A Double-blind, Placebo-controlled, Crossover Study of Magnesium Supplementation in Patients with XMEN Syndrome

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Protocol Summary

Full Title:	**A double-blind, placebo-controlled, crossover study of magnesium supplementation in patients with XMEN syndrome**
Short Title:	XMEN syndrome and magnesium
Clinical Phase:	Phase 1 and 2
Conducted by:	NIAID/LI
Principal Investigator:	Juan Ravell, MD
Sample Size:	n=20 (n=10 for each cohort)
Accrual Ceiling:	n=30
Study Population:	Patients ≥6 years with X-linked immunodeficiency with magnesium defect and increased susceptibility to Epstein-Barr virus (EBV) infection and neoplasia (XMEN) syndrome. Subjects will be divided into 2 cohorts: **Cohort 1 (high EBV):** Subjects with baseline blood EBV viral load ≥5,000 copies/mL or EBV log ≥3.7 IU/mL. **Cohort 2 (low/no EBV):** Subjects with baseline blood EBV viral load <5,000 copies/mL or EBV log <3.7 IU/mL.
Accrual Period:	4 years
Study Design:	A randomized, double-blind, placebo-controlled, crossover study to evaluate the safety and efficacy of oral magnesium L-threonate in subjects with XMEN syndrome. In Part I, patients within each cohort will be randomized into 2 arms. Each arm will receive 12 weeks of either oral magnesium L-threonate or placebo followed by crossover treatment for another 12 weeks. Patients in cohort 1 who experience ≥0.5-log reduction in the number of EBV-infected B cells and patients in cohort 2 who experience ≥2-fold increase in NKG2D receptor expression on CD8 T cells during oral magnesium treatment as compared to placebo will have completed the study. Patients who do not meet these respective criteria will undergo a 2-week washout and transition to Part II in which they will be hospitalized to receive 3 days of IV $MgSO_4$. These patients will then restart escalating doses of oral magnesium and continue for 24 weeks, at which point participation will be complete.
Study Duration:	Start Date: 6/1/2015; End Date: 6/1/2020 Total length of individual subject participation: either 27 or 53 weeks

Study Agent/ Intervention Description: Part I: 12 weeks of oral magnesium L-threonate at escalating, weight-based doses ranging from 96 mg to 384 mg daily, and 12 weeks of placebo.

Part II: 3 days of IV $MgSO_4$ at 30 mg/kg/day, followed by escalating doses of oral magnesium L-threonate for 24 weeks.

Primary Objectives:

- Cohort 1: To investigate the efficacy of magnesium supplementation in patients with XMEN syndrome by comparing the absolute number of EBV-infected B cells after 12 weeks of oral magnesium and after 12 weeks of placebo.
- Cohort 2: To investigate the efficacy of magnesium supplementation in patients with XMEN syndrome by comparing NKG2D receptor expression on CD8 T cells after 12 weeks of oral magnesium and after 12 weeks of placebo.

Secondary Objectives:

- To assess the safety and tolerability of $MgSO_4$ IV infusion and oral magnesium L-threonate.
- To evaluate the effects of magnesium supplementation on intracellular free magnesium (Mg^{2+}) concentrations in peripheral blood T cell lymphocytes.
- Contingent upon patient participation in Part II, to study the effect of 12 weeks vs. 24 weeks of oral magnesium supplementation after 3 days of IV magnesium on EBV viral load and number (or percent) of EBV-infected B cells (cohort 1) or NKG2D receptor expression on CD8 T cells (cohort 2) in patients with XMEN syndrome.
- To evaluate the difference in NKG2D expression between patients with high EBV titers and patients with low or negative titers.

Exploratory Objectives:

- To assess the effect of magnesium supplementation on lymphocyte subset measures.
- To quantify absolute and relative numbers of EBV-infected B cells using an assay combining cell-surface markers via flow cytometry and fluorescent *in situ* hybridization (FISH) analysis of the EBV genome.
- To study the effects of magnesium supplementation on preexisting abnormal biological markers, i.e., NKG2D expression in natural killer (NK) cells, anemia, neutropenia, thrombocytopenia, and abnormal liver enzymes.
- Assess the effect magnesium on clinical findings and health-related outcomes.

Primary Endpoints:

- Cohort 1: Difference between the absolute number of EBV-infected B cells (by EBV FISH) after 12 weeks of oral magnesium supplementation and after 12 weeks of placebo.
- Cohort 2: Difference between NKG2D expression in CD8 T cells after 12 weeks of oral magnesium supplementation and after 12 weeks of placebo.

Secondary Endpoints:

- Incidence and severity of adverse events (AEs) throughout the study.
- Contingent upon patient participation in Part II, differences in EBV viral load and number (or percent) of EBV-infected B cells (cohort 1) or NKG2D expression in CD8 T cells (cohort 2) by duration (12 weeks of IV+oral versus 24 weeks of IV+oral) of magnesium supplementation regimen.
- Change in the intracellular pool of free Mg^{2+} by duration of magnesium supplementation regimen.
    - evaluate the difference in NKG2D expression between patients with high EBV titers and patients with low or negative titers.

Exploratory Endpoints:

- Change in CD4 T cell counts, B cell counts (or percent), and/or in NKG2D expression on NK (CD3-CD56+) cells.
- Quantification of EBV-infected B cells as determined by flow cytometry and FISH.
- Percent of anemia, neutropenia, thrombocytopenia, and abnormal liver enzymes.
- Difference in number and severity of infections between placebo and magnesium treatment periods.
- Difference in number of health-related days of missed school/work between placebo and magnesium treatment periods.
- Difference in number of emergency room visits and hospital admissions between placebo and magnesium treatment periods.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CBC	Complete Blood Count with Differential
CMV	Cytomegalovirus
CNS	Central Nervous System
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C-Reactive Protein
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr Virus
EKG	Electrocardiogram
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FISH	Fluorescent *In Situ* Hybridization
GCP	Good Clinical Practice
HHV8	Human Herpesvirus 8
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
ICH	International Conference on Harmonization
IDMRS	Investigational Drug Management Research Section
IRB	Institutional Review Board
IU	Inconvenience Unit
IV	Intravenous
LI	Laboratory of Immunology
LID	Laboratory of Infectious Diseases
MAGT1	Magnesium Transporter 1
MFI	Mean fluorescent intensity
Mg^{2+}	Magnesium Ion
$MgSO_4$	Magnesium Sulfate
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer (cells)
NKG2D	Natural Kill Cell Group 2-D Receptor
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PT	Prothrombin Time
PTT	Partial Thromboplastin Times
RNA	Ribonucleic Acid
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Serum Magnesium Concentration
TCR	T Cell Receptor
UP	Unanticipated Problem

XMEN	X-linked immunodeficiency with Magnesium deficiency, Epstein-Barr Virus infection and Neoplasia

Précis

X-linked immunodeficiency magnesium defect, Epstein-Barr virus (EBV) infection and neoplasia (XMEN) syndrome is a primary immunodeficiency caused by the loss of expression of the magnesium transporter 1 (MAGT1). This syndrome is associated with CD4 lymphopenia, chronic EBV infection in most patients, and EBV-related lymphoproliferative disorders. The loss of MAGT1 leads to impaired T cell activation and decreased expression of the activator receptor, NKG2D on natural killer (NK) cells and CD8 T cells, leading to decreased EBV-specific cytolytic function of these cells. Results of previous studies suggest that magnesium supplementation may be a viable therapeutic option for patients with XMEN.

The proposed study has 2 parts, and patients will be divided into 2 cohorts. Patients in cohort 1 (high EBV group) will have baseline blood EBV viral load ≥5,000 copies/mL or EBV log ≥3.7 IU/mL. Patients in cohort 2 (low/no EBV group) will have baseline blood EBV viral load <5,000 copies/mL or EBV log <3.7 IU/mL. Part I is a randomized, double-blind, placebo-controlled, crossover study to evaluate the safety and efficacy of oral magnesium L-threonate in patients with XMEN syndrome. Within each cohort, patients will be randomized to receive escalating doses of either placebo or oral magnesium L-threonate for 12 weeks. Patients will then receive the crossover treatment (magnesium or placebo) for an additional 12 weeks. For patients who experience a 0.5-log decrease in the number of EBV-infected B cells (cohort 1) or a ≥2-fold increase in NKG2D receptor expression on CD8 T cells (cohort 2) with oral magnesium as compared to placebo, the study will be complete. Patients who do not meet this efficacy outcome will undergo a 2-week washout period and proceed to Part II, an open-label, non-randomized evaluation of intravenous magnesium sulfate ($MgSO_4$) followed by oral magnesium L-threonate. These patients will be hospitalized to receive 3 days of intravenous $MgSO_4$ in 3 daily doses totaling 30 mg/kg/day. They will then restart escalating doses of oral magnesium L-threonate and continue for the remaining 24 weeks of Part II. If conducted, Part II will allow for secondary analyses to compare different durations of magnesium supplementation.

1 Background Information and Scientific Rationale

1.1 Background and Summary of Relevant Pre-clinical Studies

Mg^{2+} is essential for many physiological processes. The majority of the intracellular Mg^{2+} (14 to 20 mM) is bound to nucleotides and proteins and is essential for energy metabolism, DNA transcription and protein synthesis [1]. The remaining unbound, intracellular free Mg^{2+} (0.5-1 mM) is tightly regulated. Under normal conditions, the magnesium transporter, MAGT1, participates in the intracellular Mg^{2+} homeostasis and facilitates a transient Mg^{2+} influx into T cells, which is a necessary step in the activation of those cells [2]. The loss of MAGT1 expression causes an immunodeficiency named XMEN syndrome associated with CD4 lymphopenia, chronic EBV infection, and EBV-related lymphoproliferative disorders [2-4]. XMEN patients may present with an inverted CD4:CD8 ratio and a reduced number of CD31+ cells in their naïve CD4 T cell population, suggesting decreased thymic output [2, 3]. Additionally XMEN is associated with B cell lymphocytosis due to chronic EBV infection [3]. The loss of MAGT1 leads to impaired T cell activation and decreased expression of the activator receptor, NKG2D on NK cells and CD8 T cells, leading to decreased EBV-specific cytolytic function of these cells [2-4]. These individuals may have reduced immunoglobulin production or a defective specific antibody response secondary to T lymphocyte dysfunction, but B cells are generally unaffected [2-4]. In addition to EBV infection, some XMEN patients have recently been found to have increased susceptibility to disease associated with other viral infections, including human herpesvirus 8 (HHV8) and herpes simplex virus (HSV). Similarly, non-EBV driven malignancies (eg, sarcoma) have been reported. **Figure 1** summarizes the main clinical and molecular features of XMEN disease.

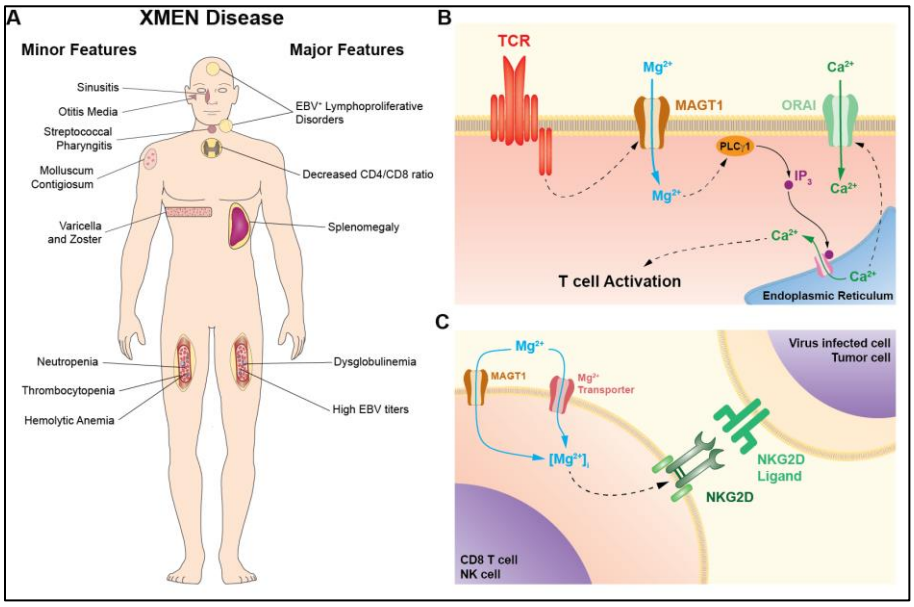


Figure 1: XMEN disease. (**A**) XMEN disease clinical features. Major features are present in almost all XMEN patients, while minor features are only found in some. (**B**) Model of the role of MAGT1 in T Cell Receptor (TCR) activation. In normal T cells, stimulation of the TCR triggers a Mg^{2+} flux through MAGT1. This transient increase of intracellular Mg^{2+} is required for the activation of phospholipase C γ1 (PLCγ1) required for the downstream generation of Ca^{2+} flux through ORAI by promoting the release of Ca^{2+} from the endoplasmic reticulum via Inositol 1,4,5-Triphosphate (IP_3) and its receptor. (**C**) Model of role of MAGT1 in Mg^{2+} homeostasis and NKG2D expression. In XMEN patients, the absence of MAGT1 leads to the chronic reduction of intracellular free Mg^{2+}, which is required to maintain the expression of NKG2D. Adapted from [3]

We analyzed null mutations in the X-linked gene encoding for MAGT1 in a group of 8 subjects. These mutations lead to the loss of MAGT1 protein expression. At a cellular level, the loss of MAGT1 has 2 main consequences: i) the loss of a TCR-induced transient Mg^{2+} influx required for optimal T cell activation and ii) a chronic decrease in intracellular free Mg^{2+} without affecting the total or bound intracellular Mg^{2+}. This latter defect is linked to the loss of expression of the NKG2D receptor on NK and CD8 T cells. *In vitro* studies of XMEN patients' cells show that supplementation of the tissue culture medium with 1 mM, 2 mM, or 5 mM of $MgSO_4$ leads to a partial but significant recovery of NKG2D expression and better cytotoxic function in both NK and CD8 T cells. Preliminary observational *in vivo* studies in 4 patients with XMEN have shown oral magnesium L-threonate to be a safe and well-tolerated therapeutic option. These patients experienced improvement in intracellular magnesium concentrations, NK and CD8 T cell function, and NKG2D expression, decreased EBV viral loads and a decrease in the number of EBV-infected B cells as determined by FISH **(Figure 2)**. Furthermore, in our experience, EBV-naïve patients who have started magnesium supplementation have also reported less frequent infections.

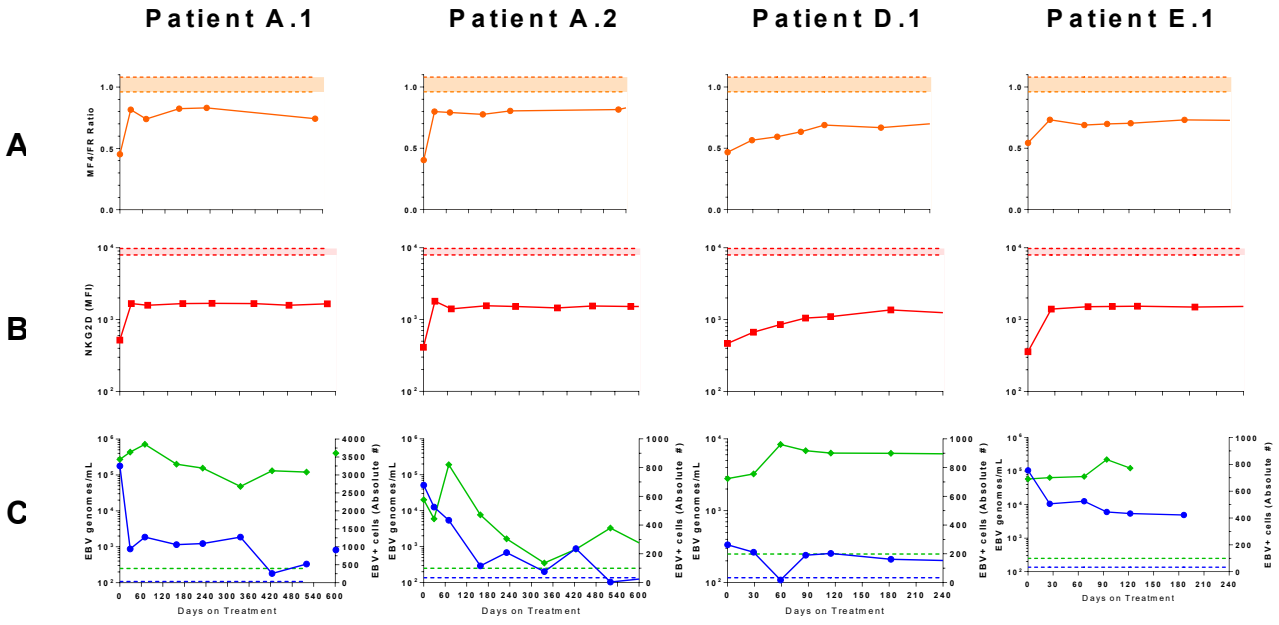


Figure 2. Effect of Mg^{2+} supplementation with Mg^{2+} L-threonate in 4 XMEN patients

(**A**) Quantification of the intracellular free Mg^{2+} with MF4/FR ratio. MF4/FR increased with oral magnesium L-threonate treatment. MF4/FR refers to the ratio of the mean fluorescent intensity (MFI) of the magnesium-specific fluorescent probe MagFluo4 (MF4) to the MFI of the calcium-sensitive probe Fura Red (FR). (**B**) NKG2D expression on CD8 T cells. NKG2D expression improved with magnesium treatment. (**C**) EBV blood levels quantified by PCR (green curve) and absolute number of EBV-infected cells quantified by EBV-FISH (blue curve) during magnesium supplementation. The absolute number of EBV-infected cells decreased with treatment while the EBV viral load transiently increased to subsequently decrease for patients A.1 and A.2. For patients A.3 and A.4 EBV viral load has not changed significantly over a shorter follow-up period. Orange and red shaded areas represent normal range; blue and green dotted lines represent minimum detection levels. The decrease in the number of EBV-infected B cells correlates with improved cytotoxic function achieved by an increase in NKG2D expression. The transient elevation and delayed decline in EBV viral load determined by PCR is an expected result due to release of EBV DNA from dying infected cells into the peripheral circulation.

Improved cytotoxic function against EBV-infected cells usually leads to a transient increase in the EBV DNA measured in blood samples. This is due to EBV DNA being released from dying EBV-infected cells into the peripheral circulation as we have observed in EBV-infected patients treated with the anti-CD20 monoclonal antibody rituximab. Quantification of EBV-infected B cells would therefore better assess variations in treatment efficacy than EBV DNA determination by polymerase chain reaction (PCR). Our preliminary data indicate that the number of EBV-infected B cells as determined by FISH is a better indicator of EBV suppressive cytolytic function *in vivo*. Quantification of EBV-infected B cells by a flow cytometric FISH assay enables detection of EBV-encoded small RNA (EBER) in B cells. This direct method to quantify EBV-infected cells and simultaneously characterize B cell lymphocytes has been shown to be accurate and reproducible [5]. **Figure 3 (A, B, and C)** summarizes our validation data for quantification of EBV-infected cells by FISH. Some studies have shown correlation between the number of EBER-positive cells and the EBV DNA viral load determined by quantitative PCR in patients with active EBV infection [6]. However, improved cytotoxic function against EBV-infected cells usually leads to a transient increase in the EBV DNA viral load despite a reduction of infected cell number due to the release of viral DNA in the blood. Thus, numbers of EBV-infected B cells could more rapidly reflect the effect of magnesium supplementation treatment than EBV viral load by PCR. **(Figure 3 D, E, and F).**

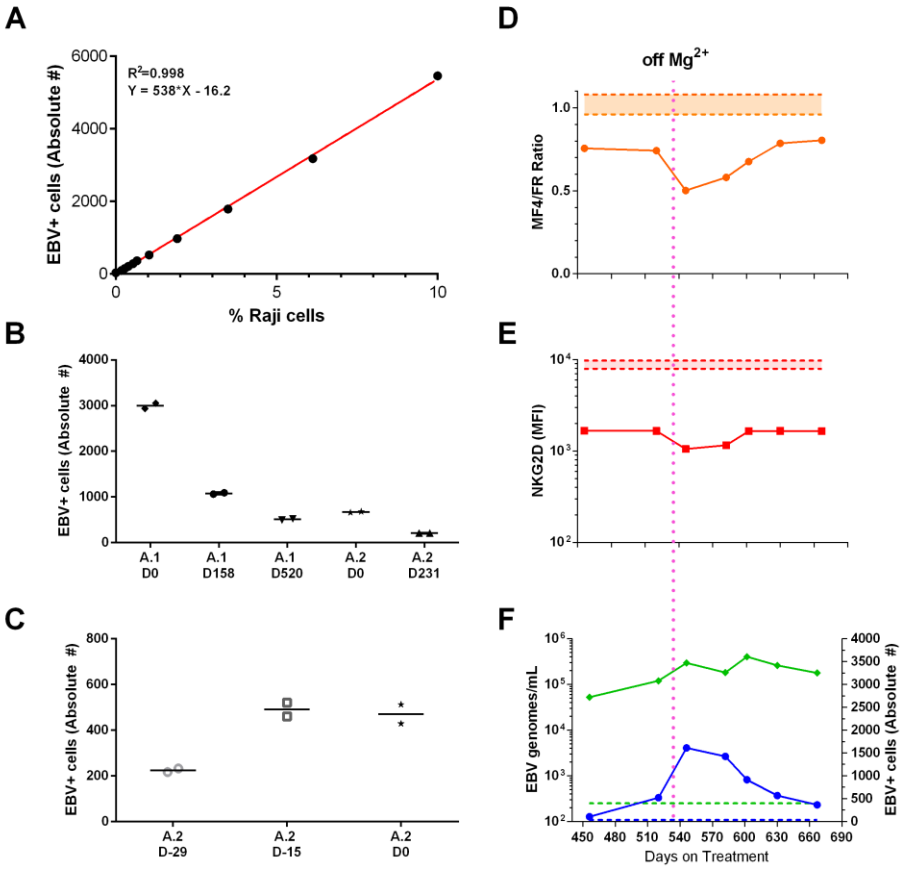


Figure 3. Quantification of EBV-infected B cells by EBV FISH. (**A**) Calibration curve of the FISH assay using a known amount of EBV+ cells (Raji) mixed with Normal PBMCs. (**B**) Examples of 2 EBV FISH measurements on the same samples performed at different time (2 symbols) for patient A.1 and A.2 at different time points after treatment. The X-axis represents days of treatment. (**C**) Examples of 2 EBV FISH measurements on the same samples performed on the same individual (A.2) from different blood draws prior to Mg^{2+} supplementation. Patient A.1 was sick for a week and could not take his Mg^{2+} capsules (pink dotted line). As a result, his intracellular Mg^{2+} (**D**), and NKG2D level (**E**) dropped, and his EBV (**F**), especially per FISH, went back up.

1.1.1 **Rationale**

Effective administration of Mg^{2+} preparations may increase intracellular free Mg^{2+} without increasing serum Mg^{2+} to supraphysiologic levels. This would lead to an improvement in NK and CD8 T cell functions and NKG2D expression, thereby reducing the EBV load. Reduced EBV load and improved cytolytic function may reduce the risk of EBV-induced lymphoma or delay its onset.

Additionally, our group has shown (in a single XMEN patient) that IV Mg^{2+} supplementation improves NKG2D expression and intracellular free Mg^{2+} while decreasing the percentage of EBV+ cells with faster kinetics than oral supplementation. **(Figure 4)**. This intervention was well tolerated with no clinically significant adverse effect noted [4].

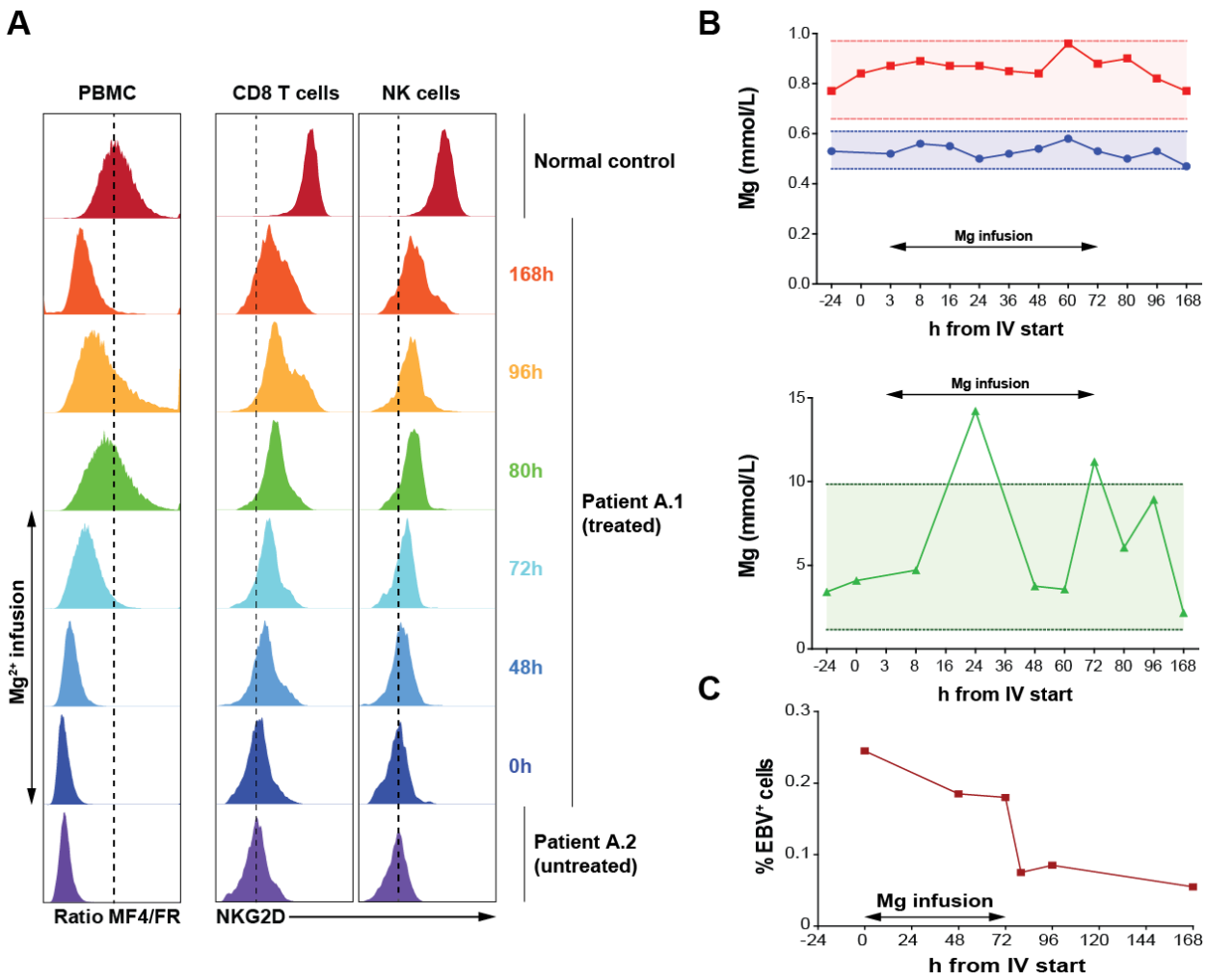


Figure 4: Intravenous Mg^{2+} infusion for 3 days (72h) is sufficient to improve basal intracellular free Mg^{2+} and NKG2D expression in patient A.1. (A) Flow cytometry profiles of MF4/FR ratios (left panel) in PBMCs and NKG2D expression on NK cells and CTLs (right panel) from a healthy control, patient A.1 (treated), and patient A.2 (untreated) during oral *in vivo* Mg2+ supplementation trial at the indicated time in hours (h). MF4/FR refers to the ratio of the mean fluorescent intensity (MFI) of the magnesium-specific fluorescent probe MagFluo4 (MF4) to the MFI of the calcium-sensitive probe Fura Red (FR). (B) Blood levels of total magnesium (red line) and ionized magnesium (blue line, upper graph) as well as urine magnesium levels (green line, lower graph) during trial. Shaded areas represent normal ranges. (C) Percentage of EBV+ cells in the treated patient's PBMCs measured by EBV-specific FISH over time shown in hours (h). The double-headed arrow shows the time of magnesium administration. Adapted from [4].

Based on these results showing that IV magnesium was more effective (faster and increased biological effect) in restoring intracellular magnesium and NKG2D expression than oral magnesium, we hypothesize that an initial IV infusion prior to oral supplementation may be more effective than oral supplementation alone.

The effects on serum magnesium concentration (SMC) after IV versus oral delivery of magnesium in cardiovascular critical care was recently reviewed by Reed and colleagues [7]. These authors found that, although consistent elevations in SMC were produced by oral magnesium delivery, IV administration resulted in greater and more rapid elevations relative to

baseline SMC. Furthermore, magnesium administered at the dose proposed in our protocol has safely been given to treat a variety of conditions in both children and adults.

2 Study Objectives

2.1 Primary Objectives

- Cohort 1: To investigate the efficacy of magnesium supplementation in patients with XMEN syndrome by comparing the absolute number of EBV-infected B cells after 12 weeks of oral magnesium and after 12 weeks of placebo.
- Cohort 2: To investigate the efficacy of magnesium supplementation in patients with XMEN syndrome by comparing NKG2D receptor expression on CD8 T cells after 12 weeks of oral magnesium and after 12 weeks of placebo.

2.2 Secondary Objectives

- To assess the safety and tolerability of $MgSO_4$ IV infusion and oral magnesium L-threonate.
- To evaluate the effects of magnesium supplementation on intracellular free Mg^{2+} concentrations in peripheral blood T cell lymphocytes.
- Contingent upon patient participation in Part II, to study the effect of 12 weeks vs. 24 weeks of oral magnesium supplementation after 3 days of IV magnesium on EBV viral load and number (or percent) of EBV-infected B cells (cohort 1) or NKG2D receptor expression on CD8 T cells (cohort 2) in patients with XMEN syndrome.
- To evaluate the difference in NKG2D expression between patients with high EBV titers and patients with low or negative titers.

2.3 Exploratory Objectives

- To assess the effect of magnesium supplementation on lymphocyte subset measures.
- To quantify absolute and relative numbers of EBV-infected B cells using an assay combining cell-surface markers via flow cytometry and FISH analysis of the EBV genome.
- To study the effects of magnesium supplementation on preexisting abnormal biological markers, i.e., NKG2D expression in NK cells, anemia, neutropenia, thrombocytopenia, and abnormal liver enzymes.
- Assess the effect magnesium on clinical findings and health-related outcomes.

3 Study Design

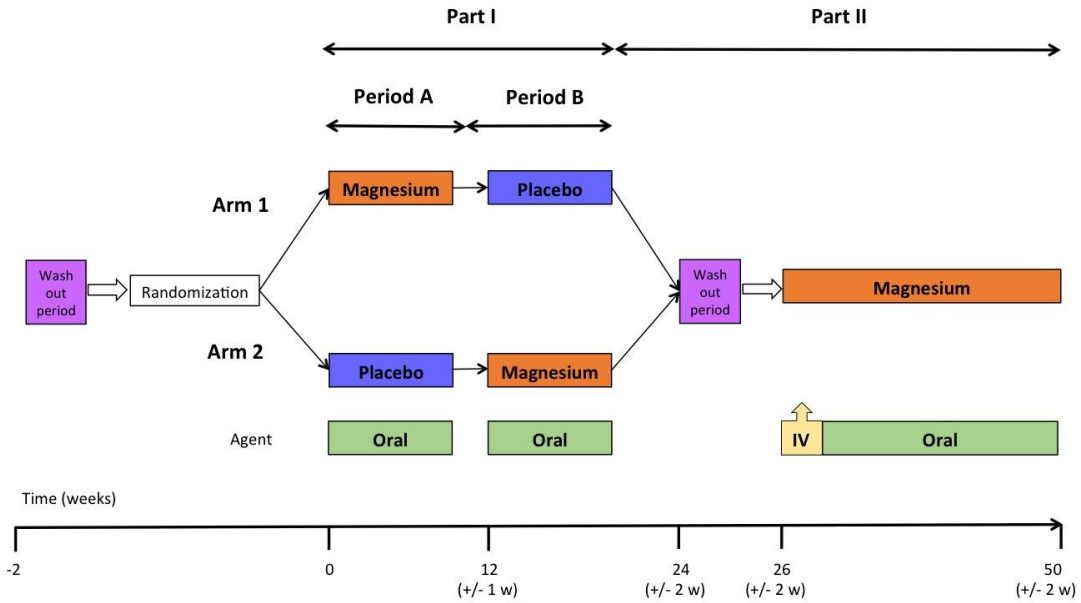
3.1 Description of the Study Design

Patients enrolled onto the study will be divided into 2 cohorts. Patients in cohort 1 (high EBV group) will have baseline blood EBV viral load ≥5,000 copies/mL or EBV log ≥3.7 IU/mL. Patients in cohort 2 (low/no EBV group) will have baseline blood EBV viral load <5,000

copies/mL or EBV log <3.7 IU/mL. The study will be conducted in 2 parts; for each patient, participation in Part II is contingent upon the outcome of Part I. Part I is a randomized, double-blind, placebo-controlled, crossover study to evaluate the efficacy of oral magnesium L-threonate compared to placebo. Following an initial washout of magnesium-containing supplements, patients within each cohort will be randomized into 2 arms **(Figure 5)**. Arm 1 will receive 12 weeks of magnesium supplementation followed by 12 weeks of placebo. Arm 2 will receive 12 weeks of placebo followed by 12 weeks of magnesium supplementation. Magnesium supplementation will consist of escalating doses of daily, orally self-administered magnesium L-threonate.

After 24 weeks of study, patients who show a 0.5-log (3.16-fold or 68.35%) reduction in the number of EBV-infected B cells (cohort 1) or or a ≥2-fold increase in NKG2D receptor expression on CD8 T cells (cohort 2) during oral magnesium treatment as compared to placebo will end study participation. Such patients may remain on oral magnesium after completion of the study under the supervision of a primary care physician. Patients in whom oral magnesium was not clearly effective will begin a 2-week washout and proceed to Part II. Such patients will be hospitalized to receive 3 days of IV $MgSO_4$ administered 3 times per day for a total daily dose of 30/mg/kg/day. Then they will restart escalating doses of orally self-administered magnesium L-threonate and continue for the remaining 24 weeks of the study.

Prior to magnesium supplementation, blood draws on 3 consecutive days will be used to establish baseline NKG2D expression on CD8+ T cells, numbers of EBV-infected B cells, EBV viral load and intrasubject variability. The number of EBV-infected B cells and other relevant measures will be recorded before and after each supplementation period. The investigator measuring NKG2D expression and the numbers of EBV-infected B cells by FISH (primary outcome measures) will be blinded to the treatment group.



3.2 Study Endpoints

Primary Endpoints

- Cohort 1: Difference between the absolute number of EBV-infected B cells (by EBV FISH) after 12 weeks of oral magnesium supplementation and after 12 weeks of placebo.
- Cohort 2: Difference between NKG2D expression in CD8 T cells after 12 weeks of oral magnesium supplementation and after 12 weeks of placebo.

Secondary Endpoints

- Incidence and severity of AEs throughout the study.
- Contingent upon patient participation in Part II, differences in EBV viral load and number (or percent) of EBV-infected B cells (cohort 1) or NKG2D expression in CD8 T cells (cohort 2) by duration (12 weeks of IV+oral versus 24 weeks of IV+oral) of magnesium supplementation regimen.
- Change in the intracellular pool of free Mg^{2+} by duration of magnesium supplementation regimen.
- Change in NKG2D expression in cohorts 1 and 2.

Exploratory Endpoints

- Change in CD4 T cell counts, B cell counts (or percent), and/or in NKG2D expression on NK (CD3-CD56+) cells.
- Quantification of EBV-infected B cells as determined by flow cytometry and FISH.
- Percent of anemia, neutropenia, thrombocytopenia, and abnormal liver enzymes.
- Difference in number and severity of infections between placebo and magnesium treatment periods.
- Difference in number of health-related days of missed school/work between placebo and magnesium treatment periods.
- Difference in number of emergency room visits and hospital admissions between placebo and magnesium treatment periods.

4 Study Population

4.1 Recruitment Plan

Patients will be recruited by referral from outside physicians and from other studies approved by NIH Institutional Review Boards (IRBs).

4.2 Subject Inclusion Criteria

All of the following inclusion criteria must be met prior to enrollment:

1. Molecular diagnosis of the MAGT1 genetic defect
2. ≥6 years years of age

3. Willingness to stop magnesium supplements (other than the study agent) and any multivitamins or over-the counter-supplements that may contain magnesium for the duration of the study
4. Willingness to go without magnesium supplementation during a 12-week placebo period and during both 2-week washout periods (pre-study and mid-study)
5. Willingness to have samples stored for future research
6. Must have a physician at home for follow-up care

4.3 Subject Exclusion Criteria

1. Chemotherapy or radiotherapy for lymphoma within 12 months prior to enrollment
2. Rituximab exposure within 6 months prior to enrollment
3. Systemic symptoms suggestive of evolving lymphoma
4. History of clinically significant cardiac arrhythmias or cardiac defects
5. Renal insufficiency (calculated creatinine clearance <50 mL/min or insufficiency requiring dialysis)
6. Advanced heart block
7. Hypermagnesemia, defined as magnesium serum concentrations >2 mmol/L (>5 mg/dL)
8. Human immunodeficiency virus (HIV) seropositivity
9. Signs or symptoms of life-threatening active microbial infection
10. History of hypersensitivity to any of the study agents
11. Any condition that, in the investigator's opinion, may substantially increase the risk associated with study participation or compromise the study's scientific objectives
12. Participation in a clinical protocol which includes an intervention that, in the opinion of the investigator, may affect the results of the current study

4.4 Justification for Exclusion of Children <6 years old

Ease and maintenance of IV access, lack of a dedicated pediatric intensive care unit, and volume of blood needed for this study will make it very difficult to treat this patient population safely at our center.

5 Study Agent/Interventions

5.1 Disposition and Dispensation

Study agent will be distributed via the NIH Pharmacy according to standard pharmacy procedures.

5.2 Formulation, Packaging and Labeling

Oral magnesium L-threonate capsules will be purchased from commercial sources. The NIH Clinical Center (CC) Investigational Drug Management Research Section (IDMRS) will make a placebo capsule to match magnesium L-threonate. The capsule will consist of a combination of Avicel PH102 and starch 1500 LM to approximately match the weight and density fill of the active ingredient capsule.

The tablets and packaging for magnesium L-threonate and placebo will be identical in appearance. Each bottle will be individually labeled with the patient ID number, dosing instructions, recommended storage conditions, and that the agent should be kept out of reach of children.

No biological test that could potentially unblind the treatment is planned in this study. See Section 13.7 for blinding and unblinding procedures.

5.3 Study Agent Storage and Stability

Magnesium L-threonate should be stored at 15°C to 30°C (59°F to 86°F). $MgSO_4$ infusions should be stored at 20°C to 25°C (68°F to 77°F). The solution should not be administered unless it is clear and seal is intact. The solution does not contain any preservative. Unused portions should be discarded.

5.4 Preparation, Administration, and Dosage of Study Agent/Intervention(s)

5.4.1 Description

Magnesium L-threonate
Magnesium L-threonate capsules are supplied in size 00 capsules that do not contain markings or engravings. Each capsule contains 48 mg of elemental magnesium for oral administration, cellulose (vegetable capsule), brown rice flour, and may contain one or both of magnesium stearate and silica.

Magnesium sulfate
$MgSO_4$ injection, USP 50% is a sterile, nonpyrogenic, concentrated solution of $MgSO_4$ heptahydrate in water for injection. It must be diluted before IV use.

Each mL contains magnesium sulfate heptahydrate 500 mg in water for injection. The pH of a 5% solution is between 5.5 and 7.0. (osmolarity: 4060 mOsmol/L [calc.]; 2.03 mM/ mL $MgSO_4$ anhydrous; 4.06 mEq/mL $MgSO_4$ anhydrous).

The solution contains no bacteriostat, antimicrobial agent, or added buffer (except for pH adjustment) and is intended only for use as a single dose injection. When smaller doses are required, the unused portion should be discarded with the entire unit.

5.4.2 Dosing and Administration

Magnesium L-threonate dose escalation
Magnesium L-threonate is an over-the-counter oral magnesium supplement. Each capsule contains 48 mg of elemental magnesium (670 mg magnesium L-threonate), which corresponds to 4 mEq of elemental Mg^{2+}. As a dietary supplement, the initial recommended dose is 144 mg of elemental Mg^{2+} taken as 2 capsules (96 mg) in the morning and 1 capsule (48 mg) in the evening. For pediatric and adult patients who are unable to swallow capsules, the capsules may be crushed and mixed with juice or food. Oral magnesium has limited bioavailability and SMCs are tightly regulated via renal excretion. Thus, oral magnesium supplementation at the recommended dose is unlikely to result in levels above 2 mmol/L.

Dose escalation for oral magnesium L-threonate will be based on the patient's weight and tolerance of the supplement. Intolerance is defined as gastrointestinal symptoms (e.g., diarrhea, nausea) after any dose. Patients experiencing intolerance will revert to the last tolerated dose. However, patients with documented renal loss may receive higher doses of magnesium up to 12 tablets a day (48 × 12 mg/day) as long as serum ionized (free) magnesium remains under 0.59 mmol/L. Patients will escalate their dose of oral magnesium every 2 weeks **(Table 1)**. Patients will be contacted prior to and after each dose escalation to assess tolerance.

In Part I, the maximum tolerated dose for periods A and B will be independent and may differ. For those who participate in Part II, the maximum dose of magnesium will be based on the highest dose tolerated in Part I.

Table 1. Dose escalation of magnesium L-threonate

Patient weight	Starting dose		2 weeks after start		4 weeks after start		6 weeks after start	
	Elemental Mg per day (milligrams)	No. capsules per day	Elemental Mg per day (milligrams)	No. capsules per day	Elemental Mg per day (milligrams)	No. capsules per day	Elemental Mg per day (milligrams)	No. capsules per day
<30 kg	96	2	144	3	192	4	240	5
≥ 30 kg	192	4	288	6	336	7	384	8

No of capsules:	Dosed as:
2	1 morning and 1 evening
3	2 morning and 1 evening
4	2 morning and 2 evening
5	2 morning, 1 afternoon, 2 evening, at ~8 hour intervals
6	2 morning, 2 afternoon, 2 evening, at ~8 hour intervals
7	3 morning, 2 afternoon, 2 evening at ~8 hour intervals
8	3 morning, 2 afternoon, 3 evening, at ~8 hour intervals

Placebo dose escalation

As investigators will be blinded to the treatment group, dose escalation for placebo will also be based on patients' weight and tolerance of the formulation (i.e., absence of gastrointestinal symptoms). The dose escalation schedule for placebo is is the same as for oral magnesium (**Table 1**).

Magnesium sulfate

IV $MgSO_4$ is approved by the Food and Drug Administration (FDA) for the treatment of magnesium deficiency, hyperalimentation, and control of convulsions (seizures). The standard dose of $MgSO_4$ for the treatment of mild hypomagnesemia in adults is 1,000 mg (8.12 mEq), which is equivalent to 14.3 mg/kg/dose for a 70-kg adult. For pediatric subjects, the starting dose for hypomagnesemia is 25-50 mg/kg/dose. IV magnesium supplementation is often used in the setting of cancer chemotherapy with cisplatinum in adults and children [8-10]. IV infusions of $MgSO_4$ are generally considered safe for use in children with moderate-to-severe acute asthma [6-15]. The National Asthma Education and Prevention Program guidelines suggest 25-

75 mg/kg (maximum 2 grams) of magnesium sulfate for severe asthma exacerbations [11]. The use of magnesium sulfate in the emergency room setting for treatment of children with asthma has been evaluated in several small studies [12-21]. In 5 randomized studies, 182 pediatric subjects with asthma (ages 1 to 18 years) were administered IV infusions of $MgSO_4$ at single doses of 25-75 mg/kg. Such doses were well tolerated with only minor AEs reported, including epigastric or facial warmth, flushing, pain and numbness at the infusion site, dry mouth, and malaise. Serious adverse effects (eg., hypotension) were not noted in any of the randomized controlled trials of magnesium sulfate in the treatment of asthma in adults or children.

The dose proposed in the current protocol is 30 mg/kg/day administered in 3 daily doses for 3 days. The total daily dose will be 30 mg/kg/day. The rate of IV injection should generally not exceed 150 mg/minute (1.5 mL of a 10% concentration or its equivalent). Solutions for IV infusion must be diluted to a concentration of ≤20% prior to administration, and the rate of administration should be slow and cautious, to avoid producing hypermagnesemia. The diluents commonly used are 5% dextrose injection, USP and 0.9% sodium chloride injection, USP.

This dose was selected based on standard replacement dosing of magnesium sulfate for hypomagnesemia, and to prevent serum concentrations from exceeding 2 mmol/L, the threshold associated with the onset of AEs. Magnesium serum concentrations will be monitored, and the dose will be adjusted not to exceed serum concentrations of 2 mmol/L. We used a similar total dose in a single-subject study of magnesium supplementation in a patient with XMEN and it was very well tolerated.

In the treatment of eclampsia with IV $MgSO_4$, the recommended initial dose ranges from 4 to 6 g intravenously administered over 15 to 20 minutes. This represents a much higher dose and faster administration rate than proposed in this protocol. The usual plasma concentration achieved in an eclampsia setting is 5 to 7 mEq/L (2.5 to 3.5 mmol/L), but higher levels are not uncommon. Even at these high doses $MgSO_4$ has been shown to be relatively safe. In a review of 7 trials comparing $MgSO_4$ to phenytoin for the treatment of eclampsia, $MgSO_4$ reduced the risk ratios for recurrent seizures, pneumonia and intensive care unit admission and improved perinatal outcomes [22]. Another review comparing $MgSO_4$ to diazepam reported reduced risks for both seizures and maternal death [23].

Administer with caution if flushing and sweating occurs. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

When repeated doses of the drug are given parenterally, serum magnesium levels will be monitored as stated in section 7.2 and subsequent doses will modified according to **Table 2**. Serum magnesium levels of 3 to 6 mg/100 mL (2.5 to 5 mEq/L) are sufficient to control convulsions. Reflexes may be absent at 10 mEq magnesium/L, where respiratory paralysis is a potential hazard.

Table 2. Dose adjustments according to serum magnesium levels

Serum Mg (>mmol/L)	IV magnesium sulfate	Oral magnesium L-threonate

≥ 2 - 3.4	Reduce dose by 25%	Dose reduction, at PI's discretion based on patient's current dose
≥ 3.5 – 4.9	Reduce dose by 50%	Dose reduction, at PI's discretion based on patient's current dose
≥ 5	Stop magnesium	Stop magnesium

Dose Adjustments/Modifications/Delays
If gastrointestinal intolerance occurs at grade 2 or higher (NCI CTCAEv4.0 ref), magnesium or placebo will be held until symptoms resolve and then restarted at previously tolerated dose.

Tracking of Dose
During the inpatient portion of the study, magnesium supplementation or placebo will be provided by NIH health care personnel. Research participants will then self-administer oral magnesium L-threonate or placebo according with the dosing scheme in Table 1. Patients will be asked to document any missed doses on the medication log so they can accurately recall the number of missed doses. They will be asked to bring back any remaining study drug at week 12 and 24.

Limitations on Prior Therapy and Use of Ancillary Medications/OTC Products
Magnesium supplements and over-the-counter vitamins or supplements that contain magnesium are not allowed 2 weeks prior to the study start and for the duration of study enrollment.

Subject Access to Study Agent at Study Closure
At the conclusion of the study, participants will have the option to begin a magnesium supplementation regimen under the care of their established primary care provider.

5.5 Study Product Accountability Procedures

Records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed will be kept by the study investigators.

Records for receipt, storage, use, and disposition will be maintained by the investigator. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

5.6 Concomitant Medications and Procedures

All concomitant prescription medications taken during study participation will be in Clinical Research Information Management System of the NIAID (CRIMSON). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in CRIMSON are concomitant prescription medications, over-the-counter and non-prescription medications taken at the time of AEs (all grades).

5.7 Drug Interactions

All medications will be reviewed by the study team to identify any potential drug interactions.

Magnesium sulfate
It has been reported that magnesium may reduce the antibiotic activity of streptomycin, tetracycline and tobramycin when administered together.

Central nervous system (CNS)—When barbiturates, narcotics or other hypnotics (or systemic anesthetics) are given in conjunction with magnesium, their dosage should be adjusted with caution because of the additive CNS depressant effects of magnesium. CNS depression and peripheral transmission defects produced by magnesium may be antagonized by calcium.

Neuromuscular blocking agents—Excessive neuromuscular block has occurred in subjects receiving parenteral $MgSO_4$ and a neuromuscular blocking agent; these drugs should be administered concomitantly with caution.

Cardiac glycosides—$MgSO_4$ should be administered with extreme caution in subjects undergoing digitalis, because serious changes in cardiac conduction resulting in heart block may occur if administration of calcium is required to treat magnesium toxicity.

6 Study Schedule

All study visits will take place at the NIH Clinical Center. A schedule of procedures is attached as Appendix B.

6.1 Screening/Enrollment (Week -5 to baseline)

Informed consent may take place at the Clinical Center or by telephone prior to the patient's arrival (see Section 13.1.2). Patients will undergo the following procedures:

- Medical history and physical examination
- Low radiation x-ray computed tomography (CT) scan to include neck, chest, abdomen, and pelvis if not done in the last 6 months
- Electrocardiogram (EKG)
- Instructions for starting a 2-week washout of all magnesium-containing supplements will be discussed during informed consent

Clinical laboratory evaluations:

- Complete blood count [CBC] with differential
- Acute care panel, mineral panel, hepatic panel
- HIV test (if not done within 1 year)
- EBV by PCR
- Cytomegalovirus (CMV) by PCR
- EBV FISH (research assay)

6.2 Baseline visit after 2 week washout period (Day -3 to Day 0):

At the end of the washout period, patients will be queried regarding recent supplement use, medications and medical history will be reviewed, and a physical exam will be performed.

Depending on which cohort subjects are assigned to, a baseline NKG2D expression on CD8 T cells and NK cells by flow cytometry or EBV FISH and EBV viral load will be determined from blood drawn on at least 3 consecutive days and averaged.

Clinical laboratory evaluations:

- 24-hour urine collection for magnesium
- CBC with differential
- Acute care, mineral and hepatic panels
- Quantitative immunoglobins
- Lymphocyte phenotyping
- Prothrombin time/partial thromboblastin time (PT/PTT)
- CMV by PCR
- EBV by PCR (will be drawn on 3 consecutive days, cohort 1)
- Erythrocyte sedimentation rate (ESR)
- C-reactive protein (CRP)
- Total and ionized serum magnesium

Research assays

- Intracellular free Mg^{2+}
- EBV FISH (will be drawn on 3 consecutive days, cohort 1)
- RNA extraction
- NKG2D expression on CD8 T cells and NK cells by flow cytometry (will be drawn on 3 consecutive days, cohort 2)

- Storage of serum, plasma and peripheral blood mononuclear cells (PBMCs)

At the conclusion of the baseline visit, patients will be provided oral study agent (magnesium L-threonate or placebo), instructions for taking it, and a medication log for recording AEs (Appendix C). The medication log will contain the study drug dosing information and will be used as a memory aide to collect information the subject would like to recall.

6.3 Part I: Oral study agent (Week 1 to Week 24)

Oral magnesium or placebo (Week 1 to Week 12)
On Day 0, patients will begin to self-administer the oral study agent according to the dose escalation scheme in section 5.4.2.

Dose escalation every 2 weeks (Weeks 2, 4, and 6, *or* until maximum tolerated dose is reached)
Prior to increasing the study drug dose the study staff will contact patients to review their medication administration, assess for AEs and approve escalation to the next dose. In addition, patients will be contacted 1 week after dose escalation to assess for AEs.

4-week follow-ups (Weeks 4 and 8 +/- 1 week)
Patients will come to NIH for clinical and research evaluations to include:

- Medical history and physical exam

Clinical laboratory evaluations:

- CBC with differential
- Acute care panel, mineral panel, hepatic panel
- EBV by PCR
- CMV by PCR
- CRP
- ESR
- Total and ionized serum magnesium
- TBNK
- Spot urine magnesium

Research assays:

- Intracellular free Mg^{2+}
- EBV FISH
- RNA extraction
- NKG2D expression on CD8 T cells and NK cells by flow cytometry
- Storage of serum, plasma, and PBMCs

Week 12 (+/- 1 week)

At the conclusion of 12 weeks of study agent patients will return to NIH for the following evaluations:

- Medical history and physical exam
- EKG

Clinical laboratory evaluations:

- 24-hour urine collection for magnesium
- CBC with differential
- Acute care, mineral and hepatic panels
- Quantitative immunoglobins
- Lymphocyte phenotyping
- CMV by PCR
- EBV by PCR
- ESR
- CRP
- Total and ionized serum magnesium

Research assays:

- Intracellular free Mg^{2+}
- EBV FISH
- RNA extraction
- NKG2D expression on CD8 T cells and NK cells by flow cytometry
- Storage of serum, plasma, and PBMCs

Crossover (Week 13-24 +/- 1 week)

To crossover (from magnesium supplementation to placebo or from placebo to magnesium supplementation), patients will return any unused study agent and begin dose escalation with a new oral agent (magnesium or placebo). Patients will be provided dosing instructions and a medication log for recording AEs (Appendix C).

Dose escalation every 2 weeks (2, 4, and 6 weeks after the start of crossover, or until maximum tolerated dose is reached)
Prior to increasing the study drug dose staff will contact patients to review their medication administration, assess for AEs and approve escalation to the next dose. In addition, patients will be contacted 1 week after dose escalation to assess for AEs.

Follow-up visits:
Patient will come to NIH at Weeks 16 and 20 for the same clinical and research evaluations as Weeks 4 and 8.

Week 24 (+/- 1 week)
At the end of 24 weeks, patients will repeat all the evaluations that were performed at the end of Week 12. This will be the last visit for patients who do not continue to Part II. Part II will be conducted only among patients in whom oral magnesium is not more effective than placebo.

6.4 Part II: Intravenous $MgSO_4$ plus oral magnesium (Weeks 25-50 +/2 weeks)

6.4.1 Washout period (Week 25 to Week 26 +/-2 weeks)

Patients proceeding to Part II will stop taking the study agent and refrain from all magnesium-containing supplements for at least 2 weeks.

6.4.2 IV infusion (Week 27 +/-2 weeks)

Patients participating in Part II will be admitted to the inpatient ward at the NIH Clinical Center to complete the following procedures between 1 and 2 days prior to initiating infusion:

- Medical history and physical exam
- EKG

Clinical laboratory evaluations:

- Spot urine magensium
- CBC with differential
- Acute care, mineral and hepatic panels
- Quantitative immunoglobins
- Lymphocyte phenotyping
- PT/PTT
- CMV by PCR
- EBV by PCR
- ESR
- CRP
- Total and ionized serum magnesium

Research assays:

- Intracellular free Mg^{2+}
- EBV FISH
- RNA extraction
- NKG2D expression on CD8 T cells and NK cells by flow cytometry
- Storage of serum, plasma and peripheral blood mononuclear cells (PBMCs)

Patients will remain hospitalized to receive IV $MgSO_4$ for 3 days. The daily dose of 30 mg/kg will be divided and infused 3 times per day (every 8 hours). During this period, patients will have a daily physical exam, be assessed for AEs, and undergo the following procedures:

12 hours (+/-3 hours) after infusion initiation

- EKG

24 hours and 48 hours (+/- 3 hours) after infusion initiation

- Begin 24-hr urine collection 48 hours after starting IV magnesium
- EKG

Blood will be drawn for the following studies:

- CBC with differential, acute care, mineral and hepatic panels
- Total and ionized serum magnesium
- Intracellular free Mg^{2+}
- NKG2D expression on CD8 T cells and NK cells by flow cytometry

Upon completion of the last IV magnesium infusion:

- EKG

Clinical laboratory evaluations:

- CBC with differential
- Acute care, mineral and hepatic panels
- Quantitative immunoglobins
- Lymphocyte phenotyping
- CMV by PCR
- EBV by PCR
- ESR
- CRP
- Total and ionized serum magnesium

Research assays

- Intracellular free Mg^{2+}
- EBV FISH
- RNA extraction
- NKG2D expression on CD8 T cells and NK cells by flow cytometry
- Storage of serum, plasma, and PBMCs

6.4.3 **Oral magnesium (open label) (Week 27-Week 50 +/-2 weeks)**

Following 3 days of infusion, patients will be discharged with a supply of oral magnesium L-threonate with instructions to begin taking the supplements and to escalate the dose every 2 weeks. Once patients reach the maximum planned dose or highest tolerated dose from Part I, they will continue that dose for the remainder of the study (24 weeks post-infusion). A medication log will be provided to record AEs and missed doses. The medication log will contain the study drug dosing information and will be used as a memory aide to collect information the subject would like to recall. Patients will return to the Clinical Center at 12 week intervals (weeks 38 and 50) after the start of oral supplementation for serum magnesium evaluation and tolerance assessment, as in Part I (see 12-week follow-ups, above).

At Week 38, patients will repeat the procedures and blood evaluations conducted at Week 12 of Part I.

6.4.4 **End-of-study follow-up (Week 50 +/- 2 weeks)**

- Medical history and physical examination
- 24-hour urine collection for magnesium
- Blood will be drawn to repeat the evaluations listed under Week 12 of Part I.
- EKG

7 Study Procedures and Evaluations

Medical history and physical examination: Each subject will be queried regarding their medical history, have vital signs and weight measured and have a physical examination. Assessment of medical history will include queries regarding missed attendance at school or work and emergency room visits since the last study visit, in addition to recent infections and other clinical manifestations (eg, rash, warts, etc.). Complete physical examinations will be conducted at enrollment and the post-study follow-up. Complaint-targeted examinations will be conducted at the other study visits. A pediatric consult will be be obtained for all patients under the age of 18 years.

Phlebotomy: Blood will be collected for routine serologic, hematologic, and clinical chemistry evaluations (as described in section 6) as well as laboratory evaluations including flow cytometry/EBV FISH, EBV PCR, quantitative serum immunoglobins, lymphocyte phenotyping and intercellular free Mg^{2+}. EBV FISH will not be performed for EBV-naïve subjects.

24-hour urine collection: The 24-hour urine Mg^{2+} test will involve collecting all urine passed during a 24-hour period. Patients will be instructed to start the 24 hr urine by urinating directly in the toilet. After urination, the time and date should be written on the container. All urine should be saved for the next 24 hours in the supplied container on ice or in a cooler. Exactly 24 hrs after the start of the test the patient should void one last time and collect the specimen. This time and date should be recorded on the container.

EKG will be performed to rule out electrical cardiac abnormalities and ensure continued eligibility to receive magnesium supplementation. A cardiologist on the study staff will address any cardiac abnormalities.

CT scan: A CT scan of the neck, chest, abdomen and pelvis will be performed on all patients to assess lymphoproliferative disease. Oral and IV contrast will be used, except in patients known to have had a reaction to IV contrast media or poor venous access. Patients will be instructed to fast for 4 hours prior to CT scan. If lymphoma is detected, patients will be referred for treatment.

Patient medication log (Appendix C) will be completed by patients to document any adverse effects of the study agent along with evaluation of drug adherence.

8 Potential Risks and Benefits

8.1 Potential Risks

$MgSO_4$ IV infusion: The AEs associated with parenterally administered magnesium are usually concentration dependent. Magnesium acts peripherally to cause vasodilation. At low doses, it causes flushing and sweating, while at higher doses, it may cause hypermagnesemia and hypotension, depressed reflexes, flaccid paralysis, hypothermia, circulatory collapse, cardiac and central nervous system depression proceeding to respiratory paralysis. The dose (30 mg/kg/day) selected for the current study is intended to prevent serum concentrations >2 mmol/L, a threshold associated with the onset of AEs. Elevations in Alanine Aminotransferase and Aspartate Aminotransferase have been seen with the administration of IV magnesium.

This product contains aluminum that may be toxic. Aluminum may reach toxic levels with prolonged parenteral administration, if kidney function is impaired. Individuals with renal impairment are not eligible to participate in this study.

Magnesium concentration-dependent toxicity, defined as SMCs >2.1 mmol/L (>5 mg/dL) are associated with decreased deep-tendon reflexes, flushing, and somnolence. Serum magnesium concentrations >4.9 mmol/L (>12 mg/dL) are associated with respiratory paralysis and heart block. Magnesium concentrations will be closely monitored in this study.

Oral magnesium L-threonate: Oral magnesium products are typically well tolerated. The most common AEs are diarrhea and nausea, which are typically dose dependent. Dosing in the current study will be based on weight. If dose-limiting AEs occur, patients will be maintained on the highest tolerated dose. Elevations in Alanine Aminotransferase and Aspartate Aminotransferase have been seen with the administration of oral magnesium.

Phlebotomy: may be associated with discomfort, bruising, local hematoma formation and, on rare occasions, infections, lightheadedness, and fainting. The amount of blood drawn for research purposes will be within the limits allowed for adult or pediatric subjects by the NIH CC (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center).

Venous line placement: Venous catheter insertion is associated with bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

CT scan: These scans are commonly used to monitor lymphoproliferative disease. Adverse reactions to iodinated contrast agent are typically mild and include metallic taste in the mouth, coldness in the arm during/after injection, headache, and nausea. These minor side effects occur in less than 3% of patients. More severe reactions, though extremely rare, include allergic reaction, shortness of breath, wheezing, flushing, hives, and decrease in blood pressure. Severe reactions including hypotensive collapse, shock, and death are reported in less than 1 in 40,000 patients. Iodinated contrast should not be used in participants who have undergone a renal transplant or who have been classified as Stage IV-V renal failure.

This research study involves exposure to radiation from 1 CT scan. Participants will be exposed to a maximum radiation amount of 1.2 rem, which is within the safety limit of 5 rem for adults, but is higher than 0.5 rem per year dose guideline for children set by the NIH Radiation Safety Committee. If participants would like more information about radiation and examples of exposure levels from other sources, they may ask the investigator for a copy of the pamphlet "An Introduction to Radiation for NIH Research Subjects."

New diagnoses: It is possible that the standard medical tests performed as part of this research protocol will result in new diagnoses. Depending on the medical findings and their implications, this could be viewed by study participants as either as a risk or a benefit. There is an additional risk that testing associated with clinical laboratory, or research laboratory testing, may lead to the identification of new medical diagnoses, or the potential for certain diseases to develop in the future. Any such information will be shared and discussed with the subject, and, if requested by the subject, it will be forwarded to the subject's primary health care provider for further evaluation and management.

8.2 Potential Benefits

Patients may derive no direct benefit from participating in this study. All patients will receive magnesium supplementation, and if magnesium is effective, they may derive benefit from improved NK and CD8 T cell functions and NKG2D expression, and, in some patients, accompanying reductions in EBV load. There may be some benefit associated with the close follow-up and additional monitoring inherent to participation in this study.

9 Research Use of Stored Human Samples, Specimens or Data

Intended use: The blood samples and data collected under this protocol may be used to study XMEN syndrome. No genetic testing will be performed. For any other research or experimental treatments done under this or other protocols, a separate informed consent will be obtained.

Storage: Access to stored samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the study investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

Tracking: Samples and data will be tracked by use of the Biological Specimen Inventory (BSI) software (by Information Management Services, Inc.).

Disposition at the completion of the protocol:

- In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to sharing any of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval.
- At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.

Reporting the loss or destruction of samples/specimens/data to the IRB:

- Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of a protocol deviation and/or compromises the scientific integrity of the data collected for the study; will be reported to the NIAID IRB.
- Additionally, patients may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done both to the subject and to the IRB. This decision will not affect the subject's participation in other protocols at NIH.

10 Assessment of Safety

10.1 Recording/Documentation

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AE's relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON.

10.2 Definitions

Adverse Event (AE)
An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Serious Adverse Event (SAE)
A Serious Adverse Event is an AE that results in one or more of the following outcomes:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life

threatening or result in death or hospitalization but they may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Package Insert or is not listed at the specificity or severity that has been observed.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; package insert or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event (UPnonAE)
Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug

Serious Unanticipated Problem: A UP that meets the definition of an SAE or compromises the safety, welfare, or wrights of subjects or others

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation: A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that
   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH-HRPP
   d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that is neither serious nor continuing.

10.3 Investigator Assessment of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All AEs thought to be related to magnesium therapy or study procedures occurring from the time the informed consent is signed through the final study visit will be documented, recorded, and reported.

The Investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

10.3.1 Severity

The Common Terminology Criteria for Adverse Events (v 4.0) will be used to evaluate the severity of AEs: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

10.3.2 Causality

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related
- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related
- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related
- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related
- does not have a reasonable temporal relationship

OR
- good evidence for a more likely alternative etiology

Not Related
- does not have a temporal relationship

OR
- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on

available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

10.4 Reporting Procedures to the NIAID IRB

10.4.1 Expedited Reporting to the NIAID IRB

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

10.4.2 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs and Deaths to the NIAID IRB

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. We expect up to 10% of doses to have been missed based on medication review at each visit; accordingly, we will only report missed doses as deviations if more than 20% are missed.

Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in XMEN syndrome. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems. Deaths related to the natural history of XMEN syndrome will be reported at the time of continuing review.

10.4.3 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs granted a waiver of reporting.
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

10.5 Follow-Up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study.

SAEs that have not resolved by the end of the study are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the SAE/UP report form.

SAEs that occur after 50 weeks of study that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related must be reported to the IRB, as described above.

10.6 Halting Criteria for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrollment until a decision is made whether or not to continue study agent administration. If 2 or more subjects experience the same or similar AE of Grade 3 or above that is thought to be related to the magnesium supplementation, the study will be halted for review of the event(s) and re-evaluation of study risks, safety measures, and possible modification of the protocol and consent as may be appropriate, and for not less than 2 weeks. The IRB or DSMB may halt the study at any time following review of any safety concerns.

The IRB or NIAID, as part of their duties to ensure that research subjects are protected, may discontinue the study at any time. Subsequent review of serious, unexpected and related AEs by the DSMB or IRB may also result in suspension of further trial interventions/administration of study agent.

10.6.1 Reporting of Study Halting

If a halting requirement is met, a description of the event(s) or safety issue must be reported by the PI within one business day to the IRB and DSMB.

10.6.2 Resumption of a Halted Study

The DSMB, in collaboration with the PI will determine if it is safe to resume the study.

10.7 Pausing Criteria for a Subject

The decision to suspend administration of the study agent(s) for a single subject requires discontinuation of study agent administrated for the study subject until a decision is made whether or not to continue study agent administration.

The pausing criteria for a single subject in this study include:

- A subject experiences an SAE or any Grade 3 or greater AE that is unexpected and is possibly, probably, or definitely related to the study agent;

OR

Any safety issue that the PI determines should pause administration of the study agent to a single subject.

IV magnesium would be stopped for a subject who experienced any of the following:

- “Abnormal” EKG values, as determined by the cardiologist on the study staff
- Development of advanced heart block
- Widening of QRS of more than 20 msec into the abnormal range (>110 msec) or more than 10 msec with QRS >120 msec
- Symptomatic hypotension
- Unexplained syncope

10.7.1 Reporting of Pausing for a Subject

If a pausing requirement is met, a description of the AEs or safety issue must be reported by the PI by fax or email within one business day to the IRB.

10.7.2 **Resumption of a Paused Study**

The DSMB, in collaboration with the PI will determine if it is safe to resume administration of the study agent to the subject. Any EKG abnormality will be assessed by a cardiologist to determine whether it would be safe to resume treatment. Grade 3 AEs that are thought to be related to the study medication must return to at least grade 1 before continuing with treatment.

10.8 **Withdrawal Criteria for an Individual Subject**

An individual subject will be withdrawn for any of the following:

- An individual subject's decision.
  (The Investigator should attempt to determine the reason for the subject's decision.)
- Any clinical AE, laboratory abnormality or other medical condition or situation such that continued participation in the study would not be in the best interest of the subject. Subjects will be followed for the duration of the study for indicated safety assessments.
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.

10.9 **Replacement for Withdrawn Subjects**

Withdrawn subjects may be replaced if eligible replacements can be identified.

11 Remuneration Plan

Eligible subjects will be compensated for travel according to the NIAID/NIH travel policy. Subjects will receive financial compensation for the time and inconvenience of study participation according to the NIH CC volunteer guidelines at $10 per inconvenience unit (IU). Total compensation will be $720 (completion of Part I only) or $1365 (completion of Part I and Part II). See Appendix D for detailed compensation amounts. Subjects will be compensated at the completion of part I, part II, or at time of withdrawal from the study (for portion of study completed).

12 Clinical Monitoring Structure

12.1 **Site Monitoring Plan**

As per International Conference on Harmonization (ICH)-Good Clinical Practice (GCP) 5.18, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID Office of Clinical Research Policy and Regulatory Operations (OCRPRO) will visit the clinical research site to monitor several aspects of the study in accordance with the appropriate regulations and the approved protocol. Only pediatric subjects will be monitored and the objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored pediatric subject; 2) to verify AEs and SAEs, including the prompt reporting of all SAEs; 3) to compare applicable CRIMSON data abstracts with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators' are in compliance with the protocol.

The investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records readily available for inspection by the local IRB, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the principal investigator and study staff prior to enrollment. The plan will outline the frequency of the monitoring visits based on factors such as study enrollment, data collection status, and regulatory obligations.

12.2 Data and Safety Monitoring Board

The NIAID Intramural Data and Safety Monitoring Board (DSMB) will review the study prior to initiation and twice a year thereafter. The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAE and UP will be reported by the PI to the DSMB at the same time they are submitted to the IRB. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB upon receipt.

13 Statistical Considerations

13.1 Study Hypotheses

Cohort 1: The absolute numbers of EBV-infected B cells in XMEN subjects with a blood EBV viral load ≥5,000 copies/mL or EBV log ≥3.7 IU/mL will be lower following treatment with IV $MgSO_4$ and oral magnesium L-threonate than following placebo administration.

Cohort 2: The surface expression of NKG2D in XMEN subjects with baseline blood EBV viral load <5,000 copies/mL or EBV log <3.7 IU/mL will be greater following treatment with IV $MgSO_4$ and oral magnesium L-threonate than following placebo administration.

Treatment with IV $MgSO_4$ and oral magnesium L-threonate will increase the intracellular free Mg^{2+} level without increasing serum Mg^{2+} to supraphysiologic levels leading to improvement in NK and CD8 T cell functions and NKG2D expression in subjects with XMEN, thereby reducing the number of EBV-infected B cells.

13.2 Statistical Analysis

Cohort 1
The primary analysis will compare the difference between the number of EBV-infected B cells at the end of the treatment and control periods. Specifically, for each patient, the number of EBV-infected B cells at the end of the placebo period will be subtracted from the number of EBV-infected B cells at the end of the treatment period. The mean of these differences in all patients will be calculated and these values will be used to form the test statistic for a paired t-test. If magnesium treatment does not work, the true mean difference will be 0. If magnesium lowers the number of EBV-infected B cells, the true mean difference will be less than 0.

To determine which participants will continue to Part II, we will evaluate the number of EBV infected B cells (by EBV FISH) in each patient after 12 weeks of oral magnesium and after 12 week of placebo. If a patient has a 3.16-fold or 68.35% reduction with oral magnesium as compared with placebo, then that patient's study participation will end. Those patients who do

not meet this outcome will proceed to Part II (IV magnesium). Participation in Part II will depend on the result for each individual patient. We have chosen a 3.16-fold (68.35%) reduction as this is equivalent to a 0.5 log decrease.

Contingent upon patient participation in Part II, secondary analysis will compare the numbers of EBV-infected B cells at the end of 12 weeks versus 24 weeks of oral supplementation.

Cohort 2
The primary analysis will compare the difference between the mean fluorescence intensity (MFI) of NKG2D on CD8+ T cells at the end of the treatment and control periods. Specifically, for each patient, the MFI for NKG2D at the end of the placebo period will be subtracted from the MFI for NKG2D at the end of the treatment period. The mean of these differences in all patients will be calculated and these values will be used to form the test statistic for a paired t-test. If magnesium treatment does not work, the true mean difference will be 0. If magnesium increased the surface expression for NKG2D on CD8+ T cells, the true mean difference will be less than 0.

To determine which participants will continue to Part II, we will evaluate the MFI for NKG2D in CD8+ T cells in each patient after 12 weeks of oral magnesium and after 12 week of placebo. If a patient has a 2-fold or greater increase with oral magnesium as compared with placebo, then that patient's study participation will end. Those patients who do not meet this outcome will proceed to Part II (IV magnesium). Participation in Part II will depend on the result for each individual patient.

Contingent upon patient participation in Part II, secondary analysis will compare the MFI for NKG2D on CD8+ T cells at the end of 12 weeks versus 24 weeks of oral supplementation.

13.3 **Sample Size Justification**

For cohort 1, the primary endpoint is the difference between the absolute number of EBV-infected B cells (EBV FISH) after 12 weeks of magnesium supplementation and after 12 weeks of placebo. With 10 subjects, there is 90% power to detect 1.15 standard deviation change by the 2-sided paired *t-test* with type I error rate of 0.05. To evaluate EBV change over time, we will compare the absolute number of EBV-infected B cells at the end of the control period and at the end of the treatment period. We will report the mean change in EBV with 95% confidence intervals.

For cohort 2, the primary endpoint is the difference between the MFI for NKG2D surface expression on CD8+T cells after 12 weeks of magnesium supplementation and after 12 weeks of placebo. With 10 subjects, there is 90% power to detect 1.15 standard deviation change by the 2-sided paired *t-test* with type I error rate of 0.05. To evaluate NKG2D change over time, we will compare the MFI for NKG2D surface expression on CD8+ T cells at the end of the control period and at the end of the treatment period. We will report the mean change in NKG2D with 95% confidence intervals.

13.4 **Power calculation**

Table 3 gives the detectable difference (EBV-infected B cells at end of magnesium – EBV-infected B cells or NKG2D surface expression at end of placebo) with various sample sizes. The differences shown in the table are functions of the standard deviation. For example, with 10 patients if the standard deviation of the differences is 1 there is 90% power to detect a true difference of 1.15. If the standard deviation is 2, there is 90% power to detect a difference of 2.3=1.15*2.The values in the table are based on using a t-test for the analysis. **Table 3** gives the ratio of the difference to the standard deviation that can be detected with various sample sizes in each group to obtain 90% power. At the end of the study a paired *t-test* will be used to determine whether the difference in the number of EBV-infected B cells or NKG2D expression in CD8+ T cells between the period treated with magnesium and treated with placebo (number of EBV-infected B cells or MFI for NKG2D expression after 3 months of magnesium treatment – number of EBV-infected B cells or MFI for NKG2D expression after 3 months of placebo treatment) is different from 0.

Table 3. Detectable differences with various sample sizes with 90% power and 2-sided type I error rates of 0.05. The differences are shown as functions of the standard deviations

Total sample size (with half of subjects randomized to each sequence)	Difference/standard deviation
10	1.15
12	1.03
14	0.94
16	0.87
18	0.81
20	0.76

13.5 **Secondary Analyses**

To compare the effect of IV magnesium followed by 12 weeks vs. 24 weeks of oral magnesium, we will calculate the difference between the number of EBV-infected cells (cohort 1) and NKG2D expression in CD8+ T cells (cohort 2) at 12 weeks to 24 weeks in Part II. We will also calculate the difference in NKG2D expression between the 2 cohorts at the same timepoints.

13.6 **Randomization within each cohort**

Patients will be allocated to Arm 1 or Arm 2 using a permuted block design with block size of 20. This means that exactly 10 patients will be randomly assigned to each arm. The study statistician will provide the randomized list of arm assignments.

13.7 Blinding and Unblinding Procedures

13.7.1 Blinding

The IDMRS staff is responsible for maintaining security of the treatment assignments. The subject, the clinical staff, and the study team will be blinded to treatment allocation. The study arm agents will be ordered according to the randomization code list maintained by the IDMRS in the electronic Investigational Drug Management System (IDMS).

13.7.2 Unblinding

13.7.2.1 Scheduled Unblinding

Unblinding will occur for every participant at the end of the 24-week period in Part I after the laboratory analysis for the primary endpoints (NKG2D and EBV by FISH) has been completed. At this point, participants will be informed about their treatment assignment in Part I.

13.7.2.2 Unscheduled Unblinding

Intentional: Any subject that is unable or unwilling to complete the 24-week period will be taken off study and unblinding will occur. To break a treatment blind, the PI, after consulting with the medical advisory investigator, will request a subject's treatment assignment from the IDMRS. If an emergency request for treatment assignment is made by an individual other than the PI and the PI is not immediately available, the request will be made to the medical advisory investigator or designee, as applicable, who will then contact the IDMRS or appropriate NIH Pharmacy after-hours contact to obtain the treatment assignment.

Unintentional: If unintentional unblinding of study treatment assignment occurs, the PI will create a plan for ongoing management of the subject(s) involved and preventing the recurrence of a similar incident, as appropriate. If the protocol team determines that the unintentional unblinding may have a significant impact on the study plan (e.g., if the treatment codes for multiple subjects or an entire cohort were accidentally broken), the need for a protocol amendment will be addressed as soon as possible.

Intentional and unintentional unscheduled unblinding will be documented in the appropriate source and/or research record and will include the reason for the unscheduled unblinding, the date it occurred, who approved the unblinding, who was unblinded, who was notified of the unblinding, and the plan for the subject. The PI will report all cases of intentional and unintentional unscheduled unblinding to the DSMB in writing within 1 business day after site awareness via email to the DSMB mailbox (niaiddsmbia@niaid.nih.gov) outlining the reason for the unblinding and the date it occurred. The report will also be submitted to the IRB.

If a serious adverse event (SAE) has resulted in unblinding, this information will be included in the SAE Report.

14 Ethics/Protection of Human Subjects

14.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation

between the human research subject and the researchers, which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research provide essential information about the study, including the purpose, duration, experimental procedures, alternatives, risks, and benefits. Subjects will be given the opportunity to ask questions and have those questions answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document signing of the consent form in the subject's medical record. The rights and welfare of subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in the study.

14.1.1 Non-English–Speaking Participants

If a non-English speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non-English Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12 and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will translate the IRB-approved English consent form verbatim and facilitate discussion between the participant and investigator.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used 3 times or more for the same language, this will be reported to the IRB immediately.

14.1.2 Telephone Consent Process

XMEN syndrome is rare and we will be required to recruit patients throughout the United States. We will obtain consent by telephone so that patients may begin the washout period at home and arrive at NIH ready to begin study participation.

a) Permission to mail or fax the consent document will be obtained from the subject or the subject's legal guardian.
b) Once consent is received, the subject is instructed to call the investigator for discussion of the study and consent document. The study will be thoroughly explained with ample time for

questions or concerns related to participation. Informed consent will be obtained by the principal investigator or a designated associate investigator on this protocol.

c) The subject will identify him/herself by name and date of birth, and state that he/she gives consent to participate in this study. The staff member will verify the subject's name and the verbal consent to participate.
d) Upon completion of the telephone call, the manner of obtaining consent and the names of the person administering and providing consent will be documented in the medical record, signed and dated.
e) The subject/legal guardian must sign and date the consent in his/her possession and have the signature witnessed in-person and dated by an adult over the age of 18 years.
f) This signed, witnessed, and dated consent must be sent to the investigator. No testing will occur without this consent.

Once received, the consent will be checked for accuracy and signed by the NIH investigator/designee. The signed consent will then be filed in the medical record.

14.1.3 Assent or Informed Consent Process (in Case of a Minor)

Assent forms will be used for all study participants between 7 and 18 years of age who are capable to give their assent to participate. Parental consent will be obtained for all study participants under the age of 18.

14.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including, but not limited to, medical records. Records will be kept locked, and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, NIAID, the Office for Human Research Protections (OHRP), or the sponsor's designee.

15 Data Handling and Record Keeping

15.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during the study visits, or they will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

15.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Study records will be maintained by the PI for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention

requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to NIAID/ OCRPRO with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Evaluations	Blood volume (ml)	Enrollment[a] (begin 2-week washout)	Baseline visit	**Part I** (randomized crossover), Weeks 1-24				WASHOUT	**Contingent upon each patient's outcome in Part I: Part II** (open label), Weeks 27-50			
				Oral Mg L-threonate or placebo		Oral Mg L-threonate or placebo			Pre-infusion	IV $MgSO_4$ (over 3 days of inpatient stay)	Oral Mg L-threonate escalation	
Study week (window)		Week -5 to Week -2	Day -3 to Day 0	Visits: Week 4 and 8 (+/-1 wk)	Week 12 evaluation (+/-1 wk)	Visits: Week 16, 20 (+/-1 wk)	Week 24 evaluation (+/-1 wk)	Week 25+26 (+/- 2 wk)	Week 27 (+/-2 wks; inpatient stay of 4 to 6 days)		Week 38 (+/-2 wks)	Week 50 (+/-2 wks)
Procedures												
Medical/medication history		X	X	X	X	X	X		X	X	X	X
Physical exam		X	X	X	X	X	X		X	X	X	X
IV magnesium										X		
EKG		X			X		X		X[b]	X[b]	X	X
CT[c]		X										
Oral supplement provision			X	X	X	X				X[h]	X	
Tolerance evaluation/ dose escalation[d]				X		X						
Laboratory evaluations												
Urine spot Mg2+				X		X			X			
24-hr urine collection Mg2+			X		X		X			X[i]	X	X
CBC w/differential	3	X	X	X	X	X	X		X	X[g]	X	X
Acute care, mineral, hepatic panels	4	X	X	X	X	X	X		X	X[g]	X	X
Quantitative immunoglobins	4		X		X		X		X	X[h]	X	X
Lymphocyte phenotyping	6		X	X[j]	X	X[j]	X		X	X[h]	X	X
PT/PTT	4.5		X						X			
CMV PCR	3	X	X	X	X	X	X		X	X[h]	X	X
EBV PCR		X	X[e]	X	X	X	X		X	X[h]	X	X
HIV	8	X										
ESR	1		X	X	X	X	X		X	X[h]	X	X
CRP	4		X	X	X	X	X		X	X[h]	X	X
Total + ionized serum Mg^{2+}	1		X	X	X	X	X		X	X[g]	X	X
Research blood	30											
Intracellular free Mg^{2+}			X	X	X	X	X		X	X[g]	X	X
EBV FISH[k]		X	X[e]	X	X	X	X		X	X[h]	X	X
RNA extraction			X	X	X	X	X		X	X[h]	X	X
NKG2D expression			Xa	X	X	X	X		X	X[g]	X	X
Stored blood[f]	12		X	X	X	X	X		X	X[h]	X	X

Abbreviations: CBC=complete blood count, CMV=cytomegalovirus, CRP=C-reactive protein, CT=computed tomography, EBV=Epstein-Barr virus, EKG=electrocardiogram, ESR=erythrocyte sedimentation rate, FISH=fluorescent in situ hybridization, HIV=human immunodeficiency virus, IV=intravenous, MgSO4=magnesium sulfate, NK=natural killer, PBMC-peripheral blood mononuclear cells, PCR=polymerase chain reaction, PT=prothrombin time, PTT=partial thromboplastin times, TBNK=T-lymphocyte, B-lymphocyte, and natural killer cells

Notes:

a Evaluations may be conducted over 3 days.
b EKG to be performed prior to infusion, 12, 24, and 48 hours after the start of the first infusion, and at completion of the infusion.
c Low radiation CT scan to include neck, chest, abdomen, and pelvis if not done in the previous 3 months.
d Patients will increase their dose of oral study agent every 2 weeks. Patients will be contacted prior to and 1 week after each dose escalation to assess for adverse events.
e Blood will be drawn on 3 consecutive days to establish baseline values and to determine intrasubject variability.
f Stored blood includes serum, plasma and PBMCs.
g To be done 24 and 48 hours after starting and at completion of magnesium infusion.
h Evaluations to be conducted upon completion of magnesium infusion.
i 24-hour urine collection to begin 48 hours after initiation of magnesium infusion.
j TBNK
k EBV FISH will not be performed for EBV-naïve patients.

Appendix C: Patient Medication Log

Month: FEBRUARY							
Date	Morning		Afternoon		Evening		Side Effects/Notes
	No. of capsules	Time	No. of capsules	Time	No. of capsules	Time	
9 March	1	8:45 am			1	9:00 pm	Example

Appendix D: Compensation Table

Compensation calculations are shown in the table below. IUs for compensation were determined using benchmarks presented by the Office of Communications, Patient Recruitment, and Public Liaison, NIH CC.

Evaluations	Enrollment[a] (Begin 2-wk washout)	Baseline visit	**Part I** (Randomized crossover) Wks 1-24						**Cumulative through part I**	WASHOUT	**Contingent upon each patient's outcome in Part I: Part II** (open label) Wks 27-50				
			Oral Mg L-threonate or placebo			Oral Mg L-threonate or placebo					Pre-infusion	IV $MgSO_4$ (over 3 days)	Oral Mg L-threonate escalation		
Study wk (+/- window indicated in study schedule)	Wk -5 to Wk -2	Day -3 to Day 0	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24		Wk 25 to 26	Wk 27 (inpatient stay of 4 to 6 days)		Wk 38	Wk 50	Cumulative through Part II
Inconvenience (2-4 IU/day)	$20	$20	$20	$20	$20	$20	$20	$20	$160		$40 x 6 days = $240		$20	$20	$440
Medical/medication history (1 IU)	$10	$10	$10	$10	$10	$10	$10	$10	$80		$10	$10	$10	$10	$120
Physical exam (2 IU)	$20		$20	$20	$20	$20	$20	$20	$140		$20	$20	$20	$20	$220
IV magnesium (4.5 IU)									$0			$45			$45
EKG (2 IU)	$20				$20			$20	$60		$20	$20	$20	$20	$140
CT[c] (5 IU)	$50								$50						$50
Urinalysis (1 IU)		$10	$10	$10	$10	$10	$10	$10	$70		$10	$10	$10	$10	$110
Blood draw (2 IU: includes evaluations, research + stored)	$20	$20	$20	$20	$20	$20	$20	$20	$160		$20	$20	$20	$20	$240
TOTAL compensated									**$720**						**$1365**

Abbreviations: IU = inconvenience unit at $10/IU, CT=computed tomography, EKG=electrocardiogram, IV=intravenous, wk = week.