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DEPARTMENT OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A Phase I/II Dose-Escalation Trial of Leflunomide in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma

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COH Amendment 07 Protocol dated 10/23/2017			07
COH Amendment 08 Title Page dated 03/11/2019			08
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SPONSOR/IND NUMBER:	City of Hope/127341
DISEASE SITE:	Multiple Myeloma
STAGE (if applicable):	Refractory or Relapsed
MODALITY:	Oral
PHASE/TYPE:	Phase I/II- Intervention

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Clinical Trial Protocol

A Phase I/II Dose-Escalation Trial of Leflunomide in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma

Protocol Version Date:	10/23/2017
Protocol Version No.:	05
COH Protocol No.:	15140
Agent:	Leflunomide, commercially available
IND#:	**127341**
IND holder:	City of Hope
Coordinating Center:	City of Hope (dcc@coh.org)

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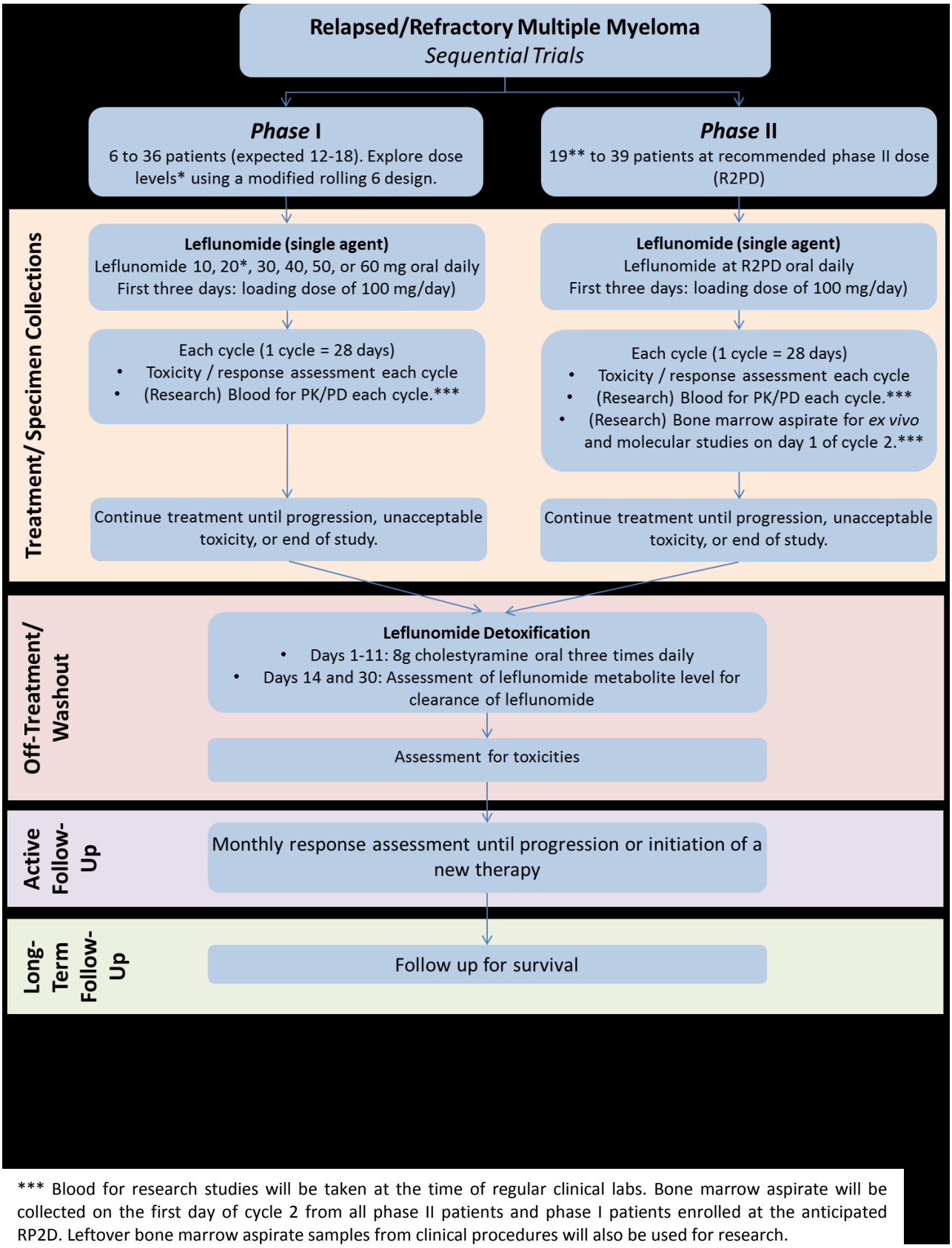
EXPERIMENTAL DESIGN SCHEMA



*** Blood for research studies will be taken at the time of regular clinical labs. Bone marrow aspirate will be collected on the first day of cycle 2 from all phase II patients and phase I patients enrolled at the anticipated RP2D. Leftover bone marrow aspirate samples from clinical procedures will also be used for research.

PROTOCOL SYNOPSIS

Protocol Title:
A phase I/II dose-escalation trial of leflunomide in patients with relapsed or relapsed/refractory multiple myeloma
Brief Protocol Title for the Lay Public (if applicable):
Leflunomide in relapsed or refractory multiple myeloma
Study Phase:
Phase I/II
Participating Sites:
Single Center: City of Hope, Duarte
Rationale for this Study:
Despite many advances in treatment, multiple myeloma remains an incurable disease and new, effective treatments are needed. Leflunomide is a commercially available oral immunosuppressive agent that has been FDA approved since 1998 for the treatment of rheumatoid arthritis (RA). The primary mechanism of action of leflunomide is inhibition of de novo pyrimidine synthesis by targeting dihydroorotate dehydrogenase (DHODH), and thus achieving anti-proliferative effect in B- and T-lymphocytes. Leflunomide has been investigated for its anti-neoplastic potential in a variety of pre-clinical tumor models. Recent pre-clinical studies have demonstrated significant anti-neoplastic activity in multiple myeloma. In addition, limited clinical experience is suggestive of a beneficial response when leflunomide is used to treat relapsed/refractory multiple myeloma. Considering the favorable toxicity profile and extensive clinical experience with leflunomide in rheumatoid arthritis, this drug represents a potential new candidate for therapy in multiple myeloma.
Objectives:
In patients with relapsed or relapsed/refractory multiple myeloma: **Phase I** **Primary Objectives:** o To determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of leflunomide, when given as a single agent. o To assess the safety and tolerability of leflunomide at each dose level by evaluation of toxicities including: type, frequency, severity, attribution, time course and duration. **Phase II** **Primary Objective:** o To evaluate the anti-myeloma activity of leflunomide, when given as a single agent, as assessed by overall response rate (ORR). **Secondary Objectives:** o To obtain estimates of:

- Response duration
- Clinical benefit response
- Overall survival and
- Progression-free survival.

Clinical Pharmacology Objectives (Phase I and II)

- To characterize the relationship between serum concentration of the active leflunomide metabolite, teriflunomide (A77 1726), and toxicity.
- To assess the relationship between serum concentration of the active leflunomide metabolite, teriflunomide A77 1726), and disease response.

Exploratory Ex-Vivo and Molecular Objectives (Phase I and II)

- To explore the relationship between polymorphisms in the *CYP1A2, CYP2C19*, or *DHODH* genes and toxicity/response. CYP1A2, CYP2C19 are the hepatic enzymes responsible for leflunomide metabolism.
- To explore the *ex vivo* cytotoxicity of leflunomide toward primary MM cells, in order to evaluate whether individual *ex vivo* leflunomide response might be a useful predictor of therapeutic response.
- To explore the potential additive or synergistic effects of combining leflunomide with other classes of FDA-approved drugs. The classes include HDAC inhibitors, immunomodulatory drugs [IMiDs], proteasome inhibitors, steroid hormones, and antibody therapeutics such as the CD38 antibody daratumumab.
- To generate a preliminary RNA/miRNA and DNA-methylation signature associated with response of MM cells to leflunomide *in vivo* and teriflunomide *ex vivo*.

Study Design:

This study will be conducted as a single center, single agent phase I/II trial.

The phase I portion will implement a modified rolling six dose escalation design, a more conservative version of the rolling six design of Skolnik, et al. [1], for enrollment with dose escalation, de-escalation, or expansion of a cohort on the basis of the occurrence of dose limiting toxicities (DLTs) during cycle 1.

The starting dose of leflunomide is 20mg daily, with the intention to test up to 60mg daily. Escalation will proceed in increments of 20mg/day (odd numbered dose levels); de-escalation will proceed in decrements of 10mg/day. Up to six doses of leflunomide will be considered:

Dose Level	Leflunomide Dose*
-1	10 mg PO daily
1	20 mg PO daily
2	30 mg PO daily
3	40 mg PO daily
4	50 mg PO daily
5	60 mg PO daily

*NOTE: Leflunomide is administered with a loading dose of 100 mg for the 1st three doses.

The highest dose level that produces ≤1/6 DLTs in cycle 1 will be the MTD. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities.

Following the phase I trial, a single arm phase II trial will be conducted using a Simon Two-Stage Optimal Design [2, 3] to evaluate the anti-myeloma activity of leflunomide, when given as a single agent, as assessed by overall response rate (ORR). The phase II portion of the study is expected to enroll a minimum of 19 and a maximum of 39 patients. The sample size is based on the desire to discriminate a promising ORR of 30% from a disappointing response rate of 15% using a type I error rate of 0.10 and power of 80%.

Patients will be treated in planned 28-day treatment cycles until disease progression, unacceptable toxicity, or other criteria for removal from study treatment are satisfied. Patients will undergo monthly evaluation for response using the International Myeloma Working Group (IMWG) criteria.

Endpoints:

Phase I: The primary endpoint is toxicity. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 4.03. Dose limiting toxicity (DLT) is defined in Section 13.1 of the protocol.

Phase II: The primary endpoint is response. Response will be categorized using the IMWG response criteria for multiple myeloma [37, 38]. The response categories and criteria are summarized in Table 11.2 of the protocol.

Sample Size:

The expected phase I sample size is 12-18 patients; allowing for 6 patients to replace inevaluable/ineligible patients. The expected phase II sample size is 19-39 patients. It is anticipated that the 6 patients from the phase I portion who are treated at the R2PD will be included in the sample size for the phase II portion (phase I/II participants).

Estimated Duration of the Study

With an expected accrual rate of approximately 1 patient enrolled per month, the phase I portion of the trial is expected to complete accrual in 12-18 months. The phase II portion is expected to accrue 2 patients per month, with completion in 17 months. The expected maximum duration of therapy is 24 months. The total study is expected to last a maximum of (18+17+24) 59 months or approximately 5 years.

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

- All subjects must have the ability to understand and the willingness to sign a written informed consent.
- Patients must be age ≥ 18 years.
- Patients must have a life expectancy of > 3 months.
- Patients must exhibit an ECOG performance status of 0-2.
- Patients must have a diagnosis of multiple myeloma.
- Patients must have measureable disease, defined as one of the following within 21 days prior to registration:
    - Serum M-protein ≥ 0.5 g/dL
    - Urine M-protein ≥ 200 mg/24 hr

  - Serum free light chain ≥ 10 mg/dL provided the FLC ratio is abnormal.
  - 10% plasma cells in bone marrow
- Patients must be relapsed or are refractory to at least 3 prior lines of therapy, including both a proteasome inhibitor and an IMiD, and for whom transplant is not recommended (induction therapy and stem cell transplant +/- maintenance will be considered as one regimen).
- At least 2 weeks from prior therapy to time of start of treatment. Prior therapy includes steroids (except prednisone or equivalent – up to 10 mg per day is allowed).
- Platelet count $\geq$ 50,000/µL. Platelet transfusions are not allowed within 14 days of platelet assessment.
- ANC ≥ 1000/mm^3. Growth factor is not permitted within 14 days of neutrophil assessment.
- AST and ALT < 2.0 x ULN.
- Total Bilirubin < 1.5 x ULN.
- Calculated creatinine clearance (CrCl) ≥ 30 mL/min per 24-hour urine collection or the Cockcroft-Gault formula.
- Negative serum or urine β-HCG test (female patient of childbearing potential* only), to be performed locally within the screening period.
- Negative for tuberculosis antigen (e.g. T-Spot test).
- Negative for hepatitis A, B, or C infection.
- Adequate pulmonary function as defined by FVC and DLCO ≥ 50% of predicted by pulmonary function testing.
- Agreement by females of childbearing potential* and sexually active males to use an effective method of contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for three months following duration of study participation. The effects of study treatment on a developing fetus have the potential for teratogenic or abortifacient effects. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately. *A female of childbearing potential is defined as a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months.

Exclusion Criteria

Prospective participants who meet any of the following criteria will not be eligible for admission into the study:

- Prior treatment with leflunomide.
- Current or planned use of other investigational agents, or concurrent biological, chemotherapy, or radiation therapy during the study treatment period. (See inclusion criterion #8 for washout times).
- Current or planned growth factor or transfusion support until after initiation of treatment. If growth factor or transfusion support is provided between screening and start of treatment, the participant will no longer be eligible.
- Prior diagnosis of rheumatoid arthritis.
- Prior Allogeneic transplant.
- Acute active infection requiring systemic therapy within 2 weeks prior to enrollment.
- Pre-existing liver disease.
- Known HIV infection.
- History of allergic reactions attributed to compounds of similar chemical or biologic

composition to leflunomide or cholestyramine.

- Non-hematologic malignancy within the past 3 years aside from the following exceptions:
  - adequately treated basal cell or squamous cell skin cancer
  - carcinoma in situ of the cervix
  - prostate cancer < Gleason Grade 6 with a stable PSA
  - successfully treated in situ carcinoma of the breast
- Clinically significant medical disease or condition that, in the investigator's opinion, may interfere with protocol adherence or the patient's ability to give informed consent.
- Pregnant women and women who are lactating. Leflunomide has potential for teratogenic or abortifacient effects. Because there is a potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued if the mother is enrolled on this study.
- Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures, e.g., infection/inflammation, intestinal obstruction, unable to swallow medication, social/ psychological issues, etc.
- Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

Investigational Product Dosage and Administration:

Leflunomide will be administered orally at an initial dose of 20 mg daily following a loading dose of 100 mg per day for the first three days of administration. For the phase I portion of the study, five daily dose levels of leflunomide may be tested (10, 20, 30, 40 or 60 mg daily) There will be no intra-patient dose escalation. The R2PD will be used as the daily dose for the phase II portion of the study. At the end of the study, all participants will receive 11 days of oral cholestyramine treatment (8 g three times daily) to reduce the plasma leflunomide levels.

Clinical Observations and Tests to be Performed:

Clinical observations will include tolerance as well as response to treatment. Baseline studies will include: history and physical, pulmonary function test, skeletal survey and bone marrow aspiration and biopsy (the bone marrow aspiration will be used for clinical assessment and leftover samples will be used for correlative laboratory studies), as well as a laboratory evaluation to include CBC with differential, comprehensive chemistry panel (to include LDH, magnesium, phosphorus and uric acid) and myeloma labs (serum protein electrophoresis, serum immunofixation, 24-hour urine protein electrophoresis, urine immunofixation, serum free light chains, and quantitative immunoglobulins). Clinical observation of response will take place on day 1 of each 28-day cycle. Subjects will be evaluated through clinic visits and physical exams as well as laboratory tests to include CBC with differential, comprehensive chemistry panel and myeloma labs. Blood tests will be performed at a minimum of every 28 days but may be done more frequently as clinically indicated. A skeletal survey may be repeated as clinically indicated. Patients will undergo toxicity assessment every two weeks for the first three cycles following initiation with leflunomide and will undergo, at a minimum, monthly toxicity assessments thereafter. For all phase II patients and phase I patients enrolled at the anticipated RP2D, a second bone marrow aspiration will be conducted for correlative laboratory studies on day 1 of cycle 2. Confirmation of complete response (CR), stringent complete response (sCR), and progressive disease (PD) will involve bone marrow aspiration and, for patients who have enrolled in the phase II portion, leftover samples from these bone marrow aspirations will be used

for correlative laboratory studies.
Statistical Considerations:
Analysis: Observed toxicities will be summarized in terms of type (organ affected or laboratory determination), severity, attribution, time of onset, duration, serum concentration of the active leflunomide metabolite, probable association with the study treatment and reversibility or outcome. Baseline information (e.g. the extent/type of prior therapy) and demographic information will be presented as well to describe the patients treated in this study. Rates and 95% Clopper Pearson binomial confidence interval (CI) will be calculated for overall response rate (patients that have confirmed sCR/CR/VGPR or PR) and clinical benefit rate (patients that have confirmed sCR/CR/VGPR/PR/MR or SD). In addition, sub-analyses will be performed where participants will be considered evaluable for response if they are confirmed eligible, receive at least 75% of leflunomide during the first cycle of therapy and have their disease re-evaluated. Response rates will also be explored based on number/type of prior therapy(ies) and serum concentration of the leflunomide metabolite (µg/mL). Time to response and survival will be estimated using the product-limit method of Kaplan and Meier. Serum trough levels of leflunomide's active metabolite, teriflunomide (A77 1726), will be measured in each patient at multiple time points throughout the study. Both total and free teriflunomide will be tested. The association between serum levels and toxicity, as well as response, will be evaluated at the conclusion of the study. Genetic polymorphisms in genes associated with the biological activity of leflunomide (e.g., DHODH, CYP1A2, and CYP2C19) have been previously described and will be explored for possible associations with response and safety outcomes. *Ex vivo* studies will be performed on bone marrow aspirate-derived cells to evaluate the IC50 for teriflunomide and the potential synergy with other anti-neoplastic agents. Correlation analyses will be performed to determine the relationship between *ex vivo* and *in vivo* response. Preliminary molecular profiles for miRNA, mRNA, and DNA methylation status will be derived from both *ex vivo* and *in vivo* treatment. These profiles will be used to identify molecular correlates of therapeutic response using pathway identification and supervised principal components analysis.
Sponsor/Licensee:
Investigator Initiated: City of Hope
Case Report Forms
Medidata RAVE® Electronic Data Capture system. Forms are detailed in Section 12.1.3.

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ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRC	Clinical Research Coordinator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLCO	Carbon Monoxide Diffusing Capacity
DLT	Dose Limiting Toxicity
DHODH	Dihydroorotate Dehydrogenase
DSMC	Data Safety Monitoring Committee
EBMT	European Bone Marrow Transplant
EOT	End of Treatment
FDA	Food and Drug Administration
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
ICG	Integrated Genomics Core
IDS	Investigational Drug Services
IMWG	International Myeloma Working Group
IND	Investigational New Drug
IRB	Institutional Review Board
MM	Multiple Myeloma
MNC	Mononuclear Cells
MR	Minimal Response
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
RP2D	Recommended Phase II Dose
RRMM	Relapsed Refractory Multiple Myeloma
RA	Rheumatoid Arthritis
SAE	Serious Adverse Event
sCR	Stringent Complete Response
SD	Stable Disease

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

In patients with relapsed or relapsed/refractory multiple myeloma (MM):

Phase I

Primary Objectives:

- To determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of leflunomide, when given as a single agent.
- To assess the safety and tolerability of leflunomide at each dose level by evaluation of toxicities including: type, frequency, severity, attribution, time course and duration.

Phase II

Primary Objective:

- To evaluate the anti-myeloma activity of leflunomide, when given as a single agent, as assessed by overall response rate (ORR).

Secondary Objectives:

- To obtain estimates of:
  - Response duration
  - Clinical benefit response
  - Overall survival and
  - Progression-free survival.

Clinical Pharmacology Objectives (Phase I and II)

- To characterize the relationship between serum concentration of the active leflunomide metabolite, teriflunomide (A77 1726), and toxicity.
- To assess the relationship between serum concentration of the active leflunomide metabolite, teriflunomide A77 1726), and disease response.

Exploratory Ex-Vivo and Molecular Objectives (Phase I and II)

- To explore the relationship between polymorphisms in the *CYP1A2, CYP2C19*, or *DHODH* genes and toxicity/response. CYP1A2, CYP2C19 are the hepatic enzymes responsible for leflunomide metabolism.
- To explore the *ex vivo* cytotoxicity of leflunomide toward primary MM cells, in order to evaluate whether individual *ex vivo* leflunomide response might be a useful predictor of therapeutic response.
- To explore the potential additive or synergistic effects of combining leflunomide with other classes of FDA-approved drugs. The classes include HDAC inhibitors, immunomodulatory drugs [IMiDs], proteasome inhibitors, steroid hormones, and antibody therapeutics such as the CD38 antibody daratumumab.
- To generate a preliminary RNA/miRNA and DNA methylation signature associated with response of MM cells to leflunomide *in vivo* (mRNA/miRNA and DNA methylation, phase II only) and teriflunomide *ex vivo* (mRNA/miRNA).

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Multiple myeloma (MM) is a plasma cell malignancy characterized by periods of remissions and relapses. Worldwide, the incidence of MM is about 86,000 cases annually with about 63,000 deaths each year [4]. In the United States, over 30,000 patients are expected to be diagnosed with MM in 2016 and more than 12,000 patients will die from the disease [5, 6].

Treatments for MM include alkylating agents, immunomodulatory (IMiD) drugs, proteasome inhibitors, anthracyclines, and glucocorticoids. Therapeutic agents are used sequentially or in combination either as double, triple or quadruple chemotherapy combinations. While the prognosis for MM patients has improved [7, 8], MM remains an incurable disease with a median survival of approximately 3 to 4 years with standard therapy[9]. The 5-year survival rate for patients with MM is estimated at 46% (Cancer Statistics 2016), with only 17% of patients surviving for 10 years[10].

Relapsed and refractory multiple myeloma (RRMM) is defined as disease that is nonresponsive while on salvage therapy, or progressing within 60 days of cessation of the most recent prior therapy in patients who have achieved minimal response (MR) or better[11]. Primary refractory myeloma is defined as disease that is nonresponsive in patients who have never achieved a minimal response or better with any therapy[11]. The overall survival for patients who have received more than 3 prior regimens and whose disease has relapsed after treatment with bortezomib and lenalidomide is estimated to be between 6 and 10 months [12, 13]. Treatment decision making in the RRMM population is particularly challenging, given underlying comorbidities and the toxic nature of available agents. Moreover, duration of response to therapies is traditionally short due to multi-drug resistance as well as changes in disease biology (e.g., development of aggressive phenotype, higher proliferation rates, and lower apoptotic rates) [14]. Patients with RRMM are in dire need of new treatments with new mechanisms of action that could be used successfully long term.

2.2 Leflunomide

Leflunomide is a commercially available oral immunosuppressive agent that has been FDA approved since 1998 for the treatment of rheumatoid arthritis (RA) as a single agent or in combination with methotrexate. It has been used in over 300,000 patients worldwide for RA treatment. Leflunomide is generally well-tolerated and may be taken over a long period of time.

The in vitro and in vivo mechanisms of leflunomide are not completely defined. The primary clinical mechanism of action is inhibiting de novo pyrimidine synthesis by targeting dihydroorotate dehydrogenase (DHODH), and thus achieving anti-proliferative effect in B- and T-lymphocytes. A secondary mechanism of action is inhibition of cytokine and growth factor receptor associated tyrosine kinase activity [15].

Leflunomide (the pro-drug) is rapidly converted to its active primary metabolite teriflunomide (A77 1726), which mediates leflunomide's pharmacologic activity. Teriflunomide serum concentrations show wide inter-patient variability; in one study, the mean serum teriflunomide concentration in patients receiving 20 mg/ daily was 42 mg/l with a range from 3-150 mg/l and a standard deviation of 35 mg/l [16]. Of note, the serum concentration of this metabolite was shown to predict RA response[16].

The FDA approved dose in adults for the treatment of RA is a loading dose of 100 mg orally (PO) once daily for 3 days, followed by a maintenance dose of 10-20 mg PO once daily. Leflunomide has been used at up to 40 mg/day in patients with Wegner's granulomatosis with a safety profile similar to the 20mg/day dose used in RA [17, 18]. Doses up to 60 mg/day have been safely used for the treatment of allograft polyoma BK virus in renal allograft patients to achieve a targeted blood level of teriflunomide

(A77 1726) of 50-100 micrograms/mL [19]; the targeted blood level was frequently achieved with a dose of 40mg/ day with no increase in toxicity reported [19].

In addition to studies that demonstrate the serum concentration of the metabolite may predict response, recent work demonstrates that polymorphisms in the gene encoding DHODH may be associated with leflunomide treatment outcome in RA patients[20, 21].

The long half-life of the metabolite (about 2 weeks) affects management of patients taking leflunomide. A loading dose of 100 mg for 3 days is used to facilitate rapid attainment of steady state levels of M1. Without a loading dose, it is estimated that attainment of steady state concentrations would require nearly two months of dosing [22]. To eliminate the metabolite, a drug elimination procedure using cholestyramine is recommended. Cholestyramine, a bile acid sequestrant, fixes the metabolite, preventing reabsorption and expediting drug elimination. Without the drug elimination procedure, it may take up to 2 years to reach non-detectable plasma levels after stopping treatment with leflunomide [22].

2.3 Preclinical Studies of Leflunomide as an Anti-Neoplastic Agent

Leflunomide has been investigated for its anti-neoplastic potential in a variety of pre-clinical tumor models. The first reports of anti-tumor activity in mouse xenograft models of glioma indicate that inhibition of pyrimidine synthesis is not sufficient for anti-tumor activity and concomitant inhibition of tyrosine phosphorylation may also play a role[23]. In chronic lymphocytic leukemia (CLL), leflunomide has been shown to inhibit the cell cycle progression in primary leukemic cells in vitro [24]. Dietrich et al showed that teriflunomide (A77 1726) affects proliferation of CLL by blocking DHODH at very low concentration (3-5 µg/ml) and by additionally inhibiting JAL/STAT pathway at intermediate concentrations (>10 µg/ml)[25]. The metabolite is also effective in fludarabine-refractory cells[25]. In addition, pre-clinical anti-tumor activity of leflunomide has been reported for melanoma [26] and prostate cancer [27].

Recent pre-clinical studies of teriflunomide (A77 1726) have demonstrated significant antineoplastic activity in myeloma [28]. Teriflunomide inhibits cell growth and induces apoptosis in common myeloma cell lines at clinically achievable concentrations (50-200 µmol/L) in a time- and dose-dependent manner. The stimulatory effect of conditioned medium of HS-5 bone marrow stromal cells on multiple myeloma cell growth is completely abrogated by teriflunomide. In addition, studies revealed additive effects when teriflunomide was combined with the genotoxic agents melphalan and doxorubicin and synergistic effects when combined with bortezomib and treosulfan[28]. Finally, the Rosen laboratory has found leflunomide to be active in both glucocorticoid sensitive and resistant myeloma cell lines (Steven Rosen, personal communication).

2.4 Clinical Experience with Leflunomide for Multiple Myeloma

Oral leflunomide was given to an 81-year-old female myeloma patient who did not have other treatment options or suitable clinical trials. This patient was diagnosed with myeloma in January of 2001 and had an extensive treatment history including Velcade, Velcade-Doxil, Revlimid-dexamethasone, Revlimid-Melphalan-Prednisone, and Velcade-dexamethasone. Her disease was refractory and progressive, with rapidly increasing IgG, M protein, and pancytopenia with transfusion-dependent anemia. She began oral daily leflunomide (20 mg per day) in February 2011. Her myeloma showed a gradual and significant improvement, with reduction of her serum IgG (from 6280 to 3890 mg/dl), M-protein, and beta-2-microglobulin levels. Her anemia improved so that she was no longer transfusion-dependent. She tolerated the treatment fairly well except for mild diarrhea managed with loperamide. She remained on leflunomide for 16 months with continued clinical response of her myeloma but unfortunately died of her other medical problems (severe cardiac valvular disease and congestive heart failure).

2.5 Background for Correlative Studies

Leflunomide is rapidly converted to its active primary metabolite teriflunomide (A77 1726), which mediates leflunomide's pharmacologic activity. Previous population-based PK studies in patients with RA have demonstrated a high degree of intersubject variability in measured steady-state total (free + albumin-bound) teriflunomide serum concentrations, resulting in coefficients of variation in CL/F estimates of >50% [29, 30]. Teriflunomide is >99% bound to serum albumin and free drug concentrations have also been reported to be highly variable, with a median of 55.8 μg/l and a range of 27.9-148.4 μg/l [31]. Moreover, measured total and free teriflunomide levels have been shown to predict response and toxicity in RA patients, leading some investigators to suggest a role for therapeutic drug monitoring and dose optimization of leflunomide [16]. It has also been reported that genetic polymorphisms in CYP1A2 and CYP2C19, the hepatic enzymes responsible for leflunomide metabolism, and DHODH, a key enzyme in the *de novo* synthesis of pyrimidines and the main target of teriflunomide, can all influence the risk of leflunomide-associated toxicity in RA patients [32-34]. DHODH is expressed in MM cells, however, the relationship between DHODH gene polymorphism and toxicity/response to leflunomide is not well understood. Therefore, a better understanding of the relationship between teriflunomide serum concentrations and toxicity/response, as well as the influence of inherited polymorphisms in the genes involved in the metabolism and mechanism of action of leflunomide is needed.

Various distinct genomic and epigenomic abnormalities have been observed in multiple myeloma, including gene mutations, chromosomal abnormalities, and DNA methylation [35]. Inhibition of tumor suppressor gene expression by DNA hypermethylation promotes survival and expansion of MM cells and clinical outcomes have been recently linked to DNA methylation status of MM cells [36]. Molecular signatures capturing such gene expression data have also recently emerged as powerful tools to identify patients who might respond particularly well to a specific treatment. Although genomic and epigenomic markers show promise for MM, there are still very few studies available and reliable markers have not yet emerged.

2.6 Overview and Rationale of Proposed Study

The pre-clinical data, along with limited although promising clinical data of leflunomide for treatment MM, and the relatively low toxicity profile seen with prolonged administration in the rheumatoid arthritis population, in total provide evidence to support the investigation of leflunomide as an anti-myeloma agent. This single agent phase I/II dose-escalation trial is designed to determine the MTD and to generate information about efficacy at theRP2D. Relapsed or relapsed/refractory multiple myeloma (RRMM) patients with documented measurable disease after at least three prior regimens will be considered for enrollment.

While the standard dose of leflunomide (20mg daily), following a loading dose of 100mg daily for 3 days, is FDA approved for continuous treatment of rheumatoid arthritis, leflunomide has been administered without an increase in the type, frequency or severity of adverse events at doses up to 40 mg/day in patients with Wegner's granulomatosis, and up to 60mg/day in a subset of patients with polyoma BK neuropathy to achieve targeted therapeutic levels [17, 18].

Because the current dosing guideline for patients with rheumatoid arthritis do not to exceed 20 mg daily maintenance doses [22], the 20mg/day dose is selected as the safe starting dose. We will attempt to escalate the dose of leflunomide to three times that approved for RA using a modified rolling six dose-escalation/ de-escalation/expansion design, a more conservative version of the rolling six design of Skolnik, et al.[1]. The starting dose of leflunomide is 20mg daily, with the intention to test up to 60mg daily. Escalation will proceed in increments of 20mg/day (odd numbered dose levels); de-escalation will proceed in decrements of 10mg/day.

Patients will be treated in planned 28-day treatment cycles. Patients may continue to receive cycles of treatment on study until disease progression or unacceptable toxicity, or other criteria for removal from study treatment are satisfied. While on active treatment patients will undergo monthly evaluation for response using the International Myeloma Working Group (IMWG) criteria [37, 38]. Following the decision to end leflunomide treatment, all patients will undergo 11 days of treatment with cholestyramine, which fixes the metabolite, preventing re-absorption and effectively lowering drug levels. Cholestyramine has been shown to decrease plasma levels of teriflunomide (A77 1726) in healthy volunteers by approximately 40% in 24 hours and by 49-65% in 48 hours [22].

Previous RA studies have shown a large variation in the serum concentration of the primary active metabolite, teriflunomide (A77 1726), and a suggested association between serum concentration of this metabolite and response [19]. Although the prior PK/PD relationship was identified in a non-oncologic patient population, it is possible that a similar association could exist for leflunomide exposures and antitumor response. Therefore, as part of the secondary objectives for this trial, enrolled RRMM patients will have blood samples collected and then processed by the Analytical Pharmacology Core Facility (APCF) to assess the possible relationship between serum concentration of the active leflunomide metabolite (teriflunomide) and toxicity/response. The natural PK variability of leflunomide administered at fixed doses will allow us to assess patients over a wide range of measured drug exposures. If a therapeutic window for leflunomide exposure can be identified, future studies based on targeted drug exposures will be considered. Additional testing will be done on collected blood samples to assess, in an exploratory manner, the potential association between germline polymorphisms in the *CYP1A2, CYP2C19,* and *DHODH* gene and either toxicity or response.

In addition to the PK/PD analysis, additional molecular correlates and *ex vivo* responses will be evaluated using bone marrow specimens, which will be collected from patients before treatment and after one month of leflunomide treatment. MM cells will be purified from the pre-treatment specimens and treated with teriflunomide to determine the *ex vivo* IC50. The goal of this study is to determine whether *ex vivo* drug response predicts *in vivo* therapeutic response. The *ex vivo* treatment concentrations will be similar to *in vivo* drug levels. Teriflunomide will also be combined with drugs from other classes (e.g., HDAC inhibitors, immunomodulatory drugs [IMiDs], proteasome inhibitors, steroid hormones, and antibody therapeutics such as the CD38 antibody daratumumab), in order to evaluate *ex vivo* synergy and potentially predict the success of combination therapies. Molecular correlate analysis of leflunomide response will include miRNA, mRNA and DNA methylation profiling. Specimens derived from before and after leflunomide treatment (after 1 month and at progression or complete response, if possible) will be analyzed, as well as pre-treatment specimens exposed to teriflunomide *ex vivo*. The miRNA, mRNA and DNA methylation profiling analysis of specimens derived from participants treated with leflunomide *in vivo* will be conducted only for participants treated at the RP2D in order to minimize extra interventions for participants who may not receive a therapeutically effective dose.

Following completion of the phase I portion of the study and identification of the RP2D, the phase II portion of the study will be initiated. Recent studies evaluating daratumumab, a CD38 monoclonal antibody, as monotherapy for relapsed/refractory multiple myeloma were recently published [39, 40] and have led to FDA approval of this agent. One study included patients with relapsed multiple myeloma treated with at least three lines of therapy while the other included patients treated with at least two prior lines of treatment. Treatment with daratumumab as a single agent lead to an overall response rate of 29.2% and 36% respectively in each of these studies. In both studies, the agent investigated was well tolerated but required IV dosing once weekly for 8 doses, followed by twice-monthly doses for 8 doses and then monthly thereafter. We suspect leflunomide to be similarly well tolerated but with a more convenient dosing schedule. Because we are including patients who have received at least three prior lines of treatment, we will target an overall response rate of 30% for the phase II portion of the study.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

Patient MRN:	Patient Initials (F, M, L):

Informed Consent

___ 1. All subjects must have the ability to understand and the willingness to sign a written informed consent.

Age Criteria, Performance Status, and Life Expectancy

___ 2. Patients must be age ≥ 18 years.

___ 3. Patients must have a life expectancy of > 3 months.

___ 4. Patients must exhibit an ECOG performance status of 0-2.

Nature of Illness and Treatment History

___ 5. Patients must have a diagnosis of multiple myeloma.

___ 6. Patients must have measureable disease, defined as one of the following within 21 days prior to registration:

- Serum M-protein ≥ 0.5 g/dL
- Urine M-protein ≥ 200 mg/24 hr
- Serum free light chain ≥ 10 mg/dL provided the FLC ratio is abnormal.
- 10% plasma cells in bone marrow

___ 7. Patients must be relapsed or are refractory to at least 3 prior lines of therapy, including both a proteasome inhibitor and an IMiD, and for whom transplant is not recommended (induction therapy and stem cell transplant +/- maintenance will be considered as one regimen).

___ 8. At least 2 weeks from prior therapy to time of start of treatment. Prior therapy includes steroids (except prednisone or equivalent – up to 10 mg per day is allowed).

Clinical laboratory parameters

___ 9. Platelet count ≥ 50,000/µL. Platelet transfusions are not allowed within 14 days of platelet assessment.

___ 10. ANC ≥ 1000/mm^3. Growth factor is not permitted within 14 days of neutrophil assessment.

___ 11. AST and ALT < 2.0 x ULN.

___ 12. Total Bilirubin < 1.5 x ULN.

___ 13. Calculated creatinine clearance (CrCl) ≥ 30 mL/min per 24-hour urine collection or the Cockcroft-Gault formula.

$$\text{CrCl (mL/min)} = \frac{\text{(140-age) x actual body weight (kg)}}{\text{72 x serum creatinine (mg/dL)}} \text{ (x 0.85 for females)}$$

Or

$$\text{CrCl (mL/min)} = \frac{\text{(140-age) x actual body weight (kg)}}{\text{0.8136 x serum creatinine (umol/L)}} \text{ (x 0.85 for females)}$$

___ 14. Negative serum or urine β-HCG test (female patient of childbearing potential* only), to be performed locally within the screening period.

___ 15. Negative for tuberculosis antigen (e.g. T-Spot test).

___ 16. Negative for hepatitis A, B, or C infection.

___ 17. Adequate pulmonary function as defined by FVC and DLCO ≥ 50% of predicted by pulmonary function testing.

Child Bearing Potential

___ 18. Agreement by females of childbearing potential* and sexually active males to use an effective method of contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for three months following duration of study participation. The effects of study treatment on a developing fetus have the potential for teratogenic or abortifacient effects. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

*A female of childbearing potential is defined as a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months.

3.2 Exclusion Criteria

Prospective participants who meet any of the following criteria will not be eligible for admission into the study:

Previous therapies

___ 19. Prior treatment with leflunomide.

Concomitant medications

___ 20. Current or planned use of other investigational agents, or concurrent biological, chemotherapy, or radiation therapy during the study treatment period. (See inclusion criterion #8 for washout times).

___ 21. Current or planned growth factor or transfusion support until after initiation of treatment. If growth factor or transfusion support is provided between screening and start of treatment, the participant will no longer be eligible.

Other illnesses or conditions

___ 22. Prior diagnosis of rheumatoid arthritis.

___ 23. Prior Allogeneic transplant.

___ 24. Acute active infection requiring systemic therapy within 2 weeks prior to enrollment.

___ 25. Pre-existing liver disease.

___ 26. Known HIV infection.

___ 27. History of allergic reactions attributed to compounds of similar chemical or biologic composition to leflunomide or cholestyramine.

___ 28. Non-hematologic malignancy within the past 3 years aside from the following exceptions:

- adequately treated basal cell or squamous cell skin cancer
- carcinoma in situ of the cervix
- prostate cancer < Gleason Grade 6 with a stable PSA
- successfully treated in situ carcinoma of the breast

___ 29. Clinically significant medical disease or condition that, in the investigator's opinion, may interfere with protocol adherence or the patient's ability to give informed consent.

___ 30. Pregnant women and women who are lactating. Leflunomide has potential for teratogenic or abortifacient effects. Because there is a potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued if the mother is enrolled on this study.

___ 31. Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures, e.g., infection/inflammation, intestinal obstruction, unable to swallow medication, social/ psychological issues, etc.

Noncompliance

___ 32. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

3.3 Inclusion of Women and Minorities

The study is open anyone regardless of gender or race/ethnicity. Efforts will be made to extend the accrual to a representative population. However, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4.0 SCREENING AND REGISTRATION PROCEDURES

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. All screening procedures and their respective windows are detailed in Section 10, Table 10, Study Activity Calendar.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record and the patient will receive a copy of the signed informed consent document. All Institutional, Federal, and State regulations concerning Informed Consent will be fulfilled.

4.3 Registration Requirements/Process

Confirming, reserving a slot and dose level assignment

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. Staff (including physicians, protocol nurses and/or CRCs) should call the DCC at (626) 256-4673, ext. 63968 to verify the current dose level and slot availability, and to reserve a slot for a specific prospective subject. Slots can only be held for a limited time.

Eligible subjects must be registered **prior** to start of protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, the subject's registration on the study may be canceled after discussion with the PI. The Data Coordinating Center should be notified of cancellations as soon as possible.

Registration Process

Once a slot at a dose level has been reserved, the signed informed consent has been obtained, all pretreatment evaluations have been performed, and subject's eligibility has been confirmed by the Data Coordinating Center, a subject will be registered on study.

To register a subject, the treating physician should contact the protocol nurse or the responsible Clinical Research Coordinator (CRC) in the Clinical Trial Office (CTO) to complete the eligibility checklist.

The protocol nurse or CRC will contact the Data Coordinating Center at City of Hope (626-256-4673, ext. 63968 or via e-mail at dcc@coh.org), scan and EMAIL a copy of the completed and signed eligibility checklist, a copy of the signed Informed Consent (including a copy of signed subject's bill of Rights and HIPAA authorization form) and copies of any required pre-study test results that are not readily available in the COH electronic medical record to dcc@coh.org.

The protocol nurse or CRC may then call the Data Coordinating Center (626-256-4673 ext. 63968) to confirm receipt of all registration documents. To complete the registration process, the Data Coordinating Center will:

- Verify and confirm the subject's eligibility.
- Assign a subject accession number (for example, COH-001, COH-002, etc.).
- Register the subject on study centrally (the City of Hope CRC assigned to the trial will still be responsible for accessioning via MIDAS).
- Assign the subject to a dose level (as applicable).
- Complete and email a Confirmation of Registration form within 24 hours to include the COH subject study number and dose level to the study team, which will include the Principal Investigator, treating physician, protocol nurse, CRC and COH IDS Pharmacy.
- Call the protocol nurse and/or CRC to verbally confirm registration.
- Enter the subject into Medidata RAVE®.
- A subject failing to meet all protocol requirements will not be registered.

Patients must begin protocol treatment within 1 week following registration date.

4.4 Screen Failures and Registered Participants Who Do Not begin Study Treatment

The reason for screen failure or failure to begin treatment for registered participants will be documented.

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

Treatment on study will be in the outpatient setting and will consist of daily treatment with leflunomide; patients will be treated in planned 28-day treatment cycles. After the decision to end study treatment has been made, participants will receive treatment with cholestyramine (Section 5.7) to eliminate leflunomide. Participants who end study treatment for reasons other than disease progression will undergo active follow-up (Section 5.9) until disease progression or the initiation of a new therapy. All participants will be followed for survival (Section 5.9).

For a detailed tabular view of the treatment, monitoring, and follow-up schedule, see the Study Calendar in Section 10.

5.2 Treatment cycle definition

The planned cycle length is 28 days. Leflunomide will be taken orally once daily. Missed or held doses will not be made up. The day count continues despite a hold in agent administration. Day 1 safety assessments must be reviewed prior to the initiation of the cycle. For a cycle to commence, the participant must be actively taking study agent. Windows for procedures are detailed in Section 10.

5.3 Phase I/II Treatment Plan

This phase I trial will implement a modified rolling six dose escalation, de-escalation, expansion design, a more conservative version of the rolling six design of Skolnik, et al. [1] to ensure safety and to allow for the evaluation of toxicities associated with leflunomide. See Section 13.3.2 for dose escalation/de-escalation/expansion rules, and Section 13.1 for the definition of dose limiting toxicity (DLT).

Up to six doses of leflunomide will be considered (see Table 5.3.1). The starting dose of leflunomide is 20mg daily, with the intention to test up to 60mg daily. Escalation will proceed in increments of 20mg/day (odd numbered dose levels); de-escalation will proceed in decrements of 10mg/day. There will be no intra-patient dose escalation; participants will undergo dose modification due to toxicity per Section 6.2.

Table 5.3.1: Dose Levels

Dose Level	Leflunomide Dose*
-1	10 mg PO daily
1	20 mg PO daily
2	30 mg PO daily
3	40 mg PO daily
4	50 mg PO daily
5	60 mg PO daily

*NOTE: Leflunomide is administered with a loading dose of 100 mg for the 1st three doses.

5.4 Phase II Treatment Plan

Following the phase I trial, a single arm phase II study will be conducted using a Simon Two-Stage Optimal Design [2, 3] to evaluate the anti-myeloma activity of leflunomide. For the phase II portion, up to 39 patients will be treated. The six patients treated at the RP2D in the phase I portion of the study will count toward the 39 patients required; given this, we expect to enroll only 33 new patients on the phase II trial.

5.5 Agents Administration

5.5.1 Leflunomide

Participants will be instructed to take each dose of leflunomide orally, once a day, at approximately the same time each day. They may take it with or without food. If a patient forgets or misses a dose for whatever reason, it should **not** be replaced or made up. Participants will be given a study calendar (pill diary) to document each dose of leflunomide that is taken or missed.

NOTE: On study visit days, participants should refrain from taking leflunomide until after blood is collected.

Leflunomide is administered with a loading dose of 100 mg per day for the first three doses (first three days).

There will be no intra-patient dose escalation; patients will undergo dose modification due to toxicity per Section 6.2.

5.5.2 Cholestyramine

Cholestyramine is not a study agent but is required for the elimination of the study agent leflunomide. See Section 5.7 for cholestyramine administration details.

5.6 Study Procedures

For a detailed list of all study procedures including timing and windows, see Section 10.

5.7 End of Treatment Evaluations and Leflunomide Detoxification

All participants will be followed until resolution or stabilization of any serious adverse events occurring during treatment and completion of the Day 30 End of Treatment assessments. See Section 10 for a list of all assessments and windows.

Because of the ability of leflunomide to be reabsorbed through the colon, resulting in a very long half-life, all participants will be treated with cholestyramine to prevent re-absorption of leflunomide after the decision to end study treatment has been made.

Participants removed from study treatment will undergo oral administration of cholestyramine 8 grams oral suspension three times daily for 11 days. It will not be a deviation if an administration of cholestyramine is delayed or if the 11 days are not consecutive. Cholestyramine administration may be adjusted per the discretion of the treating investigator. After the treatment with cholestyramine, clinical laboratory testing will be performed to verify plasma levels less than 0.02 mg/L or 0.02µg/mL by two separate tests at least 14 days apart. If plasma levels are higher than 0.02 mg/L or 0.02µg/mL, additional cholestyramine treatment will be considered. See Section 10 for a tabular view of cholestyramine administration and metabolite testing windows and days of administration.

Note: Cholestyramine can affect the absorption of other oral medications. Such medications should be taken 1 hour before the first administration or per other guidance in the package insert. Watch for bleeding abnormalities due to vitamin K deficiency for which intravenous vitamin K may be administered.

5.8 Duration of Therapy & Criteria for Removal from Study Treatment

Patients will receive study treatment until disease progression, unacceptable toxicity or other criteria for removal from study treatment are satisfied. Of note, based on the guidelines outlined by Rajkumar et al. [38], "CR patients will need to progress to the same level as VGPR and PR patients to be considered PD. A positive immunofixation alone is therefore not sufficient." Participants may be removed from treatment for any of the following reasons:

- Evidence of disease progression
- Patient is deemed intolerant to study treatment because of toxicity, despite dose modification/delay
- Intercurrent illness that prevents further administration of treatment
- Participant withdraws from the treatment phase of the study
- General or specific changes in the participant's condition, including non-compliance, which renders the participant unacceptable for further treatment in the opinion of the treating investigator.

Documentation of the reason for discontinuing therapy and the date effective should be made in the medical record and appropriate eCRF. The participant should then proceed to off-treatment procedures for safety monitoring, leflunomide elimination and follow-up procedures. The participant's status is to be modified in the MIDAS system once the off-treatment period is completed. Alternative care options will be discussed with the participant.

5.9 Active Follow Up and Long Term Follow Up

Participants who do not progress while on treatment will be in *Active Follow Up*, where they will continue to undergo monthly response assessment (myeloma labs) until disease progression or the initiation of a new therapy.

Participants who progress during Treatment Phase skip *Active Follow-Up* phase and continue directly into *Long-term Follow-up* phase.

In *Long-term Follow-Up* participants will be followed for survival information which may be obtained by reviewing the City of Hope medical record, contacting the participant and/or a review of outside medical records. Note: this is not intended to be an all-inclusive list.

The schedule and windows for assessments and data collection points are further detailed in Table 10.

5.10 Criteria for Completion of Study Participation

Participants may be removed from the study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuing study participation should be documented and may include:

- Participant completes all study procedures including all study follow-up procedures.
- Participant withdraws consent for follow-up.
- Participant is determined to be lost to follow-up. All attempts to contact the subject must be documented.

The reason for study removal and the date the participant was removed must be documented in the source documentation and the study-specific case report form (CRF). The participant's status is to be modified in the MIDAS system once the participant completes the study.

5.11 Supportive Care, Other Concomitant Therapy, Prohibited Medications

Concomitant medications and treatments that are permitted while on study treatment include allopurinol, anti-emetics, anti-diarrheals, FDA-approved bisphosphonates, erythropoietin, transfusions (as necessary), and palliative radiation.

Concurrent treatment with any other anti-neoplastic therapies will not be permitted while on study treatment. Concurrent administration of live vaccines will not be permitted while on study treatment.

During cycle 1 only: Transfusions are not permitted unless platelets <25,000/mm^3 or platelets < 50,000/ mm^3 accompanied by bleeding. Growth factors are not permitted unless ANC <500/ mm^3.

5.12 Additional Studies

See Section 9.0 for correlative studies.

6.0 ANTICIPATED TOXICITIES AND DOSE DELAYS/MODIFICATIONS FOR ADVERSE EVENTS

6.1 Anticipated Toxicities

6.1.1 Leflunomide

Per the package insert for leflunomide, the expected toxicities for leflunomide follow, where the asterisk (*) signifies a common event (10%-30% of patients), unmarked items are infrequent events (1-3% of patients), a double asterisk (**) signifies a rare but possibly serious event, and a triple asterisk (***) signifies an event that may become serious and occurs in up to 10% of patients:

Blood and lymphatic system: anemia (including iron deficiency anemia), ecchymosis, pancytopenia**, agranulocytosis**, neutropenia**, thrombocytopenia**, leukopenia**,

Cardiac: angina pectoris, migraine, palpitation, tachycardia, vasodilatation

Endocrine: diabetes mellitus, hyperthyroidism

Eye: blurred vision, cataract, conjunctivitis, eye disorder

Gastrointestinal: diarrhea*, abdominal pain, dyspepsia, nausea, vomiting, oral ulceration, anorexia, pancreatitis**, constipation, esophagitis, flatulence, gastritis, gingivitis, melena, oral moniliasis, pharyngitis, salivary gland enlarged, stomatitis (or aphthous stomatitis), tooth disorder

Hepatobillary: cirrhosis**, hepatitis**, hepatic failure**, acute hepatic necrosis**, cholelithiasis**, cholestasis**, elevated hepatic enzymes (primarily ALT and AST)

Immune system: allergic reactions, anaphylactoid reactions**

Infections: respiratory infection*, infections (including bronchitis, rhinitis, sinusitis, pharyngitis, pneumonia, and urinary tract infections, oral or vaginal candidiasis, herpes simplex, herpes zoster, and fungal dermatitis), opportunistic and/or severe infections** including sepsis, that may be fatal (especially Pneumocystis jiroveci pneumonia, tuberculosis, aspergillosis)

Metabolism and nutrition: hyperglycemia, creatine phosphokinase increased, hyperlipidemia

Musculoskeletal and connective tissue: arthrosis, bone necrosis, bone pain, bursitis, muscle cramps, myalgia, tendon rupture

Neoplasms: secondary malignancy**, cyst

Nervous system: headache*, peripheral neuropathy* (including peripheral numbness, tingling, burning, severe pain, cold sensation in the distal extremities, or extremity weakness), paresthesias, taste perversion (dysgeusia), anxiety, depression, dry mouth, insomnia, neuralgia, neuritis, sleep disorder, sweating increased, vertigo, migraine

Renal and urinary: albuminuria, cystitis, dysuria, hematuria, hypophosphaturia, hyperuricemia, increased urinary frequency

Reproductive: menstrual irregularity/disorder, menstrual disorder, vaginal moniliasis vaginal moniliasis, prostate disorder

Respiratory: interstitial lung disease** (sometimes fatal), interstitial pneumonitis**, pulmonary fibrosis**, asthma, dyspnea, epistaxis, lung disorder

Skin and subcutaneous tissue: maculopapular rash*, dry skin*, alopecia*, hair discoloration*, Stevens-Johnson syndrome**, toxic epidermal necrolysis**, erythema multiforme**, cutaneous lupus erythematosus**, acne, contact dermatitis, fungal dermatitis, hematoma, nail disorder, skin discoloration, skin disorder, skin nodule, subcutaneous nodule, skin ulcer

Vascular: varicose veins, hypertension***, vasculitis, cutaneous necrotizing vasculitis**

Miscellaneous: weight loss*, leg cramps*, jaundice**, allergy related angioedema**, fever, peripheral edema, hernia, neck pain, pelvic pain, pain, abscess, malaise

6.1.2 Cholestyramine

Per the package insert for cholestyramine, the expected toxicities for cholestyramine follow, where the double asterisk (**) signifies a common event, and a single asterisk (*) signifies a less common event, and remaining unmarked items are less likely:

Blood and lymphatic system: anemia, ecchymosis, prolonged prothrombin time, hypoprothrombinemia

Ear and labyrinth: tinnitus, vertigo

Eye: uveitis

Gastrointestinal: constipation**, abdominal pain*, anorexia*, nausea*, vomiting*, diarrhea*, flatulence*, diarrhea*, eructation,* steatorrhea,* diverticulitis, bleeding from known duodenal ulcer, dysphagia, gastrointestinal hemorrhage, hemorrhoidal bleeding,

hiccups, intestinal obstruction (rare), melena, pancreatitis, rectal pain, tongue irritation, tooth enamel damage (dental erosion), dental bleeding, dental caries, dental discoloration

Hepatobiliary: abnormal hepatic function tests, biliary colic, gallbladder calcification,

Immunologic: hypersensitivity reaction

Metabolism: hyperchloremic metabolic acidosis

Musculoskeletal and connective tissue: arthralgia, arthritis, backache, myalgia, osteoporosis associated with vitamin D deficiency

Nervous system: anxiety, dizziness, drowsiness, dysgeusia, fatigue, headache, neuralgia, paresthesia, syncope

Respiratory: asthma dyspnea and wheezing associated with hypersensitivity reaction

Renal and urinary: hematuria, dysuria, burnt odor to urine, diuresis

Skin and subcutaneous tissue: perianal skin irritation, skin irritation, skin rash, urticaria

Miscellaneous: adenopathy, increased libido, edema, bleeding tendencies due to vitamin K deficiency, vitamin deficiency (A, D, E, K), weight loss, weight gain, hematoma, hemorrhage, femoral nerve pain

6.2 Dose Modifications of Leflunomide

6.2.1 General Information

a. The study will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 to grade toxicities. A copy of the version 4.03 can be downloaded from:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

b. Intra-patient dose escalation is never permitted in this study. Rules for dose modification are found in Table 6.2.2.

c. Baseline values are from the last values obtained prior to treatment.

d. **For holds due to toxicities related to study agent, if the participant does not meet criteria to resume treatment within 14 days, the participant must permanently discontinue study treatment.**

6.2.2 Dose Modifications

The tables below detail the specific dose modifications for toxicities on single agent leflunomide (Table 6.2.2) and are to be used in agreement with the information in Section 6.2.1.

Table 6.2.2 Dose Modifications for Leflunomide Treatment

Adverse Event	Treatment modification
Hematological Toxicities:	
Febrile Neutropenia Grade 3 (ANC: <1.0 x10^9/L with a single temperature of >38.3 ˚C or a sustained temperature ≥ 38.0˚C for more than one hour.	Hold study agent. If resolves to ANC ≥ 1.0 x 10^9/L and temperature < 38.0˚C within 14 days, resume study agent at next lowest safe dose. If does not resolve within 14 days, permanently discontinue study agent.
Febrile Neutropenia Grade 4 Life threatening consequences; urgent intervention needed.	Permanently discontinue study agent.

Neutropenia (ANC) Grade 1 (1.5x 10^9/L -<LLN)	Maintain study agent.
Neutropenia (ANC) Grade 2 (1.0 –<1.5x 10^9/L)	Maintain study agent.
Neutropenia (ANC) Grade 3 (0.5 –< 1.0x 10^9/L)	Maintain study agent. Growth factor recommended after cycle 1.
Neutropenia (ANC) Grade 4 (<0.5x 10^9/L)	Hold study agent and provide supportive care. If ANC does not resolve to ≤ grade 3 (ANC ≥ 0.5 x 10^9/L) in 5 days, permanently discontinue study agent. Otherwise proceed as follows: • If resolves to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L) in ≤ 14 days, resume study agent at the next lowest safe dose. • If ANC does not resolve to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L) by 14 days, permanently discontinue study agent.
Thrombocytopenia Grade 1 (75 x 10^9/L -<LLN)	Maintain study agent
Thrombocytopenia Grade 2 (50 – <75 x 10^9/L)	Maintain study agent
Thrombocytopenia Grade 3 without bleeding (25 – <50 x 10^9/L)	Maintain study agent.
Thrombocytopenia Grade 3 with bleeding (25 – <50 x 10^9/L)	Hold study agent and provide supportive care. If bleeding does not resolve in 5 days, permanently discontinue study agent. Otherwise proceed as follows: • If bleeding resolves within 5 days and platelets resolve to ≤ grade 2 (platelets ≥ 50 x 10^9/L) in ≤ 14 days, resume study agent at next lowest safe dose. If bleeding resolves within 5 days but thrombocytopenia does not resolve to ≤ grade 2 (platelets ≥ 50 x 10^9/L) by 14 days, permanently discontinue study agent.
Thrombocytopenia Grade 4 (<25 x 10^9/L)	Hold study agent and provide supportive care. If platelets do not resolve to ≤ grade 3 (platelets ≥ 25 x 10^9/L) in 5 days, permanently discontinue study agent. Otherwise proceed as follows: • If resolves to ≤ grade 2 (platelets ≥ 50 x 10^9/L) in ≤ 14 days, resume study agent at next lowest safe dose. • If thrombocytopenia does not resolve to ≤ grade 2 (platelets ≥ 50 x 10^9/L) by 14 days, permanently discontinue study agent.
Gastrointestinal	
Diarrhea Grade 1 (2-3 stools/day >baseline)	Maintain treatment.
Diarrhea Grade 2 (4-6 stools/day > baseline)	Maintain study agent.
Diarrhea Grade 3 (7-9 stools/day > baseline)	Hold study agent and provide optimal anti-diarrheal therapy. *First Occurrence:* If resolves to ≤ grade 1 in ≤ 7 days, resume at pre-hold dose. If resolves to ≤ grade 1 in 8-14 days, resume study agent at next lowest safe dose. If does not resolve within 14 days, permanently discontinue study agent. *Recurrence:* If resolves to ≤ grade 1 in ≤ 14 days, resume at next lowest safe dose. If does not resolve within 14 days, permanently discontinue study agent.

Diarrhea Grade 4 (≥10 stools/day > baseline)	Hold study agent and provide optimal anti-diarrheal therapy. If does not resolve to Grade 3 within 48 hours, permanently discontinue study agent. If resolves to Grade 3 within 48 hours, then: *First Occurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 7 days, resume at pre-hold dose. If resolved in 8-14 days, resume study agent at next lowest safe dose. If not resolved within 14 days, permanently discontinue study agent. *Recurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 14 days, resume study agent at next lowest safe dose. If not resolved within 14 days, permanently discontinue study agent.
Vomiting or Nausea Grade 1	Maintain study agent.
Vomiting or Nausea Grade 2	Maintain study agent.
Vomiting or Nausea Grade 3	Hold study agent and provide optimal supportive care/therapy. *First Occurrence:* If resolves to ≤ grade 1 in ≤ 7 days, resume at pre-hold dose. If resolves to ≤ grade 1 in 8-14 days, resume study agent at next lowest safe dose. If does not resolve within 14 days, permanently discontinue study agent. *Recurrence:* If resolves to ≤ grade 1 in ≤ 14 days, resume at next lowest safe dose. If does not resolve within 14 days, permanently discontinue study agent.
Vomiting Grade 4	Hold study agent and provide optimal supportive care/therapy. If does not resolve to Grade 3 within 48 hours, permanently discontinue study agent. If resolves to Grade 3 within 48 hours, then: *First Occurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 7 days, resume at pre-hold dose. If resolved in 8-14 days, resume study agent at next lowest safe dose. If not resolved within 14 days, permanently discontinue study agent. *Recurrence:* Hold until resolution to ≤ grade 1. If resolved in ≤ 14 days, resume study agent at next lowest safe dose If not resolved within 14 days, permanently discontinue study agent.
Hepatic investigations	
ALT (SGPT) or AST (SGOT) ≤ 3.0 x ULN or TBili ≤ 1.5 x ULN	Maintain study agent.
ALT (SGPT) or AST (SGOT) >3.0 - 5.0 x ULN or TBili >1.5 - ≥ 3.0 x ULN	*First Occurrence:* Hold until resolution to < 3.0 x ULN for ALT or AST or < 1.5 for TBili. Resume at pre-hold dose if resolved within 14 days. If does not resolve within 14 days, permanently discontinue study agent. *Recurrence:* Hold until resolution to < 3.0 x ULN for ALT or AST or <1.5 for TBili. Resume at next lowest safe dose if resolved within 14 days. If does not resolve within 14 days, permanently discontinue study agent.
ALT (SGPT) or AST (SGOT) > 5.0 x ULN or TBili > 3.0 x ULN	Permanently discontinue study agent.
Electrolyte/metabolic toxicity	

Electrolyte/metabolic Grade 3	Provide support care. Maintain study agent per investigator discretion. If alteration persists despite aggressive replacement therapy, then dose reduce or permanently discontinue (per investigator discretion with PI consultation).
Electrolyte/metabolic Grade 4	Provide support care. Maintain study agent per investigator discretion. If resolves to Grade 1 or baseline within 48: for first event, maintain at pre-hold dose; for recurrent event, dose reduce or permanently discontinue (per investigator discretion with PI consultation). If does not resolve to Grade 1 or baseline within 48 hours, permanently discontinue study agent.
Other unspecified Non-Hem toxicities considered related to leflunomide	
Grade 1	Maintain study agent.
Grade 2	Maintain study agent.
Grade 3	*First Occurrence:* Hold until resolution to ≤ Grade 2. Resume at pre-hold dose if resolved within 7 days. If does not resolve within 7 days, permanently discontinue study agent. *Recurrence:* Hold until resolution to ≤ Grade 1. Resume at next lowest safe dose if resolved within 14 days. If does not resolve within 14 days, permanently discontinue study agent.
Grade 4	Permanently discontinue study agent.
Other unspecified Non-Hem Toxicities considered UNRELATED to study agent	
Other unspecified events of any grade considered unlikely to be related or not related to study agents.	Maintain treatment with study agents. Interruption of study agent or dose de-escalation is permitted if the investigator consults with the Principal Investigator to determine that this is in the best interest of the participant.

6.3 Dose Modifications of Cholestyramine

Cholestyramine will be administered and dose modified per the discretion of the treating investigator in agreement with standard administration practices.

7.0 DATA AND SAFETY MONITORING, UNANTICIPATED PROBLEM AND ADVERSE EVENT REPORTING

7.1 Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because the study involves COH as the IND holder and escalates the study agent to a dose above that indicated by the FDA for the agent's currently approved indication.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, Collaborating Investigator, CRC/protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

7.3 Dose Escalation

This study will utilize the Phase I Tracking Log to monitor data and safety for dose escalation. The Tracking Log will contain dose levels administered, dose limiting toxicities (DLT), DLT-defining adverse events, and any details regarding dose level escalation. The record of doses administered and resultant adverse events will be included in the PMT Report.

7.4 Adverse Events and Serious Adverse Events

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

7.5 Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.03.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been

observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

7.6 Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

7.7 COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The Office of IND Development and Regulatory Affairs (OIDRA) will assist the PI in reporting the event to the Food and Drug Administration (FDA).

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy [policy effective date: 05/14/14].

7.8 Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

7.9 Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

8.0 AGENT INFORMATION.

8.1 Leflunomide

Leflunomide is an FDA approved agent for the treatment of rheumatoid arthritis. Please refer to the Package Insert for additional details not provided in this section.

8.1.1 Description and classification

The chemical name for leflunomide is N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide. It has a molecular formula C H F N O, a molecular weight of 270.2. Leflunomide is a pyrimidine synthesis inhibitor.

8.1.2 Mode of action

Leflunomide is an isoxazole immunomodulatory agent which inhibits dihydroorotate dehydrogenase (an enzyme involved in de novo pyrimidine synthesis) and has antiproliferative activity. Several in vivo and in vitro experimental models have demonstrated an anti-inflammatory effect.

8.1.3 Toxicology

See Section 6.1.1.

8.1.4 Pharmacology

Following oral administration, leflunomide is metabolized to an active metabolite teriflunomide (A77 1726) which is responsible for essentially all of its activity *in vivo*. Following oral administration, peak levels of the active metabolite occurred between 6 to 12 hours after dosing. Due to the very long half-life of teriflunomide (~2 weeks), a loading dose of 100 mg for 3 days was used in clinical studies to facilitate the rapid attainment of steady-state levels of teriflunomide. Without a loading dose, it is estimated that attainment of steady-state plasma concentrations would require nearly two months of dosing. Biliary recycling is a major contributor to the long elimination half-life of teriflunomide. The active metabolite of leflunomide is eliminated slowly from the plasma. Use of a drug elimination procedure (cholestyramine or activated charcoal) is used to reduce the drug concentration more rapidly after stopping leflunomide therapy.

8.1.5 Storage and stability

Leflunomide tablets should be stored at 25°C (77°F); excursions are permitted to 15–30°C (59–86°F). Protect from light.

8.1.6 Preparation

Leflunomide is commercially available for oral administration as tablets containing 10, 20, or 100 mg of active drug.

8.1.7 Agent administration

Leflunomide is administered orally. Participants will be given a study calendar to track all doses taken or missed. There will be an initial loading dose of 100 mg per day for the first 3 days of doses. Participants will then receive leflunomide at the assigned dose level. See Section 5.5.1 for administration details.

8.1.8 Availability and supply

Tablets in 10 and 20 mg strengths are packaged in bottles of 30 pills per bottle. Leflunomide will be provided to patients by the study as an investigational agent. A commercial supply will be purchased and distributed as an investigational agent by the Investigational Drug Pharmacy at City of Hope.

8.2 Cholestyramine

Cholestyramine is not the study agent being evaluated for efficacy, but is required for the elimination of the study agent leflunomide. Cholestyramine is FDA approved for hypercholesterolemia. **Cholestyramine use is indicated in the leflunomide package insert to eliminate the leflunomide metabolite.**

8.2.1 Description

Cholestyramine is a strong anion exchange resin comprised of a quaternary ammonium group attached to an inert styrene-divinylbenzene copolymer. Cholestyramine removes bile acids from the body by forming insoluble complexes with bile acids in the intestine, which are then excreted in the feces.

8.2.2 Other names

Questran®, Questran Light, Cholybar, Olestyr

8.2.3 Mode of action

Cholestyramine resin adsorbs and combines with the bile acids in the intestine to form an insoluble complex which is excreted in the feces. This results in a partial removal of bile acids from the enterohepatic circulation by preventing their absorption.

8.2.4 Toxicology

See Section 6.0.

8.2.5 Storage and stability

Store between 20º-25ºC (68º-77ºF). [See USP Controlled Room Temperature]. Excursions permitted to 15º-30ºC (59º-86ºF).

8.2.6 Agent administration

Cholestyramine should be administered orally after being prepared per package insert (powder diluted in fluid). See Section 5.5 for study administration details.

8.2.7 Availability and supply

Cholestyramine will be provided to participants by the study. A commercial supply will be purchased and distributed by the pharmacy at City of Hope.

9.0 CORRELATIVE/SPECIAL STUDIES

9.1 Pharmacokinetics

All participants will undergo serial blood sampling to evaluate the steady-state systemic exposure to teriflunomide (A77 1726) the active metabolite of leflunomide. Pharmacokinetic sampling will consist of trough level monitoring throughout the entire period of active treatment with leflunomide.

9.1.1 Specimen Collection

Prior to initiation of drug administration on day 1 of cycle 1 of treatment and then at follow-up visit thereafter (see Table 10 Study Activity Calendar), venous blood will be collected into one 4-mL purple-top (EDTA) tube. Patients will be instructed not to take their leflunomide dose until after they have had their blood drawn on the days of their clinic appointment. Blood samples will be kept on ice until prompt delivery (so that samples can be processed within an hour of collection) to the Analytical Pharmacology Core Facility (APCF) for processing. Samples are to be taken to Shapiro room 1042. The APCF (Leslie Smith-Powell or her representative) will be notified in advance of sampling (ideally at least a day in advance) by e-mail to LSmith-Powell@coh.org. Any updates to the location of the laboratory or the contact information will be distributed to the study team.

9.1.2 Initial specimen processing and storage

Specimen processing and storage will occur at the APCF plasma is separated from whole blood by centrifugation at 1500 x g (within 1 hour). Plasma will then be transferred to appropriately labeled polypropylene tubes and stored at < -70°C until analysis. Each sample will be labeled with the participant's name, medical record number, date of collection, and actual sample collection time.

9.1.3 Analytical Method

Total and free teriflunomide (A77 1726) plasma concentrations will determined in the Analytical Pharmacology Core Facility (APCF) at the City of Hope using a validated LC-MS/MS method. The method is based on a previously reported assay that has been validated over a wide dynamic concentration range of 10-4000 ng/mL from a starting plasma volume of 200 µl [41].

9.1.4 Pharmacokinetic Data Analysis

Non-compartmental pharmacokinetic analyses of teriflunomide (A77 1726) will be used to determine the average steady-state total and free teriflunomide trough concentration (C_{trough}). C_{trough} results will be summarized within and between participants using means and standard deviations.

9.2 Pharmacodynamics

All participants who undergo serial blood sampling to evaluate pharmacokinetics will also have peripheral blood mononuclear cells (PBMC) isolated from their blood samples for exploratory analyses of potential pharmacodynamic (PD) markers of the effects of the active metabolite of leflunomide. Potential PD assays include the quantitative analysis of intracellular levels of dihydroorotate, orotate, as well as nucleotide pools as direct indicators of DHODH inhibition. Furthermore, the expression of lymphocyte cell surface activation markers will be assessed as an indirect measure of drug effect. Additional PD assays may also be included based on the results of the ex vivo mechanistic studies to be performed under 9.4 below.

9.2.1 Specimen Collection

Venous blood will be collected into two 4-mL purple-top (EDTA) tubes. Please refer to Sec 9.1.1 for other details of collection.

9.2.2 Initial specimen processing and storage

Any blood remaining in the three 4-mL purple-top tubes used to prepare plasma for PK above will be diluted 1:1 with Hank's Balanced Salt Solution ("HBSS", Irvine Scientific, Cat. 9228 or equivalent) and combined in a sterile 50 ml conical centrifuge tube. PBMC will then be isolated from the combined whole blood sample by Ficoll-gradient separation as described below;

- Allow Accuspin-Histopaque tubes ("Accuspin", Sigma Cat. A6929 or A0561, for 12 or 100 tubes, respectively) and HBSS to warm to room temperature. Place a Mr. Frosty container in the refrigerator and prepare the freezing media by adding 10% DMSO to fetal calf serum and chill at 4˚C or on ice.
- Prepare Accuspin tubes by centrifuging at 1000 x g for 1 minute at room temperature (RT) with brakes on. Each tube can process up to 20 mLs of whole blood; prepare the appropriate amount of tubes necessary. After centrifugation, the Histopaque reagent should be below the barrier of the tube. Add 5 mLs of HBSS to the Accuspin tube. Add up to 20 mls of whole blood to each Accuspin tube until all the blood has been distributed.
- Centrifuge the blood sample at 800 x g for 15 minutes at RT with brakes on LOW. After centrifugation, three layers should be visible above the barrier of the tube: the plasma layer at the top, a cloudy layer in the middle where the PBMC are, and a clear Histopaque reagent layer right below. Using a pipette, remove the upper plasma layer to within 2 cm of the cloudy interphase. Carefully pipette the cloudy PBMC interphase and transfer to a sterile 50 mL centrifuge tube.
- Add HBSS up to the 45 mL mark in the centrifuge tube with the PBMC and spin at 400 x g for 10 minutes at RT with brakes on. Decant the supernatant and loosen the cell pellet before adding HBSS to the 45-mL mark again for a second wash. Centrifuge at 300 x g for 10 minutes at RT with brakes on. Decant the supernatant, loosen the cell pellet and then add a known volume of HBSS to resuspend the cells for counting. Mix the cell suspension up and down with a pipette several times before removing a small aliquot for cell count.
- Centrifuge the cell suspension one final time at 300 x g for 10 minutes at RT with brakes on. PBMC should be frozen down at 0.5 – 1 x 10^7 cells/vial. Determine the volume of freezing media (fetal calf serum with 10% DMSO) needed to give a 1 x 10^7 cell/mL suspension. After the last centrifugation is complete, discard supernatant and loosen the cell pellet before adding freezing media slowly, a small volume at a time with mixing in between (vortex at low speed). Aliquot 0.5 - 1 mL of the final cell suspension into individually labeled cryovials. Transfer the cryovials into Mr. Frosty and store at -80˚C. Twenty four hours later, cryovials will be transferred to liquid nitrogen tanks for long-term storage.

9.2.3 Analytical Method

The intracellular levels of dihydroorotate, orotate, and nucleotide pools will be performed using liquid chromatography tandem mass spectrometry as previously described[42, 43]. Expression of cell surface markers of lymphocyte activation (CD25 and CD134) will be measured by flow cytometryas previously described[44].

9.3 Pharmacogenomics

All participants will undergo genetic studies to evaluate germline genetic polymorphisms in the coding exome. The potential association between germline polymorphisms in CYP1A2, CYP2C19, and DHODH and either toxicity or response will be analyzed. CYP1A2 and CYP2C19 are major leflunomide metabolic enzymes and DHODH is the main molecular target of leflunomide

9.3.1 Specimen Collection and Processing and Analysis

Cells will be obtained from the PK processing procedure (Section 9.1.2) for DNA extraction by the APCF (one sample per participant). The extracted DNA material will then be sent to the Integrative Genomics Core (ICG) for sequencing.

9.3.2 Gene Sequencing Data Analysis

Gene sequences provided by the IGC will be assessed for gene polymorphisms identified in the literature [20, 21] associated with leflunomide response in RA.

9.4 Ex vivo and Molecular Studies

9.4.1 Sample Collection, Transfer, and Initial Processing

Please see the Appendix for detailed procedures. Any updates to the laboratory locations or contact information will be distributed to the study team. Bone marrow specimens will be collected prior to drug administration, on day 1 of cycle 2 (all phase II patients and phase I patients enrolled at the anticipated RP2D), at progression (where applicable), and at confirmation of CR/sCR (where applicable). See Table 10, Study Activity Calendar. Bone marrow aspirate (10 ml) will be collected into a sodium heparin (green-top) tube. Samples will be gently inverted 8 times to mix the sample and then promptly taken at room temperature to Tinisha McDonald (Kaplan CRB, RM 3016) in Dr. Guido Marcucci's laboratory (or her representative) for processing (so that samples can be processed within an hour of collection). Tinisha McDonald (tmcdonald@coh.org) as well as the supervisors Allen Lin (alin@coh.org) and Dr. Guido Marcucci (gmarcucci@coh.org) will be notified in advance of sampling (at least 1 day in advance) by email. Each sample will be labeled with the participant's name, medical record number, date of collection, and actual sample collection time.

The Marcucci laboratory will conduct Ficoll-Paque density centrifugation and preparation of mononuclear cells (MNCs) and then transfer the sample on ice to Dr. Ralf Büttner at the Rosen laboratory (Kaplan CRB, room 1026) for enrichment CD138-positive (CD138+) plasma cells by magnetic cell sorting, according to the manufacturer's instructions. The CD138+ MNCs will be re-suspended in complete RPMI medium, counted and stored on ice for further processing.

9.4.2 Sample Storage and Distribution for Further Analysis

At least 5 million (≥5%) CD138-positive MNCs can be expected from each BM aspiration after immunomagnetic enrichment. For RNA and DNA analysis without further ex vivo treatment, 500,000 cells (RNA) and 1,000,000 cells (DNA) will be pelleted and frozen at -80 °C. The remaining CD138-enriched cells will be stored in the vapor phase of a liquid nitrogen tank in freezing medium if the cells are not processed immediately. A small aliquot of the cell population (~50,000 cells) will also be used to confirm that the enriched cell population is >95% pure. Up to three million CD138-enriched cells will be

used for *ex vivo* cell culture studies either immediately after enrichment or after thawing of the frozen cells. See Table 9.3.2 for allocation of samples to downstream assays. If insufficient cell numbers are collected for the proposed *ex vivo* and molecular studies, we will prioritize as follows: mRNAseq/miRNAseq > *ex vivo* cytotoxicity studies > DNA methylation sequencing studies > *ex vivo* synergy studies.

Table 9.3.2 Distribution of CD138-enriched bone marrow aspirate cells for research

	Ex vivo teriflunomide or control treatment			No *ex vivo* treatment
	Cytotoxicity	Synergy analysis	mRNA/miRNA profiling	mRNA/miRNA/DNA methylation profiling
Baseline/screening	X**	X**	X***	X***
1 mo. of leflunomide				X***
Progression*				X***
Complete response*				X***

*Where applicable, ** both the phase I and phase II components, *** all participants treated at the R2PD (phase II and phase I/II participants)

9.4.3 *Ex vivo* Cytotoxicity and Synergy Analysis

All cells will be grown in RPMI-1640 medium supplemented with 10% heat-inactivated FBS and 1x antibiotics/antimycotics. Primary pre-treatment MM patient-derived CD138+ cells will be co-cultured with increasing concentrations of teriflunomide. Teriflunomide will be used because conversion of leflunomide to the active metabolite teriflunomide happens primarily in the intestine. Therefore, we consider it unlikely that conversion will occur *ex vivo/in vitro*, and that use of teriflunomide is the better option. Proliferation and cytotoxicity of primary MM cells will be measured based on quantitation of the ATP present in cells, MTS assays, and Annexin V staining; IC_{50} values will be calculated. The cells will also be cultured with increasing amounts of teriflunomide and selected MM drugs. Examples from each class include the HDAC inhibitor panobinostat, the IMiDs lenalidomide and pomalidomide, the proteasome inhibitors ixazomib and bortezomib, the steroid hormone dexamethasone and the CD38 antibody daratumumab. To evaluate synergy, we will generate combination index (CI) values by combining teriflunomide and the FDA-approved agents at constant ratios based on the IC_{50}s, as described previously by Chou TC [45], and generate isolobograms to identify the ratios producing maximal synergy.

9.4.4 High Throughput Genomics Analysis

We will evaluate MM cells treated with teriflunomide *ex vivo* (as described in Section 9.3.3) and leflunomide *in vivo* (comparison of MM samples from baseline, after one month of treatment and at PD or CR, where applicable). DNA (from 1 million cells, *in vivo* treatment only) and RNA (from 100,000 cells, *ex vivo* and *in vivo* treatment) will be extracted according to the manufacturer's instructions for RNA isolation (Qiazol, Qiagen) and DNA isolation (DNAzol, Thermofisher) and submitted to the COH Integrative Genomics Core for analysis. Sequencing will be performed on an Illumina Hiseq 2500. Image and data processing will be conducted using Illumina's pipeline, as well as alignment tools such as TopHat (mRNA), Novoalign (miRNA), and Bismark (DNA methylation). Custom R scripts will be used for further processing, pathway analysis, and inter-group comparisons. Genetic loci/transcripts identified by bisulfite sequencing (DNA-methylation) and RNAseq (mRNA/miRNA sequencing) will be further validated using real time RT-PCR assays.

10.0 STUDY CALENDAR

Table 10 describes required procedures. All procedures may increase in frequency if clinically indicated or oriented following toxicity. Adjustments to the treatment cycle due to a hold in study agent are detailed in Section 5.2.

Table 10.0 Study Activity Calendar

	Screening [a]	Cycle 1, 2, 3		Cycle 4+	End of Treatment [e]				Active Follow-Up [j]	Long Term Follow-Up [k]
		Day 1[b]	Day 14[c]	Day 1[d]	Day 0[f]	Days 1-11[g]	Day 14[h]	Day 30[i]		
Informed Consent [l]	X [u]									
Inclusion/Exclusion Criteria [m]	X									
Registration [n]	X [n]									
Medical history [o]	X									
Physical exam	X	X		X				X		
Vital signs [p]	X	X		X				X		
Adverse events assessment [q]		X	X	X	X			X		
Concomitant meds review	X	X		X	X			X		
ECOG status [r]	X	X		X				X		
Pregnancy test [s]	X									
TB antigen test [t]	X									
Hepatitis A, B, C testing	X [u]									
Pulmonary function test	X [u]									
Extramedullary disease survey [v]	X [u]			X [w]	X [w]			X [w]	X [w]	
Skeletal survey	X [u]									
Clinical bone marrow aspiration/biopsy with research use of leftover samples	X [u,y]	X [y,z, aa]		X [y,z]	X [y,z]			X [y,z]	X [y,z]	
Research bone marrow aspiration		X [bb]								
CBC with differential [cc]	X	X	X	X				X	X	
Chemistry panel [dd]	X	X	X	X				X	X	
Myeloma labs [ee]	X	X [kk]		X	X			X	X	
Response assessment [ff]		X [aa]		X	X			X	X	
Research blood samples [gg]		X	X	X	X					
Teriflunomide clinical testing [hh]					X		X [ii]	X [ii]		
Leflunomide administration [jj]		Daily								
Cholestyramine [g]						X [g]				
Survival										X [k]

a. All screening procedures to be performed within 21 days of start of study agent except informed consent, acute hepatitis panel, skeletal survey, bone marrow biopsy, and pulmonary lung function test which may occur within 30 days of start of treatment (footnote u) and except for registration which must occur within 7 days from start of treatment (footnote n).

b. Day 1 assessments to be performed within 5 days (120 hours) prior to Day 1 drug (except footnote u for C1 only). Day 1 safety assessments must be resulted and reviewed prior to administration of agent for the cycle that is initiating. Screening assessments, if performed within this aforementioned time frame, may serve as C1D1 assessments. Note: items with footnote "aa" are not performed for C1D1; the windows for screening assessments still apply.

c. Day 14 evaluations have +/- 3 day window.

d. Day 1 assessments to be performed within 5 days (120 hours) prior to Day 1 drug. Day 1 safety assessments must be resulted and reviewed prior to administration of agent for the cycle that is initiating.

e. Reasons to end study treatment include disease progression and unacceptable toxicity. See Section 5.8 for all criteria.

f. End of Treatment (EOT) Day 0 is defined as the day the determination to end treatment is made. EOT Day 0 assessments to be performed as soon as feasible, especially in the event of off treatment due to unacceptable toxicity and no later than 7 days after decision to end treatment; assessments performed after last dose of study agent(s) and within 7 days of the decision to end treatment may serve as EOT Day 0 assessments.

g. EOT Day 1 is defined as the day cholestyramine administration begins; it will usually follow immediately after EOT Day 0, although it will not be deviation if this does not occur. Cholestyramine (8 grams three times daily for 11 days) will be administered according to section 5.7.

h. EOT Day 14 assessments must occur after completion of cholestyramine, and may occur on Day 14 +/- 3 day window. If the cholestyramine administration period is extended beyond 11 consecutive days, the EOT Day 14 assessments may occur outside of this defined window.

i. EOT Day 30 visit has a +/- 2 day window, however this window may be adjusted without deviation to ensure that it corresponds to the final teriflunomide clinical test (see footnote ee). All participants will be followed until resolution or stabilization of any serious adverse events occurring during treatment or starting within 30 days of last study drug administration or within the time to complete the EOT Day 30 assessments, which every occurs later.

j. Active follow-up will occur for participants who have completed EOT procedures and yet to demonstrate disease progression. The active follow-up visit will take place every 28 days (+/- 5 days) from the day of the EOT Day 30 visit. Active follow-up will continue until an alternative myeloma therapy has commenced or until disease progression.

k. Long term follow-up will occur approximately every 3 months (+/- 14 days) from the EOT Day 30 visit date for survival status. Information will be obtained by reviewing the City of Hope medical record, contacting the participant and/or a review of outside medical records. Note: this is not intended to be an all-inclusive list.

l. Informed consent process to be fully documented: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study reviewed including risks, and a copy of signed consent given to participant.

m. Inclusion/exclusion criteria are detailed in Section 3.

n. See Section 4.3 for slot reservation and registration process. Treatment must begin within 7 days of registration. Documentation providing Investigator's confirmation that all eligibility criteria are met must be available prior to registration.

o. Medical history to include review treatment history for myeloma, medical history pertaining to eligibility, and demographic information.

p. Vital signs: Weight, heart rate, blood pressure, respiration rate, temp. Height required only at baseline.

q. Adverse event (AE) reporting begins for events that occur after start of study agent. AE recording and reporting will continue until the completion of the EOT Day 30 visit or until resolution or stabilization of any serious AE occurring before the completion the EOT Day 30 assessments, which every occurs later.

r. See Appendix A for ECOG performance status criteria.

s. Serum or urine pregnancy test for women of child bearing potential only.

t. T-Spot or other TB antigen test.

u. Consent, acute hepatitis panel, skeletal survey, bone marrow biopsy, bone marrow aspiration, and pulmonary lung function test to be completed within 30 days prior to registration.

v. Extramedullary disease survey required at screening for all participants with known or suspected extra-medullary disease. May include CT scan of the abdomen/pelvis, CT or x-ray of the chest, ultrasound of the liver/spleen or abdomen. Technique used at screening/baseline (if applicable) should be used throughout the study and include tumor measurements.

w. Extramedullary disease survey required after screening/baseline only in patients with extramedullary disease present at screening/baseline. To occur every 12 weeks or upon clinical suspicion of progressive disease.

x. Skeletal survey (including skull, all long bones, pelvis and chest) with tumor measurements (if plasmacytomas are present). To be performed at baseline/screening and when clinically indicated.

y. Leftover bone marrow aspirate from screening/baseline, confirmation of complete response (CR/sCR, when applicable) and confirmation of progressive disease (PD, when applicable) (Section 9.4). Bone marrow aspirate will be collected into a 10mL or equivalent sodium heparin (green top) tube. Samples will be gently inverted approximately 8 times to mix the sample and then promptly taken at room temperature to Tinisha McDonald (Kaplan CRB, RM 3016) in Dr. Guido Marcucci's laboratory for processing (so that samples can be processed within an hour of collection). Tinisha McDonald (tmcdonald@coh.org) as well as the supervisors Allen Lin (alin@coh.org) and Dr. Guido Marcucci (gmarcucci@coh.org) should be notified in advance of sampling (at least 1 day in advance) by email. Changes in laboratory contact information will not be considered protocol deviations.

z. Perform bone marrow biopsy and/or aspirate once to confirm suspected CR, sCR, or PD.

aa. Bone marrow and response assessments are not to be taken on Cycle 1 Day 1; screening labs will be used as baseline values.

bb. Bone marrow aspiration for research on day 1 of cycle 2 (Section 9.4). **Note**: In the event that the research prep for the baseline bone marrow aspirate (footnote y) yields no useable product, this procedure will not be performed. In the event that this planned sample collection coincides with confirmation of suspected CR, sCR, or PD, then the clinical leftover samples (see footnotes y and z) will be used instead of a research-specific collection. See contact information for research sample transfer in footnote y.

cc. CBC with differential: erythrocytes (RBC), hemoglobin, hematocrit, platelets, total WBC plus absolute differential counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

dd. Serum chemistry panel: Comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, calcium, glucose, albumin, total bilirubin, alkaline phosphatase, total protein, ALT/SGPT, AST/SGOT) LDH, magnesium, phosphorus, and uric acid.

ee. Myeloma labs to include serum protein electrophoresis and immunofixation, quantitative immunoglobulins, serum free light chains and 24-hour urine studies (total protein, urine protein electrophoresis and immunofixation). Note: 24-hour urine studies to be performed at baseline for all participants, and after baseline to confirm complete response or in patients with urine-only measureable disease.

ff. See Section 11.1 for response assessment criteria.

gg. Research blood sample collection will occur at the time of blood collection for clinical labs. Venous blood will be collected into three 4-mL purple top (EDTA) tubes, gently agitated, placed on ice and brought promptly (so that the sample can be processed within the hour) to the APCF in Shapiro room 1042. Notify the APCF (Leslie Smith-Powell or her representative) by e-mail to LSmith-Powell@coh.org in advance of sampling (ideally at least a day in advance). Discarded cells from one sample will contribute to pharmacogenomic studies (Section 9.3.1). Changes in laboratory contact information will not be considered protocol deviations.

hh. Teriflunomide clinical blood assay to be ordered as "leflunomide metabolite" miscellaneous clinical test.

ii. Clinical testing must demonstrate teriflunomide plasma levels less than 0.02 mg/L or 0.02 µg/ml by two separate tests at least 14 days apart. If plasma levels are higher than 0.02 mg/L or 0.02 µg/mL, additional cholestyramine treatment will be considered.

jj. Participants will receive leflunomide orally on a daily basis which will be recorded on the pill diary. The first 3 loading doses will be given at a dose of 100 mg, after which treatment will continue at a dose of 20 or 10 mg daily. See administration details in Section 5.5.1. **NOTE: On study visit days, participants should refrain from taking leflunomide until after blood is collected.**

kk. Myeloma labs may be repeated at C1D1 per investigators discretion and used for disease response assessment

11.0 EVALUATION CRITERIA/MEASUREMENT OF EFFECT

11.1 Phase I/Primary Endpoint: Toxicity

The primary endpoint is toxicity. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 4.03. Dose limiting toxicity (DLT) is defined in section 13.1 of the protocol. The MTD will be based on the assessment of DLT during cycle 1. All patients who are not evaluable for dose limiting toxicity will be replaced.

11.2 Phase II/Primary Endpoint: Response

The primary endpoint is response. Response will be categorized using the IMWG response criteria for multiple myeloma [37, 38]. Table 11.2 summarizes the categories and criteria.

Table 11.2 Response Criteria

Response Category	Response Criteria
Complete response (CR)*	Negative immunofixation of serum & urine, AND Disappearance of any soft tissue plasmacytomas, AND < 5% plasma cells in bone marrow
Stringent complete response (sCR)*	CR (as defined above) PLUS: Normal FLC ratio, AND Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
Very good partial response (VGPR)**	Serum and urine M-component detectable by immunofixation but not on electrophoresis, OR ≥ 90% reduction in serum M-component plus urine M-component < 100mg per 24h
Partial response (PR)**	≥ 50% reduction of serum M protein and reduction in 24h urinary M protein by ≥ 90% or to < 200mg per 24h If serum and urine M protein are unmeasurable, a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M protein response criteria If serum and urine M protein are unmeasurable, AND serum free light assay is also unmeasurable, a ≥ 50% reduction in bone marrow plasma cells is required in place of M protein, provided baseline percentage was ≥ 30%. In addition to the above criteria, if present at baseline, ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
Minimal Response	• 25% but ≤ 49% reduction of serum M protein *and* reduction in 24-hour urine M-protein by 50 to 89% which still exceeds 200 mg per 24 hr

Response Category	Response Criteria
(MR)**, ***	• In addition to the above criteria, if present at baseline, 25-49% reduction in the size of soft tissue plasmacytomas is also required • No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response)
Stable disease (SD)**	Not meeting criteria for CR, VGPR, PR, or PD

Progressive disease (PD)**	Increase of 25% from lowest response value in any one or more of the following:
	• Serum M-component (absolute increase must be ≥ 0.5 g/100ml), and/or
	• Urine M-component (absolute increase must be ≥ 200mg per 24h), and/or
	• Only in patients without measurable serum and urine M-protein levels:
	  • The difference between involved and uninvolved FLC levels (absolute increase must be > 10mg/dl)
	  • Bone marrow plasma cell percentage (absolute % must be ≥ 10%)
	• Definite development of new bone lesions or soft tissue plasmacytomas OR definite increase in the size of existing bone lesions or soft tissue plasmacytomas
	• Development of hypercalcemia (corrected serum calcium > 11.5mg/100ml) that can be attributed solely to the plasma cell proliferative disorder

***** Confirmation with bone marrow assessment is required for CR and sCR. Only a single bone marrow assessment is needed. CR and sCR require no known evidence of progressive or new bone lesions if radiographic studies are performed.

** The following response categories: VGPR, PR, MR, SD, and PD require 2 consecutive assessments made at any time before the institution of any new therapy. VGPR, PR, MR, and SD require no known evidence of progressive or new bone lesions if radiographic studies are performed. The second assessment might be at the time of a planned assessment or earlier, based on the judgement of the treating physician.

*** Based on the recommendations in Rajkumar et al. [38], MR will be reported separately and will be distinguished from PR or better.

11.3 Phase II/Secondary Endpoints:

Best Overall Response:

Based on the International Myeloma Working Group (IMWG) criteria, best overall response is the best response (sCR/CR/VGPR or PR) recorded from the start of treatment until disease progression. Confirmation of sCR/CR/VGPR or PR assessed by IMWG criteria.

Response Duration:

Defined as the time interval from the date of first documented response (sCR/CR/VGPR or PR) to documented disease progression or death whichever occurs first.

Clinical Benefit Response:

Based on the International Myeloma Working Group (IMWG) criteria, clinical benefit response is the best response (sCR/CR/VGPR/PR/MR or SD) recorded from start of treatment until disease progression. Confirmation of sCR/CR/VGPR/PR/MR or SD assessed by IMWG criteria.

Overall Survival:

Defined as the time interval from date of first dose of study drug to date of death from any cause.

Progression-Free Survival:

Defined as the time interval from date of first dose of study drug to first documented disease progression or death from any cause, whichever occurs first.

12.0 DATA REPORTING/PROTOCOL DEVIATIONS

12.1 Data Reporting

12.1.1 Confidentiality and Storage of Records

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data Collection Forms and Submission Schedule

All data will be collected using electronic data collection, stored as indicated in Section 12.1.1, and will be submitted according to the timelines indicated in Table 12.1.3.

Table 12.1.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 10 calendar days of treatment administration
Adverse Event Report Forms	For cycle 1 only, within 7 calendar days of AE assessment/notification; for all other cycles, within 10 calendar days of AE assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of end of treatment/study
Follow up/Survival Forms	Within 14 calendar days of the protocol defined follow up visit date or call

Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research coordinator and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

12.2 Protocol Deviations

12.2.1 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation policy"…

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

12.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

12.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

13.0 STATISTICAL CONSIDERATIONS

13.1 Definition of Dose-Limiting Toxicity (DLT)

A dose limiting toxicity (DLT) will be defined as any of the following toxicities that are **at least possibly related** to leflunomide and occur during cycle 1:

Any toxicity or series of toxicities resulting in:

- Fewer than 21 doses during cycle 1
- Extension of Cycle 1 > 35 days

Hematologic:

- Grade 4 neutropenia (ANC <500/mm^3)
- Grade 3 or 4 febrile neutropenia
- Grade 4 thrombocytopenia (<25,000/mm^3)
- Grade 3 thrombocytopenia (<50,000/mm^3) with bleeding

Non- Hematologic:

- Grade 4 non-hematologic toxicity, with the following exceptions:
    - Diarrhea that resolves to G3 within 48 hours
    - Vomiting that resolves to G3 within 48 hours
    - Allergic reaction/hypersensitivity, or
    - Electrolyte/metabolic toxicity corrected to <Grade 1 or baseline within 48 hours
- Grade 3 toxicity despite maximal medical therapy lasting > 7 days
- Grade 2 or higher ALT (SGPT)/AST (SGOT)/Total Bilirubin elevation lasting > 14 days.
- Grade 3 or higher elevation of ALT (SGPT)/AST (SGOT)/Total Bilirubin of any duration.

13.2 Evaluable Participants and Participant Replacement

All participants will be evaluable for toxicity from the time of their first treatment with leflunomide.

Evaluable for Dose-Escalation/De-Escalation Criteria: Participants will be considered evaluable for dose-escalation criteria if they receive at least 75% of leflunomide and are followed during cycle 1, or experience a DLT. All participants who are not evaluable for dose-escalation criteria will be replaced. Note: While patients who receive <75% of the intended dose of leflunomide will be considered unevaluable for determination of MTD, if a patient discontinues leflunomide due to toxicities prior to receiving 75% of the dose, the patient will be considered evaluable and will not be replaced.

Evaluable for Response: Participants will have their response classified according to the IMWG response criteria (See Section 11). All patients included in the study will be assessed for response to treatment, even if there are protocol treatment deviations or if the patient is later determined to be ineligible. Each patient will be assigned one of the following categories: 1) complete response (CR), 2) stringent complete response (sCR), 3) very good partial response (VGPR), 4) partial response (PR), 5) minimal response (MR), 6) stable disease (SD), 7) progressive disease (PD), 8) early death from malignant disease, 9) early death from toxicity, 10) early death due to other cause, or 11) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria (with the possible of exception of those who received no study medication) should be included in initial analysis of response rate; overall response (sCR/CR/VGPR or PR) and clinical benefit response (sCR/CR/VGPR/PR/MR or SD). Patients in response

categories 7-11 should be considered to have a treatment failure. Thus, an incorrect treatment schedule or incorrect drug administration does not result in the exclusion from the analysis of the response rate.

In addition, as part of the phase II anti-myeloma activity assessment, a sub-analysis will be performed where participants will be considered evaluable for response if they are confirmed eligible, receive at least 75% of leflunomide during the first cycle of therapy and have had their disease re-evaluated (phase II primary endpoint).

13.3 Study Design

13.3.1 Phase I Design

This single agent phase I dose-escalation trial is designed to determine the MTD and RP2D. The RP2D of leflunomide will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities. Extensive pharmacokinetic and pharmacodynamic studies are also incorporated into this protocol and may be helpful in determining the RP2D.

While the standard dose of leflunomide, 20mg daily, is FDA approved for continuous treatment of rheumatoid arthritis, leflunomide has been administered without an increase in the type, frequency or severity of adverse events at doses up to 40 mg/day in patients with Wegner's granulomatosis, and up to 60mg/day in a subset of patients with polyoma BK neuropathy to achieve targeted therapeutic levels. We will therefore attempt to escalate the dose of leflunomide to three times that approved for RA using a modified rolling six dose-escalation/ de-escalation/expansion design, a more conservative version of the rolling six design of Skolnik, et al.[1]. The starting dose of leflunomide is 20mg daily, with the intention to test up to 60mg daily. Escalation will proceed in increments of 20mg/day (odd numbered dose levels); de-escalation will proceed in decrements of 10mg/day.

Table 13.3.1 Dose Levels

Dose Level	Leflunomide Dose*
-1	10 mg PO daily
1	20 mg PO daily
2	30 mg PO daily
3	40 mg PO daily
4	50 mg PO daily
5	60 mg PO daily

*NOTE: Leflunomide is administered with a loading dose of 100 mg for the 1st three doses.

13.3.2 Phase I Dose Escalation

This phase I trial will employ a modified rolling six design, a more conservative version of the rolling 6 design of Skolnik, et al [1]. In this design, at most 3 patients will be under observation for DLT (in cycle 1) on the current test dose level at any time. Patients who are not evaluable for dose escalation will be replaced. Once each patient completes cycle 1 and passes without a DLT, an additional patient *may* be accrued on that dose level for up to 6 patients. Once 3 patients have completed cycle 1 with no patient at that dose level experiencing a DLT, the dose can be escalated. Up to 3 additional patients may be treated at the current dose level. Although this design does not require that 6 patients be treated, no more than 6 evaluable patients will be accrued to any dose level during the dose finding portion of this study. If at any time, the dose level has 1 documented DLT with fewer than 6 evaluable patients, accrual will continue until 6 patients are evaluable. Escalation will terminate as soon as two or more patients

experience a DLT attributable to the study treatment. MTD will be the highest dose in which ≤ 1/6 patients experience a DLT. There will be no dose escalation within a patient. These rules are outlined in Table 13.3.2.

Table 13.3.2: Dose Escalation Rules

# Patients on Current Level			Action
With DLT^	Evaluable	Evaluable + At Risk^	
0	0	1-2	Accrue next patient at this level*
0	0	3	Hold accrual
0	1	1-3	Accrue next patient at this level
0	1	4	Hold accrual
0	2	2-4	Accrue next patient at this level
0	2	5	Hold accrual
0	3-6	3-6	Accrue next patient at the next higher level*,+
1	1	1-2	Accrue next patient at this level
1	1	3	Hold Accrual
1	2	2	Accrue next patient at this level
1	2	3-4	Hold accrual
1	3-5	3-5	Accrue next patient at this level
1	3-5	6	Hold accrual
1	6	6	Accrue next patient at the next higher level*
2**	any	any	Accrue next patient at next lower level (max 6)

^: DLT: a patient with a documented DLT

Evaluable: a patient who is either fully evaluable for toxicity for the purpose of dose escalations or has a DLT

At Risk: a patient who is on treatment and has not yet passed the evaluation period nor had a DLT

*: During the dose-escalation portion, if higher dose level is already closed, the next lower dose will accrue to a total of 6 patients, with 2 or higher DLTs requiring further dose de-escalation.

+: Although under this scenario escalating to the next higher dose level is suggested, additional patients can be accrued to the current level -up to n=6 patients.

**: Patients treated on a higher dose will have their treatment modified to the dose below the dose level with 2 DLTs, if pending patients have DLT.

Phase II Design

Following completion of the phase I portion of the study and identification of the RP2D, the phase II portion of the study will be initiated. Recent studies evaluating daratumumab, a CD38 monoclonal antibody, as monotherapy for relapsed/refractory multiple myeloma were recently published [39, 40] and have led to FDA approval of this agent. One study included patients with relapsed multiple myeloma treated with at least three lines of therapy while the other included patients treated with at least two prior lines of treatment. Treatment with daratumumab as a single agent lead to an overall response rate of 29.2% and 36% respectively in each of these studies. In both studies, the agent investigated was well tolerated but required IV dosing once weekly for 8 doses, followed by twice-monthly doses for 8 doses and then monthly thereafter. We suspect leflunomide to be similarly well tolerated but with a more convenient dosing schedule. Because we are including patients who have received at least three prior lines of treatment, we will target an overall response rate of 30% for the phase II portion of the study.

This single-center, single arm phase II trial will implement a Simon Two-Stage Optimal Design [2, 3]. to evaluate the anti-myeloma activity of leflunomide, when given as a single agent, as assessed by overall response rate (ORR), in patients with relapsed or relapsed/refractory MM. The phase II portion of the study is expected to enroll a minimum of 19 and a maximum of 39 patients. The sample size is based on the desire to discriminate a promising ORR of 30% from a disappointing response rate of 15% using a type I error rate of 0.10 and power of 80%.

At stage 1, 19 patients will be entered on the study. If ≤ 3 responses are seen, the study will be terminated. If at least 4 patients achieve response, the trial will continue to the second stage. The 6 patients treated at the RP2D in the phase I portion will be evaluated as part of this phase II group. The probability of early stopping after the first stage is 0.68.

At stage 2, 20 additional patients will be entered. At the end of stage 2, if 9 or more patients respond, leflunomide -as single agent therapy, will be considered worthy of further study. If ≤ 8 patients respond further investigation is not warranted.

13.4 Sample Size and Accrual Rate

Phase I: In general, the total sample size will depend on the number of dose levels evaluated to determine the RP2D. Assuming the highest dose tested is well tolerated (3 patients treated on dose levels 1 (20mg) and 3 (40mg); 6 patients treated on assumed MTD -dose level 5 (60mg), the phase I portion of the study would enroll and treat 12 patients. The expected sample size is 12-18 patients, allowing for 6 patients to replace inevaluable/ineligible patients. A maximum of 36 participants could be treated if 6 participants are treated at each dose level. A minimum of 6 patients could be treated if leflunomide is not well tolerated.

Assuming one patient is enrolled each month, accrual is expected to be completed in 12-18 months for the phase I portion. When the phase II portion begins, it is anticipated that the accrual rate will increase. This is a priority trial and the initiation of the phase II portion is likely to coincide with the end of other trials, increasing the pool of potential candidates. Therefore, we estimate a rate of 2 patients per month for the phase II portion, for an accrual time of 17 months. The expected follow-up time is 24 months, for an estimated maximum total study duration of (18+17+24) 59 months, or roughly five years. In the event that participants are receiving treatment at the end of the study, additional options for continuation, such as compassionate use, will be considered. With ~150 relapsed or relapsed/refractory MM patients treated at COH per year, we do not expect any problems accruing the required patient numbers for both phases of the clinical trial within 29-35 months from start of study, and therefore both phases of the clinical trial will be conducted solely at COH.

13.5 Statistical Analysis Plan

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, will be summarized using descriptive statistics. For continuous variables, descriptive statistics (number [n], mean, standard deviation, standard error, median (range) will be provided. For categorical variables, patient counts and percentages will be provided.

Analysis: Observed toxicities will be summarized, for all dose levels, in terms of type (organ affected or laboratory determination), severity, time of onset, duration, serum concentration of the active leflunomide metabolite, probable association with the study treatment and reversibility or outcome.

The overall response rate and clinical benefit response rate will be calculated, for the initial and sub-analysis for all dose levels; Clopper Pearson binomial 95% confidence intervals will be calculated for these estimates. As part of the initial analysis of response, the overall response rate (patients that have confirmed sCR/CR/VGPR or PR) and clinical benefit response rate (patients that have confirmed sCR/CR/VGPR/PR/MR or SD) will be calculated among all patients included in the study even if there are

protocol treatment deviations or if the patient is later determined to be ineligible. In addition, as part of the phase II anti-myeloma activity assessment, sub-analyses will be performed where participants will be considered evaluable for response if they are confirmed eligible, receive at least 75% of leflunomide during the first cycle of therapy and have their disease re-evaluated. Response rates will also be evaluated based on number/type of prior therapy(ies) and serum concentration of the leflunomide metabolite (μg/mL). Time to response and survival will be estimated using the product-limit method of Kaplan and Meier.

Pharmacokinetics: Because serum concentration of the metabolite, teriflunomide (A77 1726), can be variable, and because some studies have found an association between serum concentration of this metabolite and RA response, samples will be collected throughout the study for analysis by the Analytical Pharmacology Core Facility (APCF) for an exploratory analysis relating metabolite levels to safety and efficacy outcomes. At the conclusion of the study, total and free teriflunomide levels will be summarized at 2- and 4-week sampling time points. Descriptive statistics will be used to characterize possible inter-patient variability and relationship to dose, toxicity and response for future studies, including guidance with respect to RP2D selection. Additional testing and analysis will be done on collected samples to assess, in an exploratory manner, the potential association between germline polymorphisms in the DHODH gene and either toxicity or response.

Pharmacogenomics: To evaluate the independent effect of germline polymorphisms, all models will be adjusted for risk factors known to be associated with outcomes studied (e.g., age, disease status). Multiple comparisons adjustment with false discovery rate (FDR) will be conducted, and variants with FDR ≤ 0.05 will be considered significant. We will specifically look at *DHODH*, *CYP1A2* and *CYP2C19* gene polymorphisms and haplotypes identified in the literature that are associated with leflunomide response/toxicity in RA. Functional annotation of the variants will be performed using ANNOVAR. The major goal of this analysis is to identify potential SNPs that might affect the responsiveness of treatment, other than the miRNA/mRNA/methylation signature. Given the small sample size, we consider this analysis as exploratory and may not provide enough power to detect significant associations.

Ex vivo and molecular studies: The IC50 values for teriflunomide in MM cells will be calculated using Prism software (GraphPad). These values will be used for correlation analysis to determine the strength and linearity of the relationship between the *ex vivo* response and the response characteristics *in vivo*. The results will also be compared to responses from cell lines.

For the studies combining teriflunomide with other agents *ex vivo*, combination Index (CI) values will be calculated using the CalcuSyn software program (Biosoft, Ferguson) to determine which of the compounds work synergistically, if any. A combination index of < 0.9 indicates synergism, whereas CI = 0.9 to 1.1 indicates additive effects. CI > 1.1 indicates antagonism. Isobologram diagrams will be generated that show the varying combination treatments and the ratio where maximal synergy is obtained.

The miRNA/mRNA expression profile and DNA methylation data will be used to identify the miRNA/mRNA and differential methylated regions (DMRs) that show significant differences between responders and non-responders after leflunomide treatment. For miRNA/RNA profiling, fold changes >3 with an FDR <0.05 will be considered significant. Assuming the dispersion of genes across replicates is 0.2 and minimum average expression is 10 reads per gene, 10 samples in each group (responder vs. non-responder) should have 89% power to detect a 3-fold difference between groups at an FDR level of 0.05.

The major goal of RNA-seq/miRNA-seq/RRBS-seq is to predict potential responders based on pre-treatment genomic profiling. A logistic regression-based algorithm with risk score formula described previously [46] will be used to build a classifier for response prediction. Differentially expressed miRNA/mRNA and DMRs together with other known clinical risk factors will be included in the

multivariable logistic regression model. The performance of the identified classifier will be evaluated with leave one out cross validation (LOOCV). Specifically, for each iteration -as part of a random selection process, one sample will be left out as the test sample; the remaining samples will be used to build a classifier, and the classifier will be used to predict the response status of the test sample. The sensitivity and specificity of the classifier will be calculated, as well as receiver operating characteristic (ROC) curve and area under the curve (AUC) to evaluate its accuracy.

Another goal of the genomic profiling is to identify potential new pathways to complement leflunomide treatment. We will compare the miRNA/mRNA/methylation profiles between responders and non-responders. Differentially expressed genes will be used to identify pathways that are activated in non-responders, which will provide insights into possible complementary therapy. Gene ontology (GO) and KEGG pathways will be analyzed using DAVID and GSEA. Pathways and GO that are activated in non-responders will be sorted by their multiple comparison adjusted p-value. Potential miRNA targets will be identified using TargetScan and the enrichment of GO and pathways will be identified as well. The NCI connectivity map, an online resource to predict therapeutic efficacy based on pathway based gene expression profiles, will also be explored to determine the complimentary therapeutic agents for leflunomide.

14.0 HUMAN SUBJECT ISSUES

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

The multiple myeloma subjects will be recruited from patients undergoing treatment for multiple myeloma at City of Hope Cancer Center for hematological malignancies. Any recruitment materials will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.3 Study location and Performance Sites

This study will be performed at COH.

14.4 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record individual side effects to study treatment, and disease status, and these will be linked to the subject's identity using a coded study number. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.5 Financial Obligations and Compensation

The investigational agent, leflunomide, and cholestyramine will be provided free of charge by City of Hope for the duration of the trial.

The standard of care drug(s) and standard of care procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all

copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.6 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

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APPENDIX A: ECOG PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	**Criteria**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B. DETAILED METHODS FOR PHARMACOGENOMIC, EX VIVO, AND MOLECULAR STUDIES

This is a description of the detailed analytical methods at the time of protocol submission. Any changes to the procedures will be stored in the laboratory manual of procedures/standard operating procedures or in the laboratory research notes.

Pharmacogenomics

The extracted DNA material will then be sent to the Integrative Genomics Core (IGC; Director, Xiwei Wu, PhD) for sequencing. The DNA samples will be fragmented into 200 bp, and a pre-capture library will be generated following Illumina's recommended protocol. Agilent SureSelect Human all exon V6 probes will be used to capture the coding exome. The captured library will be sequenced on an Illumina Hiseq 2500 for paired-end 100 bp sequencing for a minimum of 100x coverage. The paired-end sequences will be aligned to the human genome (hg38) using Novoalign. Only reads aligned to a unique genomic location will be kept for further analysis. The aligned reads will be piled up using Samtools. Variants (SNPs and short indels) will be identified using VarScan. The quality of variants will be assessed by the concordance of variants to the dbSNP database. The prognostic significance of germline polymorphisms on toxicity risk/response will be analyzed using univariate and multivariable logistic regression. To evaluate the independent effect of germline polymorphisms, all models will be adjusted for risk factors known to be associated with outcomes studied (e.g., age, disease status). Multiple comparisons adjustment with false discovery rate (FDR) will be conducted, and variants with FDR ≤ 0.05 will be considered significant. We will specifically look at *DHODH*, *CYP1A2* and *CYP2C19* gene polymorphisms and haplotypes identified in the literature that are associated with leflunomide response/toxicicty in RA. Functional annotation of the variants will be performed using ANNOVAR. The major goal of this analysis is to identify potential SNPs that might affect the responsiveness of treatment, other than the miRNA/mRNA/methylation signature. Given the small sample size, we consider this analysis as exploratory and may not provide enough power to detect significant associations.

Ex Vivo and Molecular Studies Bone marrow aspirates will be promptly taken on ice to Tinisha McDonald in Dr. Guido Marcucci's lab for Ficoll-Paque density centrifugation and preparation of mononuclear cells and then forwarded to Dr. Ralf Büttner at the Rosen laboratory for processing. The bone marrow aspirate will first be filtered through a 40 µm mesh to avoid coagulated products. The filtrate will be carefully layered on top of Histopaque® (Sigma cat. # 1077-1) in 50 ml polypropylene centrifuge tubes. The sample will be centrifuged (with the brake set to "off") at 1500 x g for 25 minutes. The mononuclear blood cells at the interface between the Histopaque® and the top aqueous layer will be aspirated into a clean 15 ml polypropylene centrifuge tube using a glass Pasteur pipette.

The aspirated cells will then be washed twice in ice cold PBS, cells will be counted and placed on ice for enrichment of CD138-positive cells. CD138 is a transmembrane protein expressed on the surface of plasma cells. CD138-positive cells will be enriched by magnetic cell sorting according to the manufacturer's instructions (CD138 MicroBeads, Miltenyi Biotec Cat# 130-051-301). In brief, mononuclear cells obtained from the density centrifugation will be pelleted by centrifugation and incubated with CD138 magnetic beads for 15 minutes at 4 °C in MACS buffer, washed in MACS buffer and subjected to column separation. To increase the purity of the CD138-positive cells, the eluted fraction from the first column will be enriched over a second column, washed 1x in PBS, re-suspended in complete RPMI medium, counted and stored on ice for further processing. For RNA, DNA, and protein analysis of pre-treatment patient samples, 100,000 cells (RNA) and 1,000,000 cells (DNA, protein) will be pelleted and frozen at -80 °C. The remaining CD138-enriched cells will be stored in the vapor phase of a liquid nitrogen tank in freezing medium (10% DMSO, 50% FBS, 40% RPMI-1640) if the cells are not processed immediately. A small aliquot of the cell population will also be used to determine purity of the enriched cells by flow cytometry using anti-CD138-PE antibody (Miltenyi Biotec, Cat# 130-081-301). Purities of >95% will be accepted for further experiments.

All cells will be grown in RPMI-1640 medium supplemented with 10% heat-inactivated FBS and 1x antibiotics/antimycotics.

1): To determine the *ex vivo* cytotoxicity of leflunomide toward MM cell lines and primary MM cells. Based on our experience, a minimum of one-hundred million mononuclear cells (MNCs) can be expected from each bone marrow (BM) aspiration after Ficoll centrifugation. The COH Cytogenetics Core Laboratory will obtain a separate BM sample for standard karyotype analysis and to detect genetic variations frequently found in MM. At least 5 million (≥5%) CD138-positive MNCs can be expected from each BM aspiration after immunomagnetic enrichment. MM cell lines and MM patient derived CD138+ cells (20,000 cells per well of a 96-well plate; in triplicate) will be co-cultured in RPMI-1640 medium with increasing concentrations of teriflunomide (0 uM, 50 uM, 100 uM, 200 uM, 300 uM, 400 uM) for 24, 48 and 72h. Proliferation of primary MM cells will be measured based on quantitation of the ATP present in cells (CellTiter-Glo Luminescent Cell Viability Assay, Promega). IC_{50} values will be calculated using GraphPad Prism. IC_{50} values will also be determined by flow cytometry using Annexin V. The results from primary MM cells will be compared with the following human MM cell lines RPMI-8226, MM.1S, NCI-H929 and U266 and the human bone marrow stromal cell line HS-5, which were purchased from the American Type Culture Collection

2) To determine potential additive or synergistic effects when combining leflunomide with other classes of FDA-approved drugs. Primary pre-treatment CD138+ MM cells will be cultured in 96-well plates (20,000 cells/well) with increasing amounts of teriflunomide and selected multiple myeloma drugs for 72h in RPMI-1640 medium supplemented with or without conditioned medium derived from HS-5 stroma cells. Conditioned medium will be used to model the *in vivo* cytokine stimulation milieu. We will first determine the IC50s of each individual agent by adding increasing amounts of each drug to the cell culture for 72h, followed by the MTS assay (colorimetric assay, for cell lines) and ATP assay (luminescence assay, for primary cells). Once the IC50s are established using GraphPad Prism, we will repeat above cell culture experiments by combining teriflunomide and the FDA-approved agents at constant ratios based on the IC_{50}s, as described previously by Chou. Combination Index (CI) values will be calculated using the CalcuSyn software program (Biosoft, Ferguson) to determine which of the compounds work synergistically, if any. A combination index of < 0.9 indicates synergism, whereas CI = 0.9 to 1.1 indicates additive effects. CI > 1.1 indicates antagonism. Isobologram diagrams will be generated that show the varying combination treatments and the ratio where maximal synergy is obtained.

3) To generate a preliminary RNA/miRNA and DNA-methylation signature associated with response of MM cells to leflunomide. We will conduct high resolution genomic profiling (RNA-seq and miRNA-seq) to evaluate mRNA and miRNA profiles, and we will also conduct Reduced Representation Bisulfite Sequencing (RRBS-seq) for evaluation of DNA methylation profiles. We will compare the profiles of primary MM cells before and after *in vivo* leflunomide therapy and before and after *ex vivo* teriflunomide treatment. These results will be compared with *in vitro* teriflunomide treatment of human MM cell lines, in order to generate information about common molecular pathways of drug response. RNA and DNA will be extracted from primary patient samples (CD138-enriched) prior to start of treatment and 4 weeks into treatment. RNA will also be extracted from MM cell lines and CD138+ primary, pre-treatment cells treated with 300 µM teriflunomide (a concentration that can be achieved *in vivo*) or vehicle control for 24h. DNA (from 1 million cells) and RNA (from 100,000 cells) will be extracted according to the manufacturer's instructions for RNA isolation (Qiazol, Qiagen) and DNA isolation (DNAzol, Thermofisher) and submitted to the COH Integrative Genomics Core for analysis.

Illumina Hiseq 2500 will be used to profile total cellular RNA (RNA-seq) and micro-RNA (miRNA-seq). The concentration and integrity of RNA samples will be measured using Nanodrop and Bioanalyzer. Poly(A) RNA will be enriched with oligo d(T) and reverse transcribed, and cDNA will be used for mRNA library preparation following Illumina's TrueSeq protocol. Briefly, cDNA will be fragmented and ligated to

Illumina adapters. The cDNA fragments of 130-280 bp will be isolated from polyacrylamide-urea gels. In addition, all small RNAs of 15-52 nucleotides will be selected separately and used to make small RNA libraries following the Illumina's protocol. The libraries will be quantified using qPCR and loaded to cBot for clustering generation. Sequencing will be performed on a Hiseq 2500, and image processing and base calling conducted using Illumina's pipeline. For mRNA-seq, sequence reads will be mapped to the human genome (hg19) using TopHat and the frequency of Refseq genes will be counted with customized R scripts. For miRNA-seq, sequences will be adapter-trimmed and aligned to hg19 genome using Novoalign, and miRNA expression level will be counted as previously described. The raw counts will then be normalized and compared using the Bioconductor package “edgeR”. Gene ontology (GO) and pathways will be analyzed using DAVID (Database for Annotation, Visualization, and Integrated Discovery), an online bioinformatic resource for functional interpretation of large lists of genes. Pathways and GO that correlate with response will be sorted by their multiple comparison adjusted p value. Potential miRNA targets will be identified using TargetScan and their functional annotation will be done using DAVID.

The DNA Methylation of 5-methylcytosine will be profiled by reduced representation bisulfite sequencing (RRBS-seq). Briefly, purified genomic DNA will be digested by the methylation-insensitive restriction enzyme MspI to generate short fragments that contain CpG dinucleotides at the ends. After end-repair, A-tailing and ligation to methylated Illumina adapters, the CpG-rich DNA fragments (40-220 bp) will be size selected and subjected to bisulfite conversion and PCR amplification. The library will be sequenced on an Illumina Hiseq 2500 with paired end 100bp reads. The reads will be aligned to hg19 genome and methylation levels of each cytosine in the CG context will be summarized using Bismark. The methylation levels of each CG will be compared between leflunomide treated and untreated samples to identify hyper and hypo-methylated regions (DMRs). The common DMRs across the cell lines will be identified and considered as candidate regions. The location of the DMRs relative to coding genes will be annotated using R scripts and RefSeq database. The genes with promoter DMRs and also showing differential expression by mRNA-seq will be identified and their functions and pathways will be identified by DAVID on online annotation tools and KEGG database.