reflect that subjects will receive paper copies of forms • Section 8.3.2 Specimen Processing and Storage: Clarify that research samples will be stored for batch testing and destroyed once testing is complete. • Delete appendix V – *Targeted Toxicities* as replaced by a more detailed CRF	
3	02/09/2015	• Study synopsis, section 2: remove references to Pfizer as a generic form of pamidronate will be purchased for the study • Section 2.5: Clarify that at the end of study and after drug reconciliation, any remaining pamidronate will be destroyed per local institutional policy • Synopsis, sections 4, 7.3.1, and 8.1: Pamidronate administration – dose will be based on actual body weight or ideal body weight if overweight, to a maximum dose of 60 mg, give over 4 hours, previously over 3 to 4 hours, post infusion flush of 20 cc's will be given at the same rate as the pamidronate, previously over 1 hour • Section 5.2 and checklist – add history of a primary bone malignancy involving the lumbar spine as an exclusion criterion • Sections 7.3.5 and 8.1: Expand birth control options from oral only to include the contraceptive patch or other effective methods • Section 8.1: delete medical history at days 7 and 21, add record of medication usage at screen/study entry and at every study visit beginning with day 21 • Section 8.1.3: further clarify participant reimbursement and add "guidelines" to the section header as reimbursement schedule may differ between institutions • Section 8.2.3: update to match changes to 8.1 • Section 9.2: delete day 360 as no pamidronate is given • Minor edits and clarifications throughout	yes

revision #	version date	summary of revisions	consent form changed
4	09/29/2015	• Section 5.1.1 – clarify eligible diseases • Section 6 and appendix I – delete reference to eligibility checklist as an eligibility case report form is used instead • Sections 6.2, 7.32 and 7.3.5– clarify randomization time window of ±14 days and time window in association with lab tests required for randomization (within 10 days prior for vitamin D and 72 hour hours prior for creatinine, pregnancy testing) • Sections 6.2, 7.3.6, 7.5, and 7.7 – add disease relapse/progression as ineligibility for randomization and a reason to go off study and follow-up will end if in person post-transplant follow-up ends • Section 7.3.1 – add a 72 hour window prior for lab work in association with the pamidronate infusion, clarify that laboratory testing procedures (blood, urine) must be collected prior to pamidronate infusion, but consultations, day 90 DXA and day90 pQCT may occur after the infusion as long as done in the +/-14 day window • Sections 7.3.1, 8.1 footnote and 8.3.2 – remove language regarding 1st of the day timeframe for urine collection and add "A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded." • Section 8 – add a row for randomization on day 90 and refer reader to section 6.2 • Section 8.1 and 8.2.3 - add a row for height and weight only and add X under days 180 and 270, delete physical exam and anthropometrics for these days ; update text in section 8.2.3 • Section 8.1 - permit Vitamin D level to be drawn at screen or study entry • Section 8.2.4 – clarify DXA scan requirements • Section 9.5 – add a row to the required reporting table for stopping rule events • Appendix III – delete question regarding type of milk consumed and replace with a question regarding formula use	yes
5	02/01/2016	• Synopsis, Sections 4, 5 - expand enrollment to include persons ≤ 20 years of age • Section 5.2.1 - delete previous HCT from exclusion criteria • Section 12.5 – revise in response to DSMC concerns • Section 8.1 – increase window for DEXA scan to 6 weeks • Section 5.1.4 – remove language surrounding age of assent as this is a multi-center study	Yes
6	4/27/2016	• Section 5.2.9 – clarified eligibility; the intent was to exclude investigational drugs that either can interact with pamidronate or have an independent effect on the primary endpoint (bone mineral content/density), not all investigational drugs. • Section 6.2 – permit Vitamin D level to be drawn 14 days (instead of 10 days) prior to randomization to account for time for lab to send back result.	No

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7	12/02/2016	• Throughout protocol - University of Minnesota Principal Investigator and IND Holder was changed from Dr. Anna Petryk to Dr. Kyriakie Sarafoglou. • Section 7.1 and Appendix II, clarified timing of repeat Vitamin D levels. • Section 8.1 and 8.2.2 pregnancy test may be either urine or serum • Section 8.2.4 Added that it is not necessary to repeat a lumbar spine x-ray if previously performed within past 12 months • Section 10.4 and 10.5 updated link to the Masonic Cancer Center DSMP	Yes
8	05/21/2017	• Added non malignant diseases to study eligibility • Updated and clarified Vitamin D and Calcium supplementation guidelines (Section 7.3 and Appendix II) • Updated laboratory reference ranges to current standards	Yes
9	11/30/2017	• Section 5.2 added inability to complete DEXA scan at randomization • Section 6.2 clarified testing windows for randomization and randomization procedure • Clarified Vitamin D and Calcium supplementation guidelines (Section 7.3) • Section 7.5 added sedation option to DEXA scan, and risks of • Sections 8.1, 8.2.3, clarified schedule of height and weight evaluations • Section 9.2, clarified that AEs will not be reportable if they occur prior to randomization • Removed Appendix II as it was a repeat of tables already in the protocol • Section 10.3 updated data capture method • Appendix III added “completed by” line to the assessment tools	
9A	04/16/2018	• Section 9.2 revised and clarified AE reporting procedures	No
10	07/11/2019	• Section 7.3 Updated supplementation guidelines for clarity and consistency • Section 8.1 added footnote to timing of procedures	No

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Table of Contents

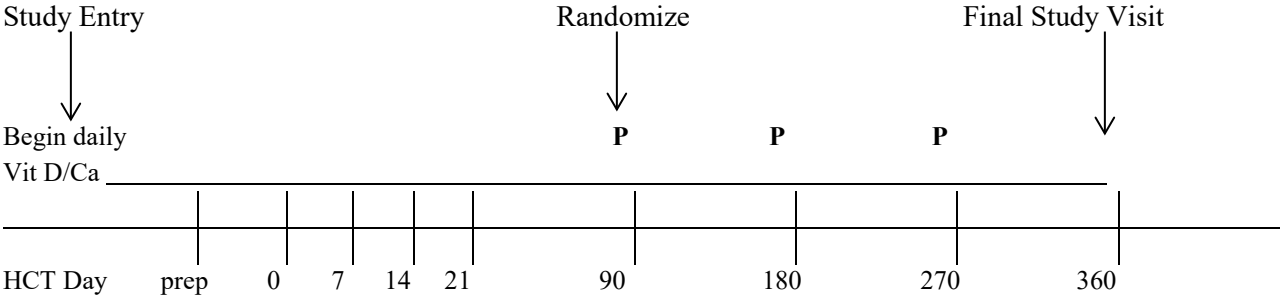
Study Summary

Prevention of Bone Loss After Pediatric Hematopoietic Cell Transplantation

IND Sponsor/ Investigator	Kyriakie Sarafoglou, MD Director, Leo Fung Center for Congenital Adrenal Hyperplasia and Disorders of Sex Development University of Minnesota Masonic Children's Hospital
Phase	Phase 2
Study Design	This is a Phase 2, open-label, randomized, controlled clinical study of pediatric subjects treated with pamidronate with calcium and vitamin D versus calcium and vitamin D alone following hematopoietic cell transplantation (HCT). Subjects randomized to pamidronate treatment will receive infusions at approximately Day 90, Day 180, and Day 270 post-HCT. Laboratory evaluations, dual-energy X-ray absorptiometry (DXA), and peripheral quantitative computed tomography (pQCT) will be performed at specified time points to evaluate safety and treatment efficacy.
Primary Objective	To test the hypothesis that subjects receiving pamidronate with calcium and vitamin D (Pamidronate Group) will have higher lumbar spine bone mineral content (LBMC; adjusted for height, age, sex, Tanner stage, and race) measured by DXA at 1 year post-HCT than subjects receiving calcium and vitamin D alone (Control Group).
Secondary Objectives:	To test the following hypotheses: • Subjects treated with pamidronate will have higher total body BMC (TBMC; excluding head; adjusted for height, age, sex, Tanner stage, and race) measured by DXA 1 year post-HCT compared to the Control Group. • Subjects treated with pamidronate will have higher total bone mineral density (BMD), cortical BMD, trabecular BMD, and estimated bone strength measured by pQCT 1 year post-HCT compared to the Control Group.
Correlative Objectives:	• Cytokine levels will increase rapidly in the first 3 weeks after HCT, preceding an increase in markers of bone resorption. • In the Control Group, increased cytokine levels and receptor activator of nuclear factor-κ B ligand (RANKL)/ osteoprogerin (OPG) ratio 3 weeks post-HCT will be associated with decreased BMC and BMD at 1 year post HCT. • Markers of bone resorption will increase in the first three months after HCT, will remain elevated until at least Day 180 in the Control Group, but will decrease after initiation of pamidronate at Day 90 in the Pamidronate Group. • Markers of bone formation, including osteocalcin (OCN) will decrease in the first three months after HCT and in the Control Group lower OCN levels at Day 90 will be associated with lower BMC and BMD 1 year post-HCT.
Duration of Study Participation	1 year from HCT
Number of Subjects	60 evaluable patients (30 per site) over 3.5 years

Main Inclusion Criteria	Subjects will be ≥1 and ≤20 years of age at the time of enrollment and receiving an allogeneic HCT for a hematologic malignancy in complete remission and using a myeloablative preparative regimen, non-malignant diseases with a myeloablative or non-myeloablative preparative regimen, or severe aplastic anemia (SAA) for which any conditioning therapy is permitted.
Standard Therapy	All Subjects - Standard supplementation with calcium and vitamin D beginning at admission to the hospital (prior to HCT) and continuing until post-HCT Day 360 visit
Study Product, Dose, Route, Regimen	Pamidronate Group Only Pamidronate disodium single 1 mg/kg intravenous infusion administered every 3 months for 3 doses - post-HCT Days 90, 180 and 270.
Endpoints	The primary study endpoint is lumbar bone mineral content (LBMC). Other endpoints are TBMC (excluding head), bone strength, total BMD, cortical BMD, trabecular BMD, levels of markers of bone turnover, cytokines, RANKL/OPG ratio, and body composition.
Statistical Methods	The primary analysis will compare the Pamidronate and Control Groups according to LBMC measured by DXA using a t-test applied to all available Day 360 measurements. A secondary analysis will use multiple imputation to impute Day 360 BMC and BMD measurements for each subject who does not provide one.

Study Schema



Key Study Time Points as related to Hematopoietic Cell Transplant (HCT):

- **Enrollment** (signing of consent, confirmation of eligibility - occurs during the pre-HCT work-up)
- **Study Entry**(DXA scan, blood and urine tests begin during pre-HCT work-up until admission or at the time of admission, daily Vitamin D/Calcium begins upon admission to the hospital)
- **Randomization** (Pamidronate versus no Pamidronate) occurs at the Day 90 post-HCT milestone visit
- **Final Study Visit** occurs at the Day 360 post-HCT milestone visit

Treatment Plan:

All subjects:

Vitamin D and Calcium Supplementation per Section 7.3 begin prior to HCT preparative regimen start (e.g. on day of admission for transplant) and continue daily through day 360 post-HCT milestone visit

Pamidronate Group

Pamidronate (P) 1 mg/kg IV (to max dose of 60 mg) over 4 hours on Day 90, Day 180, and Day 270 post-HCT

List of Abbreviations

AE	Adverse Event
AI	Adequate Intake
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Bisphosphonate
CFR	Code of Federal Regulations
CML	Chronic Myelogenous Leukemia
CNS	Central Nervous System
CPRC	Cancer Protocol Review Committee
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTSI	Clinical and Translational Science Institute
CTX	Carboxy Terminal Collagen Crosslinks
DPD	Deoxypyridinoline
DSMC	Data and Safety Monitoring Council
DSMP	Data and Safety Monitoring Plan
DXA	Dual-energy X-ray Absorptiometry
eCRF	electronic case report form
EDTA	Ethylenediaminetetraacetic Acid
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GnRH	Gonadotropin-releasing Hormone
GVHD	Graft-Versus-Host Disease
HCT	Hematopoietic Cell Transplantation
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICH	International Conference on Harmonisation
IDS	Investigational Drug Services
IL	Interleukin
IND	Investigational New Drug
iPTH	Intact Parathyroid Hormone
IRB	Institutional Review Board
IV	Intravenous
LBMC	Lumbar Bone Mineral Content
LBMD	Lumbar Bone Mineral Density
NCI	National Cancer Institute
OCN	Osteocalcin
OCP	Oral Contraceptive Pill
OH	Hydroxy
OI	Osteogenesis Imperfecta
OnCore	University of Minnesota's data capture system
OPG	Osteoprogerin
OTC	Over-the-counter
P1NP	Procollagen Type 1 Terminal Peptide
PHI	Protected Health Information

PI	Principal Investigator
PN	Parenteral Nutrition
pQCT	Peripheral Quantitative Computed Tomography
qs	Sufficient Quantity
RANKL	Receptor Activator of Nuclear Factor-κ B Ligand
RDA	Recommended Dietary Allowance
REPA	Report of External Professional Activities
Rx	Prescription
SAA	Severe Aplastic Anemia
SAE	Serious Adverse Event
SD	Standard Deviation
SST	Serum Separation Tube
TBD	To Be Determined
TBMD	Total Body Bone Mineral Density
TNF-α	Tumor Necrosis Factor Alpha
UMN	University of Minnesota
USP	United States Pharmacopeia

1 Introduction, Background and Rationale

Low bone mineral density (BMD) is common in children treated with hematopoietic cell transplantation (HCT) in both early and late follow-up (1-7). Low BMD in childhood is expected to increase the risk of early osteoporosis and increase the risk of fracture due to low peak bone mass (7-11). This has serious individual and societal implications due to impaired mobility and ability to participate in activities of daily living that occurs after fracture. In older adults, fracture has even been associated with an increased risk of death.

Bone health is dependent on a balance between bone resorption and bone formation; when this balance is disrupted bone loss and ultimately osteoporosis develop. BMD decreases in the first 6 months after HCT (1). A recent study has shown that bone resorption is significantly increased by 100 days after pediatric HCT and remains elevated through at least 180 days after HCT (12), which offers an opportunity for intervention.

1.1 Pamidronate Disodium

Pamidronate is a bisphosphonate (BP) that inhibits bone resorption and thus can increase bone density by shifting the balance towards bone formation. Although its mechanism of antiresorptive action is not completely understood, several factors are thought to contribute to this action. Pamidronate disodium adsorbs to calcium phosphate (hydroxyapatite) crystals in bone and may directly block dissolution of this mineral component of bone. In vitro studies also suggest that inhibition of osteoclast activity contributes to inhibition of bone resorption. In animal studies, at doses recommended for the treatment of hypercalcemia, pamidronate disodium inhibits bone resorption apparently without inhibiting bone formation and mineralization (13).

The Food and Drug Administration (FDA) has approved pamidronate for use in osteolytic bone metastasis associated with metastatic breast cancer or multiple myeloma, hypercalcemia of malignancy (with adequate hydration), and Paget's disease in adults. It is also frequently used to treat post-menopausal osteoporosis and has been shown to be effective in preventing post-transplant bone loss in adults (14-16).

1.2 Pamidronate in Children

Pamidronate has not been approved for use in children but clinical trials have been conducted in children with various disorders causing osteoporosis or low bone density. In children, pamidronate and other BPs have been used safely and effectively to treat the most debilitating form of childhood osteoporosis, osteogenesis imperfecta (OI) (17, 18). The first case report of beneficial effects

of BP treatment in OI was published in 1987 (19) and was followed by over 30 clinical trials (20-22). In these studies, the most commonly used BP was intravenous pamidronate at a dose of 0.6 to 3.75 mg/kg/day over 1 to 3 days every 1 to 6 months (23, 24). The patients' age was 0.04 to 18 years and follow-up lasted up to 9 years. The reported therapeutic effects included improvement in bone density (increase in lumbar spine BMD Z-score of about 2.6 or in spine BMD of 42 to 48%), grip strength, vertebral height, cortical thickness, trabecular number, decreased bone turnover, increased mobility, and decreased fracture rate. Due to these beneficial effects, pamidronate has become the standard of care in treating severe OI (25). BPs are increasingly prescribed in children with other diseases, for example neuromuscular disorders (26-28) or idiopathic juvenile osteoporosis (29, 30) with improvements noted in the spine, femoral neck, and/or total body BMD Z-scores and a reduced fracture rate. Pamidronate has also been used in pediatric burn patients to prevent bone loss (31).

There has been 1 retrospective observational study that found BP therapy improved BMD in pediatric HCT recipients (32). The study included children who survived at least 1 year after allogeneic HCT with 48 control patients receiving calcium and vitamin D and 18 patients also receiving BP therapy (17 of the 18 patients received pamidronate for the majority of the study as monthly infusions of 1 mg/kg for 6 months to 2 years). Baseline and follow- up dual energy x-ray absorptiometry (DXA) scans (mostly at the lumbar spine) were compared. The annualized increase in BMD was 10% per year for the control patients and 33% per year for patients that also received BP. To account for expected age-related increase in BMD, the interval change in BMD *Z*-scores was calculated. The annualized median change in BMD *Z*-scores per year was +0.12 among the control patients and +1.43 for the BP group. Thus, in this non-randomized retrospective study, BP-treated HCT recipients had a significantly greater increase in BMD than control patients.

Short- and long-term safety of BPs for children with compromised bone health has been recently assessed on behalf of the Drugs and Therapeutics Committee of the Pediatric Endocrine Society (25). In most studies, no serious side effects have been reported with the exception of 1 case of iatrogenic osteopetrosis in a 12 year-old boy treated with higher than recommended doses of pamidronate (2.2 to 3.4 mg/kg every 3 weeks and then less frequent intervals between 7¾ and 10½ years of age) for idiopathic bone pain with a marked elevation of alkaline phosphatase (33). More likely side effects are transient hypocalcemia (24, 34), which can be prevented/mitigated by calcium and vitamin D supplementation, and an acute-phase reaction, including influenza-like symptoms (24, 35-38), which can be mitigated by anti-pyretic/anti-inflammatory agents. Nephrotoxicity has

been reported in adults treated with pamidronate (39, 40), but no adverse effect on renal function has been reported in children treated with pamidronate or other BPs. There has been some concern about long-term effects due to deposition of BPs in the skeleton. A half-life of more than 10 years has been reported in adults (41). Long-term release of BPs has been shown in children who were treated with pamidronate for 4 to 10 years (42). Fractures have been described in OI patients following pamidronate discontinuation, at the interface between the high density (pamidronate-treated) and low density (newly formed, pamidronate-naïve) bone (43-45). It is thought, however, that this risk may be limited to patients who have persistent risk factors such as a genetic defect (e.g., collagenopathy) as opposed to transient exposures. It is unlikely that these potential long-term consequences are pertinent to this study, which requires short-term exposure to pamidronate and excludes children with any underlying genetic abnormality in bone remodeling.

2 Pamidronate Disodium (Investigational Agent)

2.1 Description

Molecular formula: $C_3H_9NO_7P_2Na_2 \bullet 5H_2O$
Molecular weight: 369.1

30 mg single use vial: 3 mg/mL pamidronate disodium, 47 mg/mL mannitol, United States Pharmacopeia (USP); water for injection, USP, sufficient quantity (qs); and phosphoric acid to adjust pH.

90 mg single use vial: 9 mg/mL pamidronate disodium, 37.5 mg/mL mannitol, USP; water for injection, USP, qs; and phosphoric acid to adjust pH.

2.2 Indications

Pamidronate disodium was approved by the FDA in 1991 for the treatment of osteolytic bone metastasis associated with metastatic breast cancer or multiple myeloma, hypercalcemia of malignancy (with adequate hydration), and Paget's disease in adults.

Pamidronate has not been FDA-approved for pediatric use but given previous data in children (refer to Section 1.2), standard safety monitoring following HCT, and the additional safety evaluations included in this protocol, the risk to subject safety is relatively low.

2.3 Study Drug Availability

For the purposes of the study vials of pamidronate disodium will be purchased by

the study through each institution's Investigational Drug Service (IDS).

Study drug inventory will be documented.

2.4 Packaging, Storage, and Preparation

Packaging and Labelling

The immediate package of an investigational drug intended for human use shall bear a label with the statement:

Caution: New Drug--Limited by Federal law to investigational use.

Storage

Vials of pamidronate disodium solution must be stored at 20 to 25°C.

Preparation for Infusion

Pamidronate disodium solution will be diluted to the recommended subject dose in sterile sodium chloride 0.45% or 0.9% or dextrose 5%. The drug will be completely dissolved before the solution is withdrawn. Parenteral drug products will be visually inspected for particulate matter and discoloration prior to administration, whenever solution and container permit. Refer to the package insert for complete details

2.5 Final Reconciliation and Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of study agents that were purchased, consumed, and remaining at the study site. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study drug.

Any leftover drug will be destroyed on site according to institutional policies.

3 Study Objectives and Hypotheses

3.1 Primary Hypothesis

The primary hypothesis is subjects treated with pamidronate and calcium and vitamin D (Pamidronate Group) will have higher lumbar bone mineral content (LBMC adjusted for height, age, sex, Tanner stage and race) measured by DXA 1 year post-HCT than subjects receiving calcium and vitamin D alone (Control Group).

3.2 Secondary Hypotheses

The secondary hypotheses are:

- Subjects treated with pamidronate will have higher total body BMC (TBMC; excluding head; adjusted for height, age, sex, Tanner stage, and race) measured by DXA 1 year post-HCT compared to the Control Group.
- Subjects treated with pamidronate will have higher total BMD, cortical BMD, trabecular BMD, and estimated bone strength measured by peripheral quantitative computed tomography (pQCT) 1 year post-HCT compared to the Control Group.
- Cytokine levels will increase rapidly in the first 3 weeks after HCT, preceding an increase in markers of bone resorption.
- In the Control Group, increased cytokine levels and receptor activator of nuclear factor-κ B ligand (RANKL)/osteoprogerin (OPG) ratio 3 weeks post-HCT will be associated with decreased BMC and BMD 1 year after HCT.
- Markers of bone resorption will increase in the first 3 months after HCT, will remain elevated until at least Day 180 in the Control Group, but will decrease after initiation of pamidronate at Day 90 in the Pamidronate Group.
- Markers of bone formation, including osteocalcin (OCN), will decrease in the first 3 months after HCT and in the Control Group lower OCN levels at Day 90 will be associated with lower BMC and BMD 1 year post-HCT.

4 Study Design

This is a Phase 2, open-label, randomized, controlled clinical study of pediatric subjects (defined as ≥1 year, but ≤20 years of age) treated with pamidronate with calcium and vitamin D versus calcium and vitamin D alone following HCT.

All subjects will receive a supplemental dose of vitamin D (based on vitamin D (D2+D3) level) beginning upon admission to the hospital for the HCT and calcium if they are not meeting the recommended dietary allowance (RDA) or if their serum calcium is low (based on ionized calcium level). Refer to Section 7.3 for supplementation guidelines.

At the Day 90 post-transplant visit, subjects will be randomized to receive one of the following treatment plans through the Day 360 post-transplant visit:

- Control Group: continue determined vitamin D per day and calcium supplementation, as appropriate to meet the RDA requirement. Refer to Section 7.1 for supplementation guidelines.
- Pamidronate Group: continue determined vitamin D per day and calcium supplementation, as appropriate to meet RDA requirement, AND 3 single IV infusions of 1 mg/kg (to a max of 60 kg) pamidronate at 3-month intervals (Day 90, 180 and 270) post-transplant. Refer to Section 7.3 for supplementation guidelines

Laboratory evaluations, DXA, and pQCT will be performed at specified time points per section 8 to monitor safety and evaluate treatment efficacy.

5 Subject Selection

This study will evaluate bone loss in pediatric patients undergoing a hematopoietic cell transplant (HCT). Study entry is open to persons ≥1, but ≤20 years of age regardless of gender, race or ethnic background. While there will be every effort to seek out and include females and minority patients, the patient population is expected to be no different than that of hematopoietic cell transplant studies for hematologic malignancies and severe aplastic anemia at the University of Minnesota and Fred Hutchinson Cancer Research Center.

Patients will be consented and screened during their pre-transplant evaluation. Vitamin D and calcium, if indicated, will be started at the time of admission for transplant.

5.1 Inclusion Criteria

All of the following criteria must be met:

5.1.1 Patients in one of the below disease categories:

5.1.1.1 Allogeneic hematopoietic cell transplant for hematologic malignancy (i.e. leukemia, lymphoma including ALL, AML, CML, NHL, HL) in complete remission; myelodysplastic syndrome (active dysplasia and/or blasts are permitted, but must not have active leukemia) who will be receiving a myeloablative preparative regimen, **OR**

5.1.1.2 Non-malignant diseases including idiopathic severe aplastic anemia (SAA) and other bone marrow failure disorders, hemoglobinopathies, adrenoleukodystrophy, immune deficiencies/dysregulation disorders who will be receiving myeloablative or reduced toxicity preparative regimens that meet the following criteria:

5.1.1.2.1 Regimens include those that are TBI based if the TBI dose is > 500cGy single dose or > 800cGy fractionated, or doses <500 cGy if combined with busulfan or treosulfan. These also include chemotherapy only based regimens that contain myeloablative doses of busulfan (>8mg/kg) or treosulfan without TBI

5.1.1.2.2 Patients with severe aplastic anemia are eligible regardless of conditioning regimen

5.1.2 Male or female ≥1 but ≤ 20 years of age at time of study enrollment

5.1.3 Patient or parent(s)/legal guardian(s) is able and willing to provide informed consent. Assent will be obtained per local institutional policy. Subjects who turn 18 during the course of the study will be consented at that time of their next visit by a member of the research staff.

5.2 Exclusion Criteria

Patients will be excluded from participation in the study if any of the following conditions exist:

5.2.1 History of a primary bone malignancy involving the lumbar spine

5.2.2 Treatment with other biphosphonates, Denosumab, or Teriparatide within one year prior to enrollment

5.2.3 Pregnancy or breastfeeding – menstruating females must have a negative pregnancy test prior to study enrollment and agree to repeat pregnancy testing and contraception use per protocol as pamidronate is Pregnancy Category D – positive evidence of human fetal risk based on adverse reaction data

5.2.4 Renal insufficiency, defined as creatinine level greater than the upper limit of normal for age

5.2.5 Hereditary metabolic bone disease or skeletal dysplasia (e.g., osteopetrosis or OI) or primary hyperparathyroidism

5.2.6 Non-malignant diseases with underlying metabolic defects that may affect bones such as Hurler's syndrome and other Mucopolysaccharidoses and osteopetrosis, or diseases where the use of hematopoietic cell transplant itself is still under investigation, primarily, epidermolysis bullosa.

5.2.7 Patients with diseases that have increased sensitivity to chemotherapy or radiation including (but not limited to) Fanconi anemia, dyskeratosis congenita.

5.2.8 Clinically significant fractures as defined by ISCD (a long bone fracture of the lower extremities, vertebral compression fracture, or two or more long bone fractures of the upper extremities) (88,89) indicated by a cast or a spine x-ray within the last 2 weeks

5.2.9 Known or suspected allergy to pamidronate or related products

5.2.10 Planned administration of an investigational study drug or agent that either can interact with pamidronate or have an independent effect on bone mineral density within the 4 weeks prior to randomization (Day 90) or planned use during study participation (Day 90 through Day 360)

5.2.11 Impending invasive dental procedure that would be expected to occur during study participation (through Day 360)

6 Study Registration and Randomization

Registration will occur after the patient or parent/guardian has signed the appropriate subject consent and eligibility is confirmed, but before any treatment has been administered. To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist case report form based on the eligibility assessment documented in the patient's medical record.

6.1 Registration with the Masonic Cancer Center Clinical Trials Office

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, the study coordinator or designee will enroll the patient into OnCore.

OnCore will automatically generate an email alerting key study personnel of the registration.

6.2 Randomization (Day 90 Post-HCT Visit +/- 14 days)

Randomization to pamidronate (Pamidronate Group) or no pamidronate (Control Group) will occur at Day 90 (+/- 14 days) by the institutional study coordinator based on the randomization schedule developed by the study statistician and entered into OnCore.

A brief review of continuing eligibility will be done and subjects will not be eligible for randomization if one or more of the following are true:

- Vitamin D (D_2+D_3) <20 ng/ml as use of pamidronate is contraindicated due to potentially higher risk of hypocalcemia. Levels should be measured within 14 days prior to randomization.**[Ŧ]
- serum creatinine is > 2 times the baseline value (at Study Entry) - perform within 14 days prior to randomization
- an investigational study drug or agent, including biphosphonates, Denosumab, or Teriparatide, was administered within the previous 4 weeks or is planned for use during study participation (Day 90 through Day 360)
- positive pregnancy test (performed within 14 days prior to randomization)
- Inability to successfully complete a DEXA scan
- the parent or adult patient refuse randomization
- disease relapse has occurred

The randomization will be stratified by age with 2 strata: < 10 years old and ≥ 10 years old.

If the patient is ineligible for or refuses randomization, no further research related procedures or treatment will occur after the Day 90 visit; however permission to continue review of the patient's medical record through the Day 360 visit for routine clinical information is embedded within the consent form document. This information will be collected only if the patient continues care at the enrolling

institution. Patient care will be at the discretion of the treating physician(s) and independent of this study.

OnCore will automatically generate an email alerting key study personnel of the randomization (or lack of).

7 Treatment Plan

7.1 Vitamin D and Calcium Supplementation

All subjects will receive a supplemental vitamin D (based on Vitamin D_2+D_3 levels beginning upon admission to the hospital for the HCT (54, 55). Vitamin D and Calcium supplementation will be determined by a combination of Vitamin D levels, ionized calcium levels, calcium intake, PTH, and TPN. See section 7.3 for supplementation guideline charts. In subjects who require parenteral nutrition (PN) and have normal vitamin D (D_2+D_3) level, vitamin D will be given as part of multivitamin supplementation.

- Adult multivitamin preparations contain 200 IU of cholecalciferol per 10 ml (or 5 mcg of ergocalciferol, equivalent to 200 IU of ergocalciferol, per 10 ml).

Pediatric multivitamin preparations contain 400 IU of cholecalciferol per 5 ml. (of 10 mcg of ergocalciferol, equivalent to 400 IU of ergocalciferol, per 5 ml)
If a subject's serum vitamin D (D_2+ D_3) is <30 ng/mL at any of the designated time points starting from study entry through Day 360 post-HCT (including 8 week rechecks for subtherapeutic levels), additional supplementation with vitamin D will be administered to maintain a serum level between 30 and 70 ng/mL according to the protocol as outlined in Section 7.

If serum vitamin D (D_2+ D_3) levels are low and supplementation is begun, levels will be re-checked after 8 weeks at the next scheduled study visit. Although the proposed regimen should normalize vitamin D level within 6-8 weeks (57), if the target range is not reached, supplementation will be continued for additional 8 weeks, and serum vitamin D level (D_2+D_3) repeated at the end of treatment. Subjects with vitamin D level (D_2+D_3) <20 ng/ml at Study Entry will be excluded from analyses of bone turnover markers until vitamin D level (D_2+D_3) normalizes but otherwise will be included in all analyses.

** Measurement of Vit (D_2+D_3) levels is recommended instead of 25-hydroxy-Vitamin D level, as they reflect vitamin D stores accurately and independently of whether the subject is on VitD_2 (ergocalciferol) or VitD_3 (cholecalciferol) supplementation. As 25-hydroxyVitamin D assay mainly measures VitD_3 stores, it cane underestimate overall vitamin D stores in patients supplemented with

ergocalciferol ($VitD_2$). However, 25-hydroxy Vitamin D assay can be used in patients who are on cholecalciferol supplementation.

ŦPer institutional practice, Fred Hutchinson Cancer Research center will measure 25-hydroxy-Vitamin D level.

7.2 Calcium

Study subjects will be assessed to determine if supplemental calcium is warranted based on dietary intake and/or ionized calcium values. At the designated time points in the study starting at admission for the HCT through Day 360, dietary calcium intake will be determined from a Food Frequency Questionnaire (FFQ) (Appendix II). Subjects who do not meet the RDA will receive additional calcium supplementation (Section 7.3).

At the designated time points in the study starting at admission for HCT through Day 360, ionized calcium or corrected calcium, and vitamin D (D_2+D_3) levels will be assessed. If the subject's level is below the normal range at any time, supplemental calcium and vitamin D supplementation will be given per Section 7.3

If a subject's total calcium level is below the normal range at any time, calculate a corrected calcium if a serum albumin level is available (may have been drawn anytime within 7 days of the calcium level) using the formula:

Corrected Ca = measured total Ca + [0.8 (4.0 - serum albumin mg/dL)]

If a serum albumin level is not available ionized calcium level should be done.

If a subject's vitamin D level (D_2+D_3) is 10-20 ng/mL and their iPTH level is >65 pg/mL or if vitamin D level (D_2+D_3) is <10 ng/mL at any time from Study Entry through Day 360, additional calcium carbonate will be administered per Section 7.3.

7.3 Vitamin D and Calcium Supplementation Charts:

For all dosing, these charts list the ideal targeted dose, however dosing will be in an acceptable range as set by available formulations or insurance barriers

ŦPer institutional practice, Fred Hutchinson Cancer Research center will measure 25-hydroxy-Vitamin D level

7.3.1 For Vitamin D Sufficient Patients

Age	All	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	sufficient	600 IU/day of Vitamin D supplementation only	No Supplementation
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	sufficient		

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	sufficient	600 IU/day of Vitamin D	600 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	sufficient	600 IU/day of Vitamin D	1,000 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	≥ 9 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	sufficient	600 IU/day of Vitamin D	1,200 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	sufficient	• 600 IU/day Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as • needed to normalize ionized or corrected calcium. • Once normalized, if dietary calcium intake is low the participant would receive calcium 600 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	sufficient	• 600 IU/day Vitamin D	• calcium supplementation at 50 mg/kg divided 3x/day day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as needed to normalize ionized or corrected calcium. • Once normalized, if dietary calcium intake is low the participant would receive calcium 1,000 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

Age	≥ 9 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	sufficient	• 600 IU/day Vitamin D	• calcium supplementation at 50 mg/kg divided 3x/day day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as needed to normalize ionized or corrected calcium. • Once normalized, if dietary calcium intake is low the participant would receive calcium 1,200 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

7.3.2 For Low vitamin D (D2+D3) Levels

Age	<9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 4000 IU/day of vitamin D supplementation x 4 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if needed • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• No supplementation
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	sufficient		

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 4000 IU/day of vitamin D supplementation x 8 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if needed • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• No supplementation
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	sufficient		

Age	1-3 Years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 4000 IU /day of vitamin D supplementation x 4 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium 600 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 4000 IU/day of vitamin D supplementation x 4 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium 1,000 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 4000 IU/day of vitamin D supplementation x 8 weeks; or 50000 IU (single dose) of vitamin D every other week x 4 doses total • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium 1,200 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	20-29	• 4000 IU/day of vitamin D supplementation x 4 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at 50 mg/kg divided 3x/day day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as .needed if low. • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant would receive calcium 600 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	20-29	• 4000 IU/day of vitamin D supplementation x 4 weeks • Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at 50 mg/kg divided 3x/day day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as needed if low. • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant would receive calcium 1,000 mg/day
ionized or corrected calcium level	low		
dietary calcium intake based on questionnaire	Low or normal		

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	20-29	• 4000 IU/day of vitamin D supplementation x 8 weeks; or 50000 IU (single dose) of vitamin D every other week x 4 doses total Vitamin D (D_2+D_3) level should be checked in 4 or 8 weeks and continue supplementation if still low • Once the Vitamin D level is normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2000 mg/day). • Check levels weekly, increase as needed if low. • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant would receive calcium 1,200 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	low		

Age	<9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ǂ	20-29	• 50000 IU (single dose) of vitamin D every other week x 2 doses total • Check Vitamin D (D_2+D_3) levels in 4 weeks • Continue vitamin D supplementation until Vitamin D levels normalize. Once normalized vitamin D would be given as part of multivitamin supplementation.	• calcium supplementation at a dose of 50 mg/kg/day (max daily calcium dose of 2000 mg/day) or the maximum allowed per TPN • Check ionized or corrected calcium level every week
ionized or corrected calcium level	low		
Patient on TPN	Yes		

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	20-29	• 50000 IU (single dose) every other week x 4 doses total • Check Vitamin D (D_2+D_3) levels in 8 weeks • Continue vitamin D supplementation until Vitamin D levels normalize. Once normalized vitamin D would be given as part of multivitamin supplementation.	• calcium supplementation a dose of 50 mg/kg/day (max daily calcium dose of 2000 mg/day) or the maximum allowed per TPN • Check ionized or corrected calcium level every week
ionized or corrected calcium level	low		
Patient on TPN	Yes		

7.3.3 Vitamin D Deficiency

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 4000 IU/day of vitamin D supplementation x 8 weeks. Vitamin D (D_2+D_3) level should be checked in 8 weeks • continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2000 mg/day) for 4 weeks • After 4 weeks of calcium supplementation, if dietary calcium is low, the participant will receive calcium 600 mg/day.
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	normal/low		
PTH*	>65		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-20 with normal ionized or corrected calcium and normal or low dietary calcium intake, calcium supplementation at a dose of 50mg/kg/day 3 times daily is not needed, otherwise follow the same supplementation guidelines.

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 4000 IU/day of vitamin D supplementation x 8 weeks. Vitamin D (D_2+D_3) level should be checked in 8 weeks • continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2000 mg/day) for 4 weeks • After 4 weeks of calcium supplementation, if dietary calcium is low, the participant will receive calcium 1,000 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	normal/low		
PTH*	>65		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-20 with normal ionized or corrected calcium and normal or low dietary calcium intake, calcium supplementation at a dose of 50mg/kg/day 3 times daily is not needed, otherwise follow the same supplementation guidelines.

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 50000 IU (single dose) every week x 8 doses total • Vitamin D (D_2+D_3) level should be checked in 8 weeks • continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • After 4 weeks of calcium supplementation, if dietary calcium is low, the participant will receive calcium 1,200 mg/day
ionized or corrected calcium level	normal		
dietary calcium intake based on questionnaire	normal/low		
PTH*	>65		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-20 with normal ionized or corrected calcium and normal or low dietary calcium intake, calcium supplementation at a dose of 50mg/kg/day 3 times daily is not needed, otherwise follow the same supplementation guidelines.

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 4000 IU/day of vitamin D supplementation x 8 weeks • Vitamin D (D_2+D_3) level should be checked in 8 weeks • continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium level every week and increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant will receive calcium 600 mg/day
ionized or corrected calcium level	low		
dietary calcium intake	normal or low		
PTH*	>65		

**** When PTH is <65 and Vitamin D level (D_2+D_3) is 10-19 with low ionized or corrected calcium and normal or low dietary calcium intake, follow the same supplementation guidelines.***

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 4000 IU/day of vitamin D supplementation x 8 weeks • Vitamin D (D_2+D_3) level should be checked in 8 weeks • continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium level every week and increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant will receive calcium 1,000 mg/day
ionized or corrected calcium level	low		
dietary calcium intake	normal or low		
PTH*	>65		

**** When PTH is <65 and Vitamin D level (D_2+D_3) is 10-19 with low ionized or corrected calcium and normal or low dietary calcium intake, follow the same supplementation guidelines.***

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	10-19	• 50000 IU (single dose) every week x 8 total doses • Vitamin D (D_2+D_3) level should be checked in 8 weeks • Continue Vitamin D supplementation if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation at a dose of 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium level every week and increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium is low, the participant will receive calcium 1,200 mg/day
ionized or corrected calcium level	low		
dietary calcium intake	normal or low		
PTH*	>65		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-19 with low ionized or corrected calcium and normal or low dietary calcium intake, follow the same supplementation guidelines.

Age	<9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ŦŦ	10-19	• 50000 IU (single dose) of vitamin D every other week x 4 total doses. • Check Vitamin D (D_2+D_3) levels in 8 weeks • Continue vitamin D supplementation until Vitamin D levels normalize. Once normalized vitamin D would be given as part of multivitamin supplementation.	• calcium supplementation a dose of 50 mg/k/day (max daily calcium dose of 2.000 mg/day) or the maximum allowed per TPN • Check ionized or corrected calcium level every week
ionized or corrected calcium levels	normal or low		
PTH*	>65		
Oral intake	No		
Patient on TPN	Yes		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-19 with normal ionized or corrected calcium, calcium supplementation at a dose of 50mg/kg/day 3 times daily is not needed, otherwise follow the same supplementation guidelines.

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ŦŦ	10-19	• 50000 IU (single dose) every week x 8 total doses • Check Vitamin D (D_2+D_3) levels in 8 weeks • Continue vitamin D supplementation until Vitamin D levels normalize. Once normalized vitamin D would be given as part of multivitamin supplementation.	• calcium supplementation a dose of 50 mg/kg/day (max daily calcium dose of 2.000 mg/day) or the maximum allowed per TPN • Check ionized or corrected calcium level every week
ionized or corrected calcium levels	normal or low		
PTH*	>65		
Oral intake	No		
Patient on TPN	Yes		

When PTH is <65 and Vitamin D level (D_2+D_3) is 10-19 with normal ionized or corrected calcium, calcium supplementation at a dose of 50mg/kg/day 3 times daily is not needed, otherwise follow the same supplementation guidelines.

7.3.4 Severe Vitamin D Deficiency

Age	1-3 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	<10	• Vitamin D 50000 IU (single dose) ; then 4000 IU/day starting day 2 for 8 weeks • Vitamin D (D_2+D_3) should be checked in 8 weeks and continue vitamin D if still low • Once Vitamin D normalized the participant would receive 600 IU/day of Vitamin D.	• calcium 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium every week and if low increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium intake is low, the participant will receive calcium 600 mg/day
dietary calcium intake			
PTH			
Ionized or corrected Calcuim			

Age	4-8 years	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	<10	• Vitamin D 50000 IU (single dose); then 4000 IU/day starting day 2 for 8 weeks • Vitamin D (D_2+D_3) should be checked in 8 weeks and continue vitamin D if still low • Once Vitamin D normalized the participant would receive 600 IU/day of Vitamin D.	• calcium 50 mg/kg divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium every week and if low increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium intake is low, the participant will receive calcium 1,000 mg/day
dietary calcium intake			
PTH			
Ionized or corrected Calcuim			

Age	≥9	Vitamin D	Calcium (elemental)
Vitamin D level (D_2+D_3) ₸	<10	• 50000 IU (single dose) on day 1; and then starting day 2, give 50000 IU (single dose) every week for 8 weeks x 9 doses total • Vitamin D (D_2+D_3) should be checked in 8 weeks and continue vitamin D if still low • Once Vitamin D normalized the participant would receive 600 IU/day of Vitamin D.	• calcium 50mg/kg/day divided 3x/day (max daily calcium dose of 2.000 mg/day) for 4 weeks • Check ionized or corrected calcium every week and if low increase supplementation as needed to normalize ionized or corrected calcium • Once ionized or corrected calcium is normalized, if dietary calcium intake is low, the participant will receive calcium 1,200 mg/day
dietary calcium intake			
PTH			
Ionized or corrected Calcuim			

Age	<9	Vitamin D	Calcium (elemental)
TPN	No Oral Intake	• give 50000 IU (single dose) of vitamin D on day 1; and then starting day 2, 50000 IU (single dose) every other week x 5 doses total • Vitamin D (D_2+D_3) should be checked in 8 weeks and continue vitamin D if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation 50 mg/kg/day or the maximum allowed per TPN

Age	≥9	Vitamin D	Calcium (elemental)
TPN	No Oral Intake	• give 50000 IU (single dose) of vitamin D every week x 8 doses total • Vitamin D (D_2+D_3) should be checked in 8 weeks and continue vitamin D if still low • Once normalized the participant would receive 600 IU/day of Vitamin D	• calcium supplementation 50mg/kg/day or the maximum allowed per TPN

If a subject's total calcium level is below the normal range at any time, calculate a corrected calcium if a serum albumin level is available (may have been drawn anytime within 7 days of the calcium level) using the formula:

Corrected Ca mg/dl = measured total Ca mg/dl+ [0.8 (4.0 - serum albumin g/dL)]

If a serum albumin level is not available ionized calcium level should be done.

VITAMIN D PREPARATIONS:

Over-the-counter (OTC): Cholecalciferol (preferred) or Ergocalciferol 400 IU, 800 IU, 1,000 IU, 2,000 IU, 4,000 IU, or 400 IU/ml

Prescription (Rx): Cholecalciferol (preferred) or Ergocalciferol 50,000 IU gel capsules or tablets prescribed for once weekly administration, Ergocalciferol 8,000 IU/ml

CALCIUM PREPARATIONS:

Over-the-counter (OTC): Calcium Carbonate: e.g., TUMS 500 mg (200 mg elemental calcium,), TUMS 750 mg (300 mg elemental calcium), TUMS 1,000 mg (400 mg elemental calcium), or TUMS kids 750 mg (300 mg elemental calcium) cherry flavored.

Rx: Calcium Carbonate: Oral suspension 1,250 mg/5 ml (elemental calcium = 500 mg/5 ml)

7.4 Pamidronate

7.4.1 Treatment Plan

Subjects randomized to the Pamidronate Group will receive 3 single doses of pamidronate at 3-month intervals (Day 90 +/-14 days, Day 180 +/-14 days, and Day 270 +/-30 days).

Note: All time point specific laboratory testing procedures (sections 8.1 and 8.3) must be collected prior to the pamidronate infusion (except those specifically designated post infusion); however for ease of scheduling the consultations as well as the Day 90 DXA and Day 90 pQCT may be performed after the infusion as long as done within the +/-14 day window. Ionized calcium or corrected calcium, and Vitamin D levels (D_2+D_3) will be drawn prior to pamidronate infusions. If either are low, the subject should receive supplemental calcium and/or Vitamin D per Section 7.3. If the subject is receiving supplemental calcium, an ionized or corrected calcium level should be drawn after the pamidronate infusion end but prior to discharge from clinic on the day of the pamidronate.

Prior to each infusion the patient will be assessed and pamidronate will be held if:

- Serum creatinine is > 2 times the baseline (Study Entry) level – refer to section 7.3.2 for monitoring plan – the pre-treatment creatinine may be done anytime within 72 hours prior to the planned pamidronate administration

- Pregnancy test is positive (subject will permanently discontinue study drug) per section 7.3.5 – pregnancy testing may be done anytime within 72 hours prior to the planned pamidronate administration

Pamidronate 1 mg/kg (to a max dose of 60 mg) will be administered via a single IV infusion over 4 hours using a line separate from all other drugs. Dosing will be based on the patient's actual weight or, if overweight, on ideal body weight (IBW). This will be followed by a normal saline flush of 20 cc total given at the same rate as the pamidronate.

Premedication: Subjects will be given 15 mg/kg acetaminophen (or maximum of 500 mg) prior to pamidronate infusion then every 4 hours as needed for a maximum of 24 hours.

Post-Pamidronate Infusion Laboratory Tests: A serum calcium level will be done after the pamidronate infusion end but prior to discharge from clinic on the day of the pamidronate.

A serum creatinine and a spot urine albumin and creatinine will be scheduled for the morning after each pamidronate infusion in the outpatient lab; however, some flexibility in timing is permitted and it will not be considered a deviation if testing is done within 7 days post-infusion. A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded. If done at an outside lab, the institutional study coordinator will oversee the necessary arrangements for testing and receipt of the results.

7.4.2 Treatment Plan Modification for Elevated Serum Creatinine

If at the time of a planned pamidronate infusion, the patient's serum creatinine level is >2 times that of the baseline level (done at Study Enrollment or Study Entry), pamidronate will be held.

If the creatinine level decreases to ≤ 2 times the baseline level within 2 weeks, the pamidronate may be given as planned.

If it remains elevated for more than 2 weeks, the subject will skip the scheduled dose of pamidronate. If at the time of the next pamidronate dose (if applicable) the subject's creatinine level has not returned to <2 times that of the baseline value pamidronate will be permanently discontinued, but the subject will continue vitamin D and calcium supplementation.

7.4.3 Expected Toxicities

The most common side effects in adults receiving pamidronate are hypertension, injection site reaction, hypocalcemia, hypokalemia, hypomagnesemia, hypophosphatemia, loss of appetite, nausea, vomiting, anemia, urinary tract infectious disease, cough, dyspnea, fatigue, and malaise. Other serious adverse effects that have been observed in adults are arthralgia, aseptic necrosis of bone of jaw, bone pain, myalgia, seizure, deteriorating renal function, focal segmental glomerulosclerosis, and nephrotoxicity.

In studies of pamidronate in children, hypocalcemia is also a common side effect. Brief acute phase reactions, including fever, headache, nausea, vomiting, rash, tachycardia, myalgia, and bone pain have also been noted on first exposure in pediatric patients. Uncommon side effects are nephrotoxicity, anterior uveitis, and atrial fibrillation. Due to these possible side effects, subjects will have laboratory evaluations performed to monitor for hypocalcemia or nephrotoxicity. Furthermore, any safety issue that might arise will be quickly recognized and treated as these subjects will be undergoing intensive monitoring following HCT.

Potential long-term side effects are radiographic metaphyseal bands, iatrogenic osteopetrosis, fractures after discontinuation of drug in growing children, delayed healing at osteotomy sites, esophageal cancer, and osteonecrosis of the jaw.

Long-term side effects are unlikely after a short-term treatment with pamidronate in patients without an underlying metabolic bone disease. There have been no reports of either esophageal cancer or osteonecrosis of the jaw in children after treatment with pamidronate.

Refer to the prescribing information for additional information.

7.4.4 Management of Treatment Related Side Effects

The research staff will monitor, as well as educate the subject and care providers, on early signs of potentially serious side effects. At each visit beginning at Day 90 post-HCT an assessment for adverse events will be completed per section 9.2.

Included are the following known risks of pamidronate:

1. Allergic reaction most commonly appearing as skin rash with or without itching
2. Anterior uveitis by subject's report (blurred vision, eye pain, redness,

sensitivity to light, irregular pupil)
3. Atrial fibrillation by the subject's report (palpitations, chest pain, shortness of breath)
4. Osteonecrosis of the jaw by subject's report (jaw pain, swelling, redness, or other signs of infection in the gums, gums or sockets that don't heal after dental work, numbness or a heavy feeling in the jaw, draining of pus, visible bone in the mouth, roughness of the gums in a particular area) and/or physical examination at study visits

Medical confirmation of diagnosis and appropriate intervention including, if indicated, referral to a specialist for further care, would be performed.

In the case of CTCAE grade 4 toxicity, no further pamidronate would be given per section 7.3.6.

7.4.5 Pregnancy Testing and Contraception Use

Since pamidronate is in the category D pregnancy risk group, a pregnancy test will be done within 72 hours prior to each dose and no further pamidronate will be given in the event of a positive result. Refer to section 9.3 regarding the reporting of pregnancy during study treatment. Post-menarchal subjects randomized to pamidronate will be put on an oral contraceptive pill (Loestrin or Orthocept or other OCP that contains 30 mcg ethinyl estradiol) or a birth control patch (e.g. Ortho Evra) or other effective means of birth control.

7.4.6 Criteria for Discontinuation

Subjects receiving pamidronate will discontinue pamidronate if any of the following conditions exist:

- Clinically significant allergic reaction (tongue or facial swelling or wheezing) to pamidronate
- Subsequently meets any of the study exclusion criteria (section 5.2) except for clinically significant current fracture, in which case pamidronate will be continued
- If creatinine level increases to > 2 times baseline level and a dose of pamidronate must be skipped for a 2nd time per section 7.3.2.
- Unacceptable toxicity (e.g. osteonecrosis of the jaw)
- Positive pregnancy test - Refer to section 9.3 regarding the reporting of pregnancy during study treatment.
- Disease relapse
- Subject's well-being, in the opinion of the Investigator, would be compromised by continuation of pamidronate administration.

These subjects will continue to be enrolled in the study, receiving vitamin D/calcium and participating in study procedures as planned unless contraindicated. An exception to this plan is for those patients who have a worsening of their disease (relapse) and discontinue routine post-HCT follow-up at the enrolling institution. In this situation, patients would be taken off study with no further study procedures.

Subjects who discontinue pamidronate treatment after the first dose (Day 90) will be included in the statistical analyses as members of the Pamidronate Group under the principle of intention-to-treat.

7.5 Expected Risks of Study Related Procedures

The risks of **venipuncture** are transient pain, bleeding, lightheadedness, bruising, possible vasovagal reaction, and infection. Only trained certified nursing assistants, nurses, nurse practitioners, or physicians will be allowed to perform venipuncture to minimize the risk of complication.

The **DXA and pQCT scans** will expose the subject to ionizing radiation. The level of exposure for this study will be approximately 3.4 millirem which is approximately 1% of that received from natural sources of radiation by a northern US resident in 1 year. Trained staff will perform the scans to minimize the risk of additional exposure. Children unable to sit still through the procedure may be given the option of mild sedation. The minor side effects of sedation include nausea, vomiting, mild allergic reactions, headache and dizziness.

The risks of **completing the questionnaires** may include feelings of sadness due to inability to have an active lifestyle and/or good food intake. Subjects and/or care providers will have the right to refuse to complete any questions that make them uncomfortable. Subjects and/or care providers may also refuse to complete one or more of the questionnaires.

7.6 Prohibited Concomitant Therapy

Prior and/or concomitant medical therapy with other bisphosphonates, Denosumab, or Teriparatide will not be permitted during the study.

Treatment with an investigational agent will also be prohibited within the 4 weeks prior to randomization (Day 90) and for the duration of the study (Day 90 through Day 360).

All other therapies are permitted.

7.7 Duration of Study Therapy

Refer to section 7.3.6 for regarding criteria for early discontinuation of pamidronate.

All subjects will receive calcium and vitamin D supplementation per protocol through the Day 360 post-HCT visit unless one of the following occurs:

- Subject voluntarily withdraws or their parent(s)/legal guardian(s) voluntarily withdraws them from the study
- Subject is not eligible for randomization at the Day 90 visit
- Subject requires treatment with a prohibited medical therapy per section 7.5
- Pregnancy
- Non-compliance
- Subject's well-being, in the opinion of the Investigator, would be compromised by continuation of pamidronate administration
- Disease relapse occurs and post-HCT follow-up ends
- The study ends early

Note: if a participant assigned to the Control Arm experiences a clinically significant fracture after Day 90, if medically appropriate, pamidronate treatment may be started (independent of the study and not covered by research funds). The participant could remain on study with analysis within the original study assignment (control).

Subjects discontinued from the study therapy but are considered evaluable for analysis (i.e. has been randomized and, if applicable has received at least 1 dose of pamidronate) will continue study participation per section 8 unless consent is withdrawn.

If a subject withdraws after an adverse event (AE), they will be followed until resolution of the issue or until their condition is stable.

In the event that a subject becomes pregnant, discontinuation, reporting, and follow-up procedures outlined in Section 9.3 will be followed.

7.8 Duration of Study Participation

Study participation will end at the Day 360 post-HCT visit (1 year post-HCT) unless the subject voluntarily withdraws or their parent(s)/legal guardian(s) voluntarily withdraws them from the study or disease relapse occurs and patient discontinues the planned post-HCT follow-up.

If a subject or their parent(s)/legal guardian(s) withdraws consent to participate in the study or care is no longer provided by the enrolling institution, attempts will be made to obtain survival data through 1 year post-HCT.

8 Study Related Tests and Procedures

8.1 Schedule of Tests and Procedures

	Screen[a]	Study Entry[b]	Day 0	Day 7 (±2)	Day 14 (+ 2)	Day 21 (±2)	Day 90 (±14)	Day 180 (±14)	Day 270 (±30)	Day 360 (±30)
Informed Consent / Assent	X or X									
Physical Examination	X[c] or X[c]									X[c]
Weight	X	X					X	X	X	X
Height		X								X
Medical History	X or X						X	X	X	X
Medication Usage	X or X					X	X	X	X	X
Creatinine	X or X						X	X	X	
Urine or serum Pregnancy Test[d,e]	X or X						X	X[l]	X[l]	X
Diet & Activity Questionnaire		X					X	X	X	X
Adverse Event Assessment							X	X	X	X
Ionized/ or corrected Calcium	X or X						X[f]	X[f]	X[f]	
Vitamin D (D_2+D_3)	X or X						X[g]	X	X	X
iPTH		X					X			X
CTX, P1NP, & OCN		X		X	X	X	X	X		X
Urinary DPD and Creatinine		X		X	X	X	X	X		X
RANKL & OPG		X		X	X	X	X			
IL-6, IL-7, & TNF-α		X		X	X	X	X			
DXA[h]		X					X			X
pQCT[i]							X			X
HCT			X							
Vitamin D & Calcium Administration[j]		X----------								X
Randomization – refer to section 6.2							X			
Pamidronate Administration[k]							X	X	X	
End of infusion (same day) serum calcium							X[l]	X[l]	X[l]	
Post infusion (next a.m.) [m] serum creatinine and spot urine for albumin and creatinine							X[l]	X[l]	X[l]	
Adverse event assessment by telephone 1 week post infusion (+/-3 days)							X[l]	X[l]	X[l]	
CTX = carboxy-terminal collagen crosslinks; DXA = dual-energy x-ray absorptiometry; DPD = deoxypyridinoline; HCT = hematopoietic cell transplantation; IL = interleukin; iPTH = intact parathyroid hormone; OCN = osteoclacin; OPG =										

osteoprotegerin; P1NP = procollagen type 1 amino-terminal propeptide; pQCT = peripheral quantitative computed tomography; RANKL = receptor activator of nuclear factor-κ B ligand; TNF-α = tumor necrosis factor alpha.

[a] Screening will occur during the pre-HCT work-up.

[b] Evaluations and tests only in the Study entry column are performed only after consent is signed and must be completed within 4 weeks (6 weeks for DEXA) prior to beginning pre-HCT conditioning regimen. If DEXA cannot be completed prior to conditioning, this will not be considered a protocol deviation

[c] Including Tanner staging of pubertal development.

[d] Females >10 years only.

[e] If documentation of a negative pregnancy test within 7 days prior to DXA scan is available, the pregnancy test does not need to be repeated.

[f] If result is below the lower limit of normal refer to section 7.2 and 7.3.

[g] Subjects who have vitamin D levels (D_2+D_3) <20 ng/ml at Day 90 will not be eligible for randomization per section per section 6.2.

[h] L1-L4 and total body scans with body composition.

[i] Subjects at the University of Minnesota (UMN) only.

[j] All subjects will begin taking supplemental vitamin D per day and calcium, if needed, to meet recommended dietary allowance (RDA) requirement upon admission to the hospital for the HCT and continue through Day 360. If a subject has low vitamin D (D_2+D_3), dietary calcium intake is below RDA, or the ionized/or corrected calcium level is below the normal range, additional vitamin D and/or calcium supplementation will be given per Section 7.3.

[k] For subjects randomized to the Pamidronate Group, 1 mg/kg (60 mg max) will be administered via intravenous (IV) infusion over 4 hours. Subjects will be given 15 mg/kg acetaminophen (or maximum of 500 mg) prior to infusion then every 4 hours as needed for a maximum of 24 hours. Postmenarchal subjects randomized to pamidronate will be put on an oral contraceptive pill (Loestrin or Orthocept or other OCP that contains 30 mcg ethinyl estradiol), birth control patch or other effective method of birth control. The results of the serum creatinine and pregnancy test (if applicable) must be reviewed before drug administration.

[l] Pamidronate patients only

[m] Serum creatinine level and spot urine collected the morning after each pamidronate infusion; however if not feasible at that time may be done anytime within 7 days after pamidronate infusion. A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded.

8.1.1 Study Protocol Compliance/Treatment Adherence

All doses of pamidronate will be administered at the clinical site.

For all subjects, adherence to the calcium and vitamin D regimen described in Section 7 will be evaluated by querying subjects every 3 months either at study visits or by telephone.

8.1.2 Deviations from the Clinical Protocol

When a deviation from the protocol is necessary for an individual subject, the Investigator must contact the Sponsor prior to the deviation (unless the deviation is safety related). The subject may continue in the study by mutual agreement of the Sponsor and the Investigator. A description of the deviation from the protocol and justification must be recorded on the Protocol Deviation Form.

8.1.3 Subject Compensation Guidelines

A$50 gift card will be given to each participant at study enrollment and at the Day 90 and 360 visits to partially compensate for the extra time required for study related tests and procedures on days of routine transplant associated visits.

Travel reimbursement (gas, parking, meals) will be offered at Day 270 study visit as it does not coincide with an institution's standard post-HCT follow-up.

Reimbursement (lodging, meals) will be offered to patients assigned to pamidronate at Day 180 and 270 visits for an overnight stay and meals in order to obtain the study related 24 hour post-infusion lab work.

It is recognized that this is a multi-center trial and the reimbursement days may differ between institutions. Therefore the above is a considered a guideline. Each institution's treatment consent will document the specific visits for their site which will be eligible for reimbursement.

Reimbursements will be made following individual institutional usual practice.

8.2 Clinical Procedures

8.2.1 Medical History

A study physician and/or study coordinator will review the following with the subject/parent(s) or legal guardian(s):

- Current and past prescription medications and doses (including calcium, vitamin D, oral contraceptives, estrogen or testosterone replacement, gonadotropin-releasing hormone [GnRH] analogs, L-thyroxine, glucocorticoids, and growth hormone),
- current and past medical history (with particular attention to fracture history, age at menarche, date of the last menstrual period, history of delayed puberty, and chronic inflammatory diseases), and
- family history

The subject's medical history will be reviewed at each study visit. This will include medical chart review and query of the subject and parent(s) or legal guardian(s) (as applicable) regarding any changes since the last study visit.

Pre-transplant treatment exposure data will be abstracted from the subject's medical records, including pre-transplant chemotherapy (agent and total cumulative doses, including glucocorticoids) and radiation therapy (sites and total cumulative doses with particular attention to cranial radiation). Transplant treatment exposure data is maintained in the electronic database of the Blood and Marrow Transplant programs at both sites and includes specific chemotherapy agents utilized in the transplant preparative regimen, dose of TBI (if given), and prophylaxis and/or treatment (glucocorticoids) for graft- versus-host disease (GVHD).

If a subject assigned the control arm is unwilling to return to the clinical site

for any visits not associated with a routine post-HCT follow-up, the subject or their parent(s)/legal guardian(s) will be contacted via telephone to discuss any changes in health since their last study visit.

8.2.2 Laboratory Evaluations

At the time points described in Section 8.1, blood will be collected for analysis of creatinine, ionized or corrected calcium, Vitamin D (D_2+D_3), iPTH, CTX, P1NP, OCN, RANKL, OPG, IL-6, IL-7, and TNF-α. Urine will also be collected for analysis of DPD, creatinine, and albumin. Analysis will be performed as described in Section 8.3.

For female patients > 10 years of age, a urine or serum pregnancy test will be performed at Screening and within 72 hours before administration of each dose of pamidronate for subjects randomized to the Pamidronate Group.

8.2.3 Medical Examination and Anthropometrics

A physical examination will be performed by a trained pediatric provider at study screen/entry and at the day 360 visit per section 8.1. Height will be recorded at study entry and on day 360. Weight will be recorded at study screening/entry and on days 90, 180, 270and 360. Tanner staging of pubertal development, including breast and pubic hair development in girls (52), and pubic hair development in boys (53) will be performed at each physical exam. Height will be measured standing without shoes with a wall-mounted stadiometer. Weight will be measured with subject in light clothing or dressing gown with shoes removed. These measurements will be used to calculate BMI as weight (kg) divided by height (m^2).

8.2.4 DXA and pQCT

Subjects will have DXA scans of L1-L4 and total body with body composition performed at the time points specified in Section 8.1 to compare BMC and body composition before and after study treatment. For the DXA scan, at least 2 vertebrae are required (i.e. L3-L4) and the same 2 vertebrae must be included on all follow-up exams.

If results from the DXA image suggest a compression fracture, the study PI or coordinator will confirm that the participant's primary care team knows of the results and appropriate follow-up (i.e. lumbar spine x-ray) is done. Note that there is no need to repeat an x-ray if (1) one was performed within the previous 12 months for the evaluation of the same vertebra with the highest BMD Z-score, and (2) was negative for fracture.

For subjects enrolled at the UMN, pQCT of the left tibia and non-dominant radius

will also be performed at the time points specified in Section 8.1.

8.2.5 Diet and Activity Questionnaire

A food frequency questionnaire (FFQ) to determine dietary calcium intake (Appendix II) and a physical activity questionnaire (Appendix III) will be completed by the subjects or their parent(s)/legal guardian(s) at the time points described in section 8.1.

If a subject is unable to return to the clinical site for Day 180 and/or Day 270 study visits, the study coordinator will send the questionnaires to the participant and/or parent/guardian with the intent to complete them via telephone at a pre-arranged time.

8.2.6 Unscheduled Visits

Additional visits will be scheduled as needed throughout the study period to address concerning signs or symptoms reported by subjects.

8.3 Laboratory Testing Procedures

During the study, creatinine, total calcium, Vitamin D (D_2+D_3), and intact parathyroid hormone (iPTH) will be analyzed to monitor subject safety.

Additionally, levels of markers of bone formation (OCN and P1NP) and resorption (CTX and DPD), cytokines (IL-6, IL-7, and TNF-α), RANKL, and OPG will be analyzed in batches or at the end of the study.

8.3.1 Laboratory Testing Procedures

Specimens for analysis of safety parameters, markers of bone turnover, cytokines, RANKL, and OPG will be collected as described in Table below at the time points indicated in section 8.1. Venipuncture or blood collection from a venous catheter (if the patient has one in place) will be performed by trained certified nursing assistants, nurses, nurse practitioners, or physicians and collection tubes will be handled per the manufacturer's recommendations.

Laboratory Analytes and Collection Information

Analyte	Matrix	Tube Type	Volume
Carboxy terminal collagen crosslinks (CTX)[a]	Plasma	K_2EDTA (di-potassium ethylenediaminetetraacetic acid; purple-top tube)	3 mL blood (minimum 700 µl plasma)
Procollagen type 1 amino terminal propeptide (P1NP)[a]	Plasma	K_2EDTA (purple-top tube)	
Osteoclacin (OCN)[a]	Plasma	K_2EDTA (purple-top tube)	
Receptor activator of nuclear factor-κ B ligand (RANKL)[a]	Plasma	K_2EDTA (purple-top tube)	
Osteoprogerin (OPG)[a]	Plasma	K_2EDTA (purple-top tube)	

Analyte	Matrix	Tube Type	Volume
Interleukin (IL)-6[b]	Plasma	K_2EDTA (purple-top tube)	3 mL blood (minimum 700 µl plasma)
IL-7[b]	Plasma	K_2EDTA (purple-top tube)	
Tumor necrosis factor alpha (TNF-α)[b]	Plasma	K_2EDTA (purple-top tube)	
Intact parathyroid hormone (iPTH)	Plasma	K_2EDTA (purple-top tube)	1 mL blood (minimum 300 µl plasma)
Urinary deoxypyridinoline (DPD)[d]	Urine	Urine specimen container	1 mL
Urine Creatinine[d]	Urine	Urine specimen container	

[a] Single sample for CTX, P1NP, OCN, RANKL, and OPG analysis collected in the same tube.
[b] Single sample for IL-6, IL-7, and TNF-α analysis collected in the same tube.
[c] Single sample for total calcium, vitamin D (D2+D3), and creatinine analysis collected in the same tube.
[d] Single sample for DPD and creatinine collected in the same container.

8.3.2 Specimen Processing and Storage

Research samples collected for the purpose of this study will be processed and stored frozen until the time of batch testing at the University of Minnesota, unless otherwise specified below. Upon completion of batch testing and verification of results at the University of Minnesota, any remaining samples will destroyed.

Vitamin D (D2+D3), Total Calcium, and Creatinine:
This testing will be done by the institutional lab with the results recorded in OnCore for the purposes of this study. Vitamin D will be measured by mass spectrometry.

PTH, intact
This testing will be done by the institutional lab with the results recorded in OnCore for the purposes of this study.

Plasma samples (CTX, P1NP, OCN, RANKL, OPG, IL-6, IL-7, and TNF-α):
Collect blood in 2 purple-top (ethylenediaminetetraacetic acid [EDTA]) tubes (3 mL each).

Refer to the Affiliate Lab Manual for sample processing and storage instructions.

Urine sample for DPD and Urine Creatinine:
Collect at least 1 mL of urine in a preservative-free urine specimen container. A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded. Collect urine mid-stream, voided directly into a sterile container (if possible). Avoid prolonged exposure to light, especially sunlight. During routine processing, samples are not affected by normal, artificial laboratory lighting.

Refer to the Affiliate Lab Manual for sample processing and storage instructions.

End of Pamidronate Infusion Serum Calcium:
A serum calcium level will be done after the pamidronate infusion end and prior to discharge from clinic on the day of the pamidronate per section 7.3.1.

Post Pamidronate Spot Urine for Albumin and Creatinine and Serum Creatinine:
Ideally the morning after each pamidronate infusion, the participant will return to the study center for a routine serum creatinine and a spot urine for albumin and creatinine to calculate albumin to creatinine ratio. A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded. The samples will be processed in the site's clinical laboratory but charged to research.

If the participant is unable or unwilling to return to the study center the morning after the pamidronate infusion, the samples may be collected within 7 days after the infusion. A morning collection is preferred, but not mandatory. The time of the urine collection must be recorded. Arrangements for the testing will be done on a per patient basis (i.e. at the study center on a different day or at an outside facility).

8.3.3 Specimen Processing and Storage - University of Minnesota

Fairview Acute Care Lab will process and store frozen (within 30-60 minutes of processing) per the study lab slips all research samples until the time of batch transfer to the Cytokine Lab (Dr. Angela Panoskaltsis-Mortari) for analysis.

8.3.4 Specimen Shipping – Fred Hutchinson Cancer Research Center

Samples collected at Fred Hutchinson Cancer Research Center will be processed at a local laboratory as detailed in the affiliate manual, then frozen in aliquots for CTX, P1NP, OCN, RANKL, OPG, IL-6, IL-7, TNF-α, and urinary DPD and creatinine and stored at frozen until the time of batch shipping to the UMN.

The samples are to be shipped Monday through Thursday only on dry ice in a Styrofoam container inside a cardboard sleeve. Send the samples via FedEx Priority Overnight to the address below:
Angela Panoskaltsis-Mortari Laboratory
Cytokine Lab
13-127 PWB
516 Delaware St. SE
Minneapolis, MN 55455
Phone: 612-626-7057

8.3.5 Laboratory Testing: Normal Values

Reference ranges for standard analytes are presented in section 8.3.1.

Laboratory Reference Ranges

	University of Minnesota (UMN)		Fred Hutchinson Cancer	
Creatinine (mg/dL)	0-21d	0.33-1.01	2 months-2 years	0.1-0.4
	22 days-8 years	0.15-0.53		
	9-14 years	0.39-0.73	2-10 years	0.1-0.6
	15-19y	0.5-1.0	10-14 years	0.2-0.9
	20 y and older, F	0.52-1.04	≥14 years	0.2-1.1
	20 y and older, M	0.66-1.25		
Calcium ("corrected calcium"?)	Age	mg/dL	8.7-10.7	
	0-11 mo	8.5-10.7		
	1-18 yr	9.1-10.3		
	19 yr & older	8.5-10.1		
Ionized Calcium	Age	mg/dL		
	0-11 mo	5.1-6.3		
	1 yr and older	4.4-5.2		
Vit D (D2 + D3) / 25-hydroxy (OH) vitamin D (ng/mL)	20-75		30-100	
Intact parathyroid hormone (iPTH) (pg/mL)	12-72pg/mL		12-75	
Interleukin (IL)-6 (pg/mL)	≤3.0		≤3.0	
IL-7 (pg/mL)	0.66-9.2		0.66-9.2	
Tumor necrosis factor alpha (TNF-α) (pg/mL)	≤8.8		≤8.8	
[a] IL-6, Il-7, and TNF-α samples from Fred Hutchinson Cancer Research Center will be analyzed at UMN.				

Laboratory normal ranges for CTX, DPD, P1NP, OCN, RANKL, and OPG vary with age. Urinary DPD will be adjusted for urinary creatinine content.

If total calcium or vitamin D (D2+D3)/25-OH vitamin D is below the normal range, supplementation will be administered per Section 7.3.

If creatinine level for a subject randomized to receive pamidronate becomes elevated during the study (creatinine level >2 times the baseline value at Study Entry) refer to section 7.3.2 for treatment modification and monitoring.

9 Event Monitoring, Documentation and Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

9.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Treatment-Emergent Adverse Event: Any event not present prior to the initiation of the treatment or any event already present that worsens in either intensity or frequency following exposure to the treatment. A treatment emergent AE refers to an event temporally related to the study treatment regardless of the causality assessment by the investigator.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

If either the IND sponsor or the investigator believes the event is life-threatening or serious, the event must be evaluated by the sponsor for expedited reporting (21CRF 312.32(a)).

Event Attribution Categories:
CTCAE does not define an AE as necessarily 'caused by a therapeutic intervention. The clinical investigator must assign attribution for an adverse event after naming and grading of the event.

Attribution	Description
Unrelated	The AE is clearly NOT related to the intervention
Unlikely	The AE is doubtfully related to the intervention
Possible	The AE may be related to the intervention
Probable	The AE is likely related to the intervention
Definite	The AE is clearly related to the intervention

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, adverse events that occur as part of the transplant or underlying disease are considered unexpected; however, they will not be reportable per section 9.4.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 9.4.

9.2 Documentation of Adverse Events

The goal of AE monitoring will be to focus on events that are related directly to study participation, not the transplant procedure or the disease under treatment unless study participation exacerbates the event. Due to the complex medical condition of the post-transplant patient, monitoring for adverse events will focus on events beginning with the 1st dose of pamidronate through the Day 360 visit.

For patients assigned to pamidronate, a toxicity assessment will be done in conjunction with each infusion (Day 90, 180, and 270) at the following time points:

- Prior to the pamidronate infusion - within 72 hours before the infusion
- 1 week (+/- 3 days) after each pamidronate infusion via telephone interview
- At any unplanned visit

At each of these time points the worst grade of the targeted toxicity since the previous assessment will be recorded in addition to any unexpected toxicities felt at least possibly related to pamidronate.

Of special concern are rare, but potentially serious risks associated with pamidronate which, if serious enough will lead to the discontinuation of the study drug per section 12.5. These targeted toxicities (based on CTCAE v4) will be collected on all patients (control and pamidronate) are:

- Anterior uveitis by subject's report (blurred vision, eye pain, redness, sensitivity to light, irregular pupil)
- Atrial fibrillation by the subject's report (palpitations, chest pain, shortness of breath)
- Osteonecrosis of the jaw by subject's report (jaw pain, swelling, redness, or other signs of infection in the gums, gums or sockets that don't heal after dental work, numbness or a heavy feeling in the jaw, draining of pus, visible bone in the mouth, roughness of the gums in a particular area) and/or physical examination at study visits.

All targeted toxicities, regardless of grade, and other events, grade 3 or greater that are felt at least possibly related to study treatment or cannot be explained by another cause (i.e. unexpected, not related to transplant, disease for which the transplant was done, underlying medical condition, etc.) will be recorded in OnCore for all patients.

After completion of the targeted toxicity form at day 360 formal collection of adverse events ends; however the sponsor/investigator must report upon knowledge any treatment related event meeting the expedited reporting criteria found in section 9.1 regardless of timing to pamidronate.

From signing of consent through the Day 360 visit any unexpected adverse event that is felt to be <u>at least possibly related</u> to the vitamin D and calcium and/or study procedures (research specimen collection, DXA) will be recorded in the study's case report forms.

9.3 Documentation of Pregnancy

If a female subject becomes pregnant during the study, she will be withdrawn from the study, the reason recorded in OnCore, and the study Sponsor/Investigator (Dr. Sarafoglou) notified. The pregnancy will be reported to the IRB according to local institutional reporting guidelines.

Though the subject will be withdrawn from the study, permission to record survival data for the mother to the end of the pregnancy and of the baby at birth will be requested

9.4 Required Reporting of Fred Hutchinson Cancer Research Center to UMN (IND Sponsor)

Events during the study period meeting the definition of serious, regardless of attribution or expectedness must be reported within 24 hours of knowledge to the University of Minnesota Study Coordinator.

Any event meeting the definition of an early study stopping event per section 12.5 must be reported within 24 hours of knowledge to the University of Minnesota Study Coordinator.

The UMN Study Coordinator will facilitate reporting to the University Of Minnesota IRB, Masonic Cancer Center, and the FDA as required.

University of Minnesota (contact information on page 5) with follow-up reporting to MCC Study Coordinator using the Event Report found in OnCore.

In addition, Fred Hutchinson Cancer Research Center will be responsible for submitting reportable events to their institutional IRB and any other required local regulatory entities.

9.5 UMN Required Reporting: FDA, IRB, and MCC's SAE Coordinator

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy to:
UMN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xraI0-sh	Within 5 business days of event discovery	Report Form	irb@umn.edu	MCC SAE Coordinator mcc-saes@umn.edu
FDA	Unexpected and fatal or life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Day	FDA prefers MedWatch 3500a Form however use of the UMCC SAE form is acceptable	Submit as an amendment to IND With copy to the affiliate PI	
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 Calendar-Day			
	Note: Events due to the disease under treatment or the transplant will not require expedited reporting to the FDA for the purposes of this study				
MCC SAE Coordinator	Stopping Rule Event – any grade 4 or 5 study-related toxicity in the pamidronate group or in either group (pamidronate or control) grade 3 or higher of the following selected toxicities: uveitis, atrial fibrillation or osteonecrosis of the jaw (ONJ)	at time of form completion	Event Form	mcc-saes@umn.edu	**n/a**

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports.

The SAE Coordinator will provide the Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

10 Study Status Updates, Data Collection and Monitoring

10.1 Study Status Updates

During the trial's period of active enrollment a weekly summary report will be generated from OnCore by the University of Minnesota summarizing enrollment and toxicity activity to date, as well as, any relevant clinical information. Sites will be expected to keep OnCore current to meet the demands of this real time summary reporting.

In addition, at least monthly teleconferences will be initially held between the PI (Dr. Sarafoglou), UMN study personnel, and key representatives of the affiliate site to discuss enrollment, treatment tolerability and toxicity, sample collection, as well as other relevant issues. Depending on the speed of enrollment and the level of patient issues, these teleconferences may be held more frequently or cancelled if there are no issues to discuss.

10.2 Data Management

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database utilizing study specific electronic case report forms. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore forms. Patient demographics, patient specific study treatment calendars, adverse events, reporting of deaths, and other information required for IND annual reporting will be placed in OnCore and other research databases maintained by MCC IT.

Subject questionnaire recording will be recorded in Oncore.

10.3 Data Capture Methods

All data will be collected at the site level by trained research study staff or site principal investigators using medical record documents, interviews, self-report instruments, lab reports, treatment strategies, and all related reports of outcome evaluations. Data from records abstraction, interviews, and participant self-reports will be entered by the research coordinator or other trained study staff at each site first onto paper CRFs and then into a secure, password-protected online database (OnCore). Copies of these paper CRFs will be retained along with other source documents in a participant file.

Participant data will be entered from the paper CRFs into electronic case report forms (e-CRFs). Both the paper and the e-CRFs will be approved by the study's

Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRFs based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

10.4 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp .

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will assess the study's progress at least quarterly.
- The S/I will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The S/I will oversee the submission of all reportable events per the definition of reportable in section 9.4 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND sponsor (Dr. Sarafoglou) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

10.5 Affiliate Site Monitoring

The PI (Dr. Sarafoglou) with the CTO has oversight responsibility for trial monitoring at affiliate sites. The affiliate site must self-monitor following the University of Minnesota Masonic Cancer Center Data and Safety Monitoring Plan (DSMP - http://z.umn.edu/dmsp) and the CTO Affiliate and Satellite Site Monitoring SOPs.

The investigator will permit study-related monitoring, audits, and inspections by the study's S/I and/or any designees, the local IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory

documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10.6 Record Retention

The investigator will retain study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient. Please contact the CTO before destroying any study related records.

11 Endpoints

11.1 Primary Clinical Endpoint

The primary endpoint is Lumbar BMC measured by DXA at 1 year after HCT (adjusted for height, age, sex, Tanner stage and race).

11.2 Secondary Clinical Endpoints

Other endpoints are:

- TBMC measured by DXA (adjusted for height, age, sex, Tanner stage and race).
- Estimated bone strength, total BMD, cortical BMD, and trabecular BMD of the left tibia and non-dominant radius measured by pQCT.
- Levels of markers of bone resorption (carboxy-terminal collagen crosslinks [CTX] and deoxypyridinoline [DPD]), and bone formation (procollagen type 1 N-terminal propeptide [P1NP] and OCN)
- RANKL, OPG, and RANKL/OPG ratio.
- Levels of cytokines including interleukin (IL)-6, IL-7, and tumor necrosis factor-alpha (TNF-α).
- Body composition: body mass index (BMI) percentile, lean body mass, and body fat mass (from DXA) adjusted for height.

12 Statistical Considerations

12.1 Sample Size

Sixty (60) subjects total (30 each in the Control and Pamidronate groups) gives 80% power to detect a difference between groups of 0.75 standard deviations (SDs)

describing variation in LBMC within study group after adjusting for height, age, sex, Tanner score, and race. Adjusting for age and sex, this SD is 5-8g (46, 47); adjusting further for race and height should reduce this SD by about half (48-50). This gives a within-group SD of about 2.5-4g and a detectable difference of 2-3g. To have 60 evaluable subjects, 192 need to be screened to allow for a 50% consent rate, 10% death rate by Day 90, 20% death rate between Day 90 and Day 360, and 10% drop out after randomization.

Anticipated Enrollment Over Duration of Study

	Screen	**Study Entry Day -14[a]**	**Day 7**	**Day 14**	**Day 21**	**Day 90**	**Day 180**	**Day 270**	**Day 360**
Total	192	96	96	96	96	86	86	86	60
University of Minnesota (UMN)	96	48	48	48	48	43	43	43	30
Fred Hutchinson Cancer Research Center	96	48	48	48	48	43	43	43	30
[a] Day 0 is the day of hematopoietic cell transplantation (HCT).									

According to data from 2011 to 2012, 36 allogeneic transplants have been performed at UMN and 37 have been performed at Fred Hutchinson Cancer Research Center. It is expected that screening 28 patients per year will be feasible for both sites.

It anticipated that enrollment will take 3.5 years.

12.2 Subject Population(s) for Analysis

All randomized subjects will be included in the primary analysis according to their randomized treatment assignment, consistent with the principle of intention-to-treat.

12.3 Randomization Scheme

The randomization will be stratified by age with 2 strata, < 10 years old and ≥ 10 years old, with 2/3 of the planned enrollment in the < 10 stratum. These fractions roughly reflect the proportion of each age group in the target study population. This choice of age groups is also based on our recent data which patients <10 years are more likely to be more severely affected (51). Each site's randomization schedule will use permuted blocks of lengths 2 and 4 within each stratum, so the study groups at a site never differ in sample size by more than 2 patients in

each stratum. The block lengths of 2 and 4 are alternated at random to make it harder to predict future treatment assignments. Randomized treatment assignments will be issued on Day 90 using a method that conceals the assignment until it is issued.

12.4 Statistical Methods

The primary analysis will compare the Pamidronate and Control groups according to LBMC measured by DXA using all available Day 360 measurements and adjusting for height, age, sex, Tanner score, and race. The analysis will be a multiple linear regression; the primary predictor will be randomized treatment assignment and adjusters listed above. If preliminary analysis shows that LBMC measurements have a grossly non-Gaussian (non- normal) distribution, a transformation of LBMC will be analyzed, most likely $\log_{10}$ LBMC Secondary analyses will include the following:

- The hypothesis that subjects randomized to pamidronate will have higher total BMD, cortical BMD, trabecular BMD, and estimated bone strength measured by pQCT at 1 year compared to subjects randomized to control (Aim 1 Hypothesis 2) will be tested by applying the foregoing analyses to outcomes measured by pQCT, namely bone strength, total BMD, cortical BMD, and trabecular BMD, but excluding height from the adjusters.

- The hypothesis that cytokine levels will increase rapidly in the first 3 weeks after HCT, preceding an increase in markers of bone resorption (Aim 2 Hypothesis 1) will be tested, for each cytokine, by using all available data for each child (up to 4 measurements from Day -14 to Day 21) and fitting a mixed linear model with a random intercept and time trend for each child and testing whether the average time trend differs from zero.

- The hypothesis that in the Control Group, increased cytokine levels and receptor activator of nuclear factor-κB ligand/osteoprotegerin ratio at 3 weeks will be associated with decreased BMC and BMD at 1 year after HCT (Aim 2 Hypothesis 2) will be tested using linear regressions with dependent variables being each of the BMC and BMD measures at Day 360 and predictors being changes in each of the cytokine levels and RANKL/OPG ratio (from Day -14 to Day 21).

- For the hypothesis that markers of bone resorption will increase in the first 3 months after HCT, will remain elevated until at least Day 180 in the

Control Group, but will decrease after initiation of pamidronate at Day 90 (Aim 3 Hypothesis 1), the test of changes up to Day 90 will use the same type of analysis as for Aim 2 Hypothesis 1 but with up to 5 measurements (Day -14 to Day 90). Tests of changes after Day 90 will include all available data for each subject and use a mixed linear model to account for correlation of measurements within subject; changes over time will be tested separately in the Pamidronate and Control groups using contrasts and compared between groups by testing the interaction of group and measurement time (i.e., Day 180, Day 360).

For the hypothesis that markers of bone formation, including osteocalcin (OCN) will decrease in the first three months and in the Control Group lower OCN levels at Day 90 will be associated with lower BMC and BMD 1 year post-HCT (Aim 3 Hypothesis 2), the test of change in OCN to Day 90 will use all available data and the same type of analysis as for Aim 2 Hypothesis 1; the test of change from Day 90 to Day 360 will use control subjects only and simple linear regression with outcome each of the BMC and BMD measures at Day 360 and predictor OCN level at Day 90.

For analyses of Aims 2 and 3 and Aim 1 hypothesis 2, further secondary analyses will use multiple imputation to impute missing measurements. Finally, exploratory analyses will explore risk factors for bone loss, including sex, BMI percentile, LBM adjusted for height, calcium and vitamin D intake, CNS radiation, TBI vs. no TBI in conditioning regimen, donor stem cell source (cord blood vs. other), physical activity, and glucocorticoid exposure for prophylaxis or treatment of GVHD. Subjects who experience a bone fracture after HCT will be excluded from analyses of bone turnover markers but otherwise will be included in all analyses.

12.5 Early Study Stopping Rule For Excessive Toxicity

Any grade 4 or 5 study-related toxicity in pamidronate-treated participants will result in a pause in study randomization for a re-evaluation of the treatment plan.

In addition to the foregoing, three toxicities will be monitored in participants in both the pamidronate and control groups: uveitis, atrial fibrillation, and osteonecrosis of the jaw (ONJ) of grade 3 or higher (as these toxicities will be recorded only if they are of grade 3 or higher). These toxicities will be monitored at interim analyses by comparing the two groups using a Lan-DeMets stopping rule with an O'Brien-Fleming alpha-spending function. For this monitoring purpose,

the outcome for each participant will be the count of AEs of any of these three types, and the P-value for comparing the groups will be computed using a permutation test that permutes groups labels on participants, with test statistic the difference between groups in their average numbers of AEs. Interim analyses will be performed every 6 months.

13 Ethical and Regulatory Considerations

13.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 Inclusion of Minorities

There are no opportunities for any active recruitment or outreach efforts that may increase minority participation beyond that of the pool of transplant patients that is referred to us.

The ethnic representation of the population within the catchment area in the states of Washington and Minnesota according to 2010 census is as follows:

- American Indian/Alaska Native 1-2%
- Asian 6-7%
- Native Hawaiian or Other Pacific Islander 0.04%
- Black or African American 4-8%
- White 79-81%
- More than one race 3-6%

The ethnic representation of children who received transplant at both institutions over the last 2-6 years is as follows:

- American Indian/Alaska Native 1-3%

- Asian 7-11%
- Native Hawaiian or Other Pacific Islander 0-1.5%
- Black or African American 1.5-9%
- White 72-77%
- more than one race 3-4%

This study has no exclusions based on race or ethnicity and will be presented to every patient who is referred for HCT who meets the eligibility criteria. Our efforts will focus on making sure that potential study subjects who are in the minority groups are enrolled in the study. We do expect minority representation in the study as indicated in the enrollment table based on the ethnic distributions of patients referred to both transplant centers in Minnesota and Washington. We will employ the following specific efforts to make sure that every ethnic minority patient with a hematologic malignancy or severe aplastic anemia who is referred for HCT will be given an opportunity to participate in the study (as listed in the enrollment table, particularly 7 Asians and 6 African Americans):

- At each transplant center the standard practice is that every patient who is referred for transplant is reviewed by a transplant coordinator for all open research studies that they would potentially be eligible for. Since a transplant coordinator is aware of all incoming patients, a transplant coordinator will then notify the study coordinator and a study PI (either myself or one of the study's co-investigators) about the appointment time for a pre-HCT evaluation who will be on site at the time of the visit. The study will be reviewed with the patient/family by a nurse coordinator for general overview information and this is then followed up with a meeting led by an attending transplant physician and a study PI (either myself or one of the study's co-investigators) where specific details of each study are presented, risks/benefits explained, and informed consent obtained.

    - Since each transplant program has access to culturally diverse healthcare interpreters, we will make sure that they are present at the time of the visit to assist in explaining the study and reviewing the consent forms for any non-English speaking minority patients as well as those who speak English, but are not native English speakers. To ensure a culturally sensitive approach in the recruitment of minority subjects who may have English as their first language, we will consult with or include in the consent meeting an impartial staff member (RN, advanced practice provider, social worker) of the same minority as the subject who would be able to facilitate verbal communication and interpretation of culturally sensitive issues even if language is not a barrier. We have confirmed that both institutions have staff members in the transplant programs

that represent at least individuals of African American, Hispanic and Asian descent.

- Patients and/or their parents/guardians will be given the option of taking copies of the consent documents with them with a phone number for the interpreter, and the study coordinator who will contact the patient or parents/legal guardians within a few days to schedule a screening visit if they are interested in participating.

- Fear and mistrust about clinical research is not uncommon, especially among minorities. Study staff and nurses in the clinic will take care to spend time educating each potential participant about clinical research and the goals of this study.

13.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant (parents/guardians for minors with assent for minor 7 years and older) decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

13.5 Protocol Amendments After Study Initiation

Should changes in the study plan or protocol become necessary in the course of the clinical trial, those specific changes will be discussed and agreed upon by the Sponsor, Investigator, and appropriate IRB approval obtained before the changes are implemented. All changes must be documented as protocol amendments. As a study conducted under an Investigational New Drug (IND), FDA notification may be required in addition to the IRB approval.

13.6 Anticipated Duration of the Clinical Investigation

Each subject will be enrolled in the study for approximately 1 year. It is expected that each of the study sites will screen 28 patients per year for 3.5 years with the final subject completing study participation 4.5 years after study initiation. The final study report will be completed approximately 5 years after study initiation.

If the study is terminated prior to completion, a closeout report will be filed with the Institutional Review Board (IRB) within 3 months of study termination.

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Appendix I – Eligibility Checklist

Refer to the Eligibility Checklist Case Report Form (CRF)

Appendix II – Food Frequency Questionnaire

Food Frequency Questionnaire

Name/Age:

_____Date:_____

We would like to know about some of the foods you eat. For each food listed please fill in how often you usually eat a portion of the size stated. If you eat the food:

- Every day or more than once a day, fill in how many times you have it per day
- Less than once a day but more than one a week, fill in the times per week
- Less than once a week, but more than once a month, fill in the times per month
- Less often than once a month, or never eat it, put an "X" under "do not eat".

Example: Janice has a glass of orange juice every morning, along with two slices of toast. She usually has two sandwiches at lunch, and eats French fries about 3 times per week. She almost never eats cauliflower.

	Per Day	Per Week	Per Month	Don't Eat
Orange juice, 1 cup	1			
French fries, regular serving		3		
Cauliflower, ½ cup				X
Bread or toast, 1 slice	6			

NUMBER OF TIMES I EAT THIS FOOD

	Per Day	Per Week	Per Month	Don't Eat
Bread or toast, 1 slice or 1 roll				
Muffin, 1 large				
Pizza, 1 medium slice				
Cheeseburger or veggie burger				
Cheese: 1 slice processed OR 1 piece hard cheese (plain or in sandwich)				
Broccoli, ½ cup				
Ice Cream, ½ cup				
Frozen yogurt, ½ cup				
Milkshake, 11 oz				
Cottage cheese, ½ cup				
Yogurt, 8 oz (small carton)				
Soft drink, 12oz				
Tofu, 2 oz				
Milk on cereal, 1 cup				
Orange juice, 1 cup				

Milk (any type), 1 cup				
Macaroni & cheese, 1 cup				

Is your orange juice fortified with calcium?
_____No
_____Yes

Formula Intake
_____Not Applicable
_____Formula Information: ⟶ Name/Brand: _____

Volume/Day _____ or _____
(oz) (ml)

Are you allergic to any foods?
_____No
_____Yes: what foods?_____

THANK YOU!

Completed By: _____

Date: _____

Appendix III - Physical Activity Questionnaires

Outdoor Playtime Recall Questionnaire

Think for a moment about a typical weekday for your child **in the past 7 days**.

1. How much time would you say your child spends playing outdoors on a typical weekday?

_____ Hours _____Minutes

Now think about a typical weekend day for your child **in the last month**.

2. How much time would you say your child spends playing outdoors on a typical weekend day?

_____ Hours _____Minutes

3. How many hours per day, **during the week,** would you say your child spends watching television in each season of the year?

_____Too young, does not watch TV

_____Spring _____Summer _____Fall _____Winter

4. On Saturday and Sunday, how many hours per day, does your child spend watching television in each season of the year?

_____Too young, does not watch TV

Saturday:

_____Spring _____Summer _____Fall _____Winter

Sunday:

_____Spring _____Summer _____Fall _____Winter

5. How many hours per day, **during the week**, does your child recreationally use the computer, play video games or participate in other screen-related activities in each season of the year?

_____Too young, does not watch TV

_____Spring _____Summer _____Fall _____Winter

6. On Saturday and Sunday, how many hours per day does your child recreationally use the computer, play video games or participate in other screen-related activities in each season of the year?

_____Too young, does not watch TV

Saturday:

_____Spring _____Summer _____Fall _____Winter

Sunday:

_____Spring _____Summer _____Fall _____Winter

Godin Leisure-time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

Times per Week

a. **STRENUOUS EXERCISE**
(HEART BEATS RAPIDLY) _____
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

b. **MODERATE EXERCISE**
(NOT EXHAUSTING) _____
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

c. **MILD EXERCISE**
(MINIMAL EFFORT) _____
(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snowmobiling, easy walking)

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	SOMETIMES	NEVER/RARELY
1. [ ]	2. [ ]	3. [ ]

3. How many hours per day, **during the week,** do you watch television in each season of the year?

_____Spring _____Summer _____Fall _____Winter

4. **On Saturday and Sunday,** how many hours per day, do you watch television in each season of the year?

Saturday:

_____Spring _____Summer _____Fall _____Winter

Sunday:

_____Spring _____Summer _____Fall _____Winter

5. How many hours per day, **during the week**, do you recreationally use the computer, play video games or participate in other screen-related activities in each season of the year?

_____Spring _____Summer _____Fall _____Winter

6. **On Saturday and Sunday,** how many hours per day do you recreationally use the computer, play video games or participate in other screen-related activities in each season of the year?

Saturday:

_____Spring _____Summer _____Fall _____Winter

Sunday:

_____Spring _____Summer _____Fall _____Winter