NCI Protocol #: N/a

DF/HCC Protocol #: .15-197

DF/HCC Biomedical Protocol Template: June 20, 2014

TITLE: A Phase II study of lenalidomide for adult histiocyte disorders

Coordinating Center: n/a

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NCI-Supplied Agent(s): n/a
Other Agent(s): Lenalidomide

Study Exempt from IND Requirements per 21 CFR 312.2(b).

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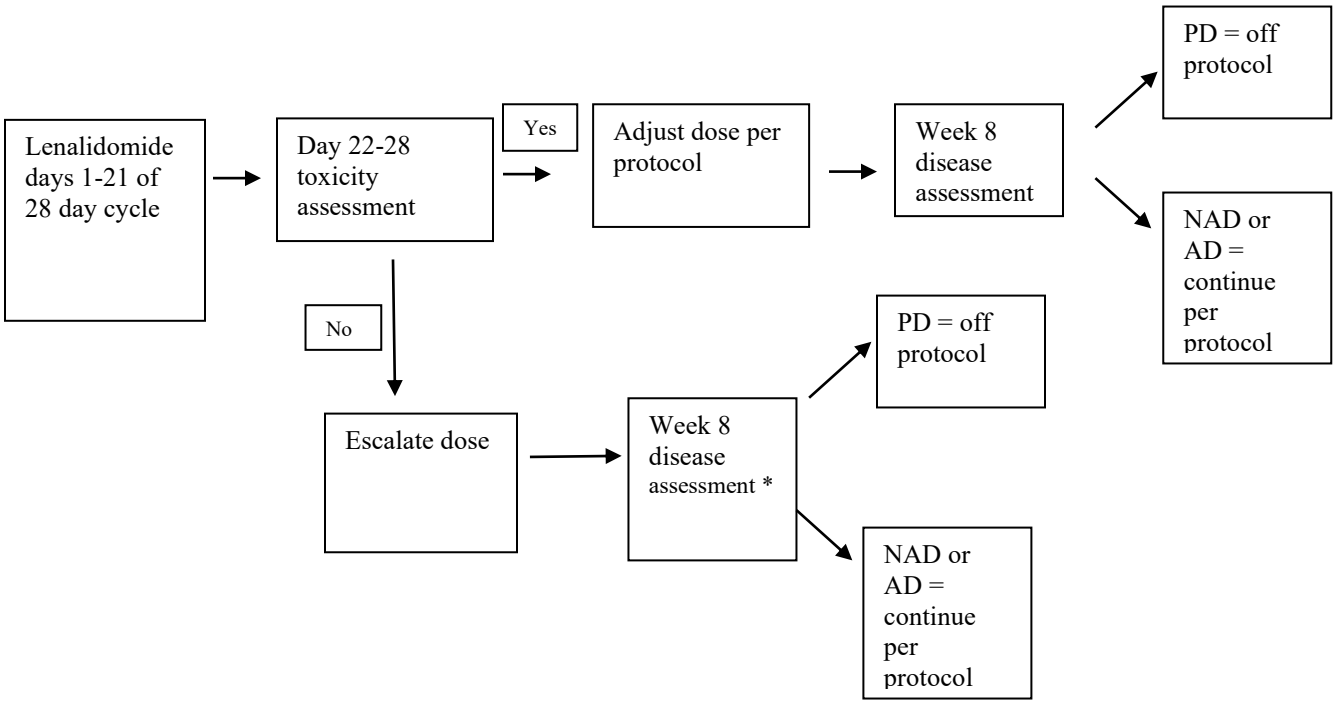
SCHEMA


Lenalidomide days 1-21 of 28 day cycle
Day 22-28 toxicity assessment
Yes
Adjust dose per protocol
Week 8 disease assessment
PD = off protocol
NAD or AD = continue per protocol
No
Escalate dose
Week 8 disease assessment *
PD = off protocol
NAD or AD = continue per protocol

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1. OBJECTIVES

This is an open-label phase II study to evaluate the efficacy and safety of lenalidomide in participants with relapsed/refractory ECD or LCH and in any patient with HS requiring systemic therapy. Patients will receive up to 12 cycles of lenalidomide with treatment occurring on days 1-21 of each 28 day cycle. In cycle one patients will receive lenalidomide 10 mg daily due to the unknown incidence of tumor flare in this population (tumor flare has been observed in other diseases such as chronic lymphocytic leukemia). Patients who tolerate the 10 mg dose well will be escalated to 25 mg for future cycles. There will also be the possibility of dose reduction for patients who do not tolerate the 10 mg starting dose. Dosage thereafter will be adjusted based upon toxicity. Lenalidomide is known to increase the risk of venous and arterial thromboembolic events. Patients not already receiving systemic anticoagulation for other clinical indications will be required to take aspirin 325 mg daily for the duration of the protocol or for one month following treatment discontinuation, whichever is longer.

Disease assessments will occur at baseline and then again at cycle 3 day 1, cycle 6 day 1, cycle 9 day 1 and cycle 12 day 1 as well as every 3 months thereafter until month 24 from study initiation when disease assessment will occur every 6 months until month 36 from the initiation of treatment. Disease assessments will consist initially of an MRI of the brain/pituitary and PET/CT (although a CT of the chest, abdomen and pelvis along with a bone scan is allowed if PET/CT is rejected by insurance). Clinical documentation of skin lesions with photography is highly encouraged. Measurement and documentation of all skin lesions is required even if photography is not performed. A repeat MRI is not required beyond baseline if the MRI at baseline is normal and there is no clinical suspicion of CNS or pituitary involvement. A bone marrow biopsy is only required if the patient has at least one cytopenia of grade 2 or greater at baseline or has evidence of bone marrow involvement on PET/CT. The bone marrow biopsy only needs to be repeated if abnormal at baseline.

Patients will be followed for 3 years (thirty-six months) from the initiation of therapy or until they initiate a new therapy for their underlying disease, whichever occurs first.

1.1 Study Design

This is a single agent, phase II open-label study of single agent lenalidomide in patients with relapsed/refractory ECD, LCH, or HS.

1.2 Primary Objectives

To estimate the response rate of patients with relapsed/refractory ECD, LCH, and HS with single agent lenalidomide.

1.3 Secondary Objectives

- To define the progression free survival (PFS) after treatment with lenalidomide
- To define the overall survival (OS) after treatment with lenalidomide
- To describe the safety profile of lenalidomide in LCH, ECD, and HS
- To assess quantitative changes in urinary and plasma cell free DNA harboring the BRAF V600E mutation and correlate the presence or absence of the BRAF V600E mutation with response
- To assess serial changes in TNF-alpha levels in patients treated with lenalidomide
- To assess the mutational landscape of these diseases using Oncopanel

2. BACKGROUND

2.1 Study Disease(s)

Langerhans Cell Histiocytosis (LCH) is a rare disease (1-10 cases per million) of unknown etiology distinguished by the clonal proliferation of pathologic histiocytes derived from bone marrow dendritic cells (1). BRAF mutations have been identified in approximately 50% of cases but the inciting event remains unclear (2). Clinical manifestations are related to the pattern of infiltration or organ involvement which is heterogeneous, can involve single or multiple organs, and can be associated with varying, often unpredictable outcomes ranging from spontaneous regression to multiple episodes of reactivation, long term debilitating sequelae, rapid progression, and death.

The natural history of LCH is ill defined in adults. Studies of disease outcomes have been primarily undertaken in the pediatric population [3]. Assessments of adult patients with LCH have reported death events in 28-32% of patients with 4-6.5 year follow up [4,5]. There is no agreed standard of care in adults for either first-line or reactivation disease. As such, there is an unmet need for a well-tolerated, efficacious agent to treat adult LCH.

A recently completed international adult study (LCH-A1) attempted to define and implement a uniform treatment approach in adults with respect to smoking cessation and the use of prednisone, vinblastine, and 6-mercaptopurine. Although final analysis of the data have not been completed at the present time, preliminary analysis indicates that many adult patients were unable to tolerate the standard pediatric multi-agent regimen of prednisone and vinblastine, due to neuropathy attributable to the latter agent. This was corroborated at Baylor College of Medicine where a study of vinblastine in LCH was terminated early when 5 or the first 7 adults treated developed Grade 3-4 toxicity at some point during therapy, and only 2/7 patients were able to complete the treatment course as prescribed by the protocol. Within a median of 6 months after starting therapy, 3/6 patients were in remission and 3/6 had active disease [6].

Reported disease reactivation rates after frontline therapy in adults vary widely. Multisystem LCH reactivation has been reported in 26-43% of patients [4, 7, 8] treated with a variety of

steroid and cytotoxic regimens. Nucleoside analogues such as cladribine and cytarabine have proven to be effective in this setting but are not without issue. Cytarabine can have significant hematologic toxicity (9). Cladribine cannot be administered for longer than six months due to concerns about secondary myelodysplasia (MDS) while studies have suggested that outcomes are better when LCH is treated with a given agent for at least 12 months (10,11). Clofarabine has proven to be effective in patients with cladribine and cytarabine-resistant disease, but causes significant hematologic and hepatic toxicity as well as cause hand-foot syndrome (12). The BRAF inhibitor vemurafenib can be effective in cases of LCH that harbor the BRAF mutation but there are significant concerns about the potential for secondary skin cancers (13). Monitoring of BRAF mutational burden in cell free DNA may be a useful marker for disease progession in this patient population, however (14).

Histiocytic sarcoma is an aggressive neoplasm deriving from non-Langerhans dendritic cells. When localized, surgery and/or radiation can be effective. However, disseminated HS responds poorly to chemotherapy and is typically fatal within six months (15). Therefore, new therapies are required for all patients with multifocal HS and for patients of relapsed/refractory LCH.

Erdheim-Chester disease (ECD) is a rare histiocyte disorder characterized most commonly by distal symmetric osteosclerosis of the long bones and retroperitoneal soft tissue infiltration. However, the disease also frequently affects periorbital soft tissue and can cause cardiac, neurologic, and pulmonary dysfunction. The optimal therapy for ECD remains to be defined, though interferon is used frequently in the front-line setting (16). BRAF V600E mutations occur in approximately 50% of patients and can BRAF inhibitors such as vemurafenib are efficacious in the treatment of ECD but skin toxicity can be limiting (17). Recent evidence has implicated TNF-alpha in the pathogenesis of ECD (18).

TNF-alpha is thought to promote the proliferation and survival of dendritic cells and histiocytes and thalidomide inhibits TNF-alpha production by affecting the gene promoter as well as other anti-cytokine effects (19). Thalidomide has reported activity in histiocyte disorders, including HS and LCH. Thalidomide has several drawbacks, including the common side effects of neuropathy and somnolence. Lenalidomide is potentially a more potent TNF-alpha inhibitor and does not cause neuropathy, making it potentially a more useful agent for prolonged administration. There are case reports describing the activity of lenalidomide in a variety of histiocyte disorders (20, 21). There is also emerging evidence on the important role of activation of RAS-PI3K-AKT signaling in histiocyte disorders and the AKT inhibitor afuresertib has been studied in LCH patients (22, 23). Lenalidomide potentially has inhibitory effects on AKT as well (24). We therefore planned a broader study to more systematically evaluate the efficacy of lenalidomide and describe the toxicity of lenalidomide in a population of patients with LCH and HS. Given the putative mechanism of action we seek to correlate serum TNF-alpha levels with response. In patients with a known BRAF V600E mutation we also wish to explore the possibility of using BRAF mutation burden assessed in urine and plasma cell free DNA as a biomarker of disease activity.

2.2 Study Agent

Lenalidomide is an immunomodulatory compound (IMiD) compound, a class of agents that have both immunomodulatory and anti-angiogenic properties. Lenalidomide has been demonstrated to possess anti-angiogenic activity through inhibition of bFGF, VEGF and TNF-alpha induced endothelial cell migration, due at least in part to inhibition of Akt phosphorylation response to bFGF (20). In addition, lenalidomide has a variety of immunomodulatory effects. Lenalidomide stimulates T cell proliferation, and the production of IL-2, IL-10 and IFN-gamma, inhibits IL-1 beta and IL-6 and modulates IL-12 production (25).0 Upregulation of T cell derived IL-2 production is achieved at least in part through increased AP-1 activity (26). 0

Although the exact antitumor mechanism of action of lenalidomide is unknown, a number of mechanisms are postulated to be responsible for lenalidomide's activity. The majority of this information has been derived from studied in multiple myeloma. Lenalidomide increases T cell proliferation, which leads to an increase in IL-2 and IFN-gamma secretion. The increased level of these circulating cytokines augment natural killer cell number and function, and enhance natural killer cell activity to yield an increase in multiple myeloma cell lysis. In addition, lenalidomide has direct activity against multiple myeloma and induces apoptosis or G1growth arrest in multiple myeloma cell lines and in multiple myeloma cells of patients resistant to melphalan, doxorubicin and dexamethasone[5].

Indications and Usage:

Lenalidomide is indicated for the treatment of patients with transfusion-dependent anemia due to Low- or Intermediate-1-risk myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities. Lenalidomide is approved in combination with dexamethasone for the treatment of patients with multiple myeloma. Lenalidomide is also approved in patients with mantle cell lymphoma whose disease has relapsed or progressed after two therapies, one of which included bortezomib

Adverse Events

The most frequently reported adverse events reported during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, dehydration, rash, itching, infections, sepsis, pneumonia, UTI, Upper respiratory infection, atrial fibrillation, congestive heart failure, myocardial infarction, chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, CVA, convulsions, dizziness, spinal cord compression, syncope, disease progression, death not specified and fractures.

Second New Cancers

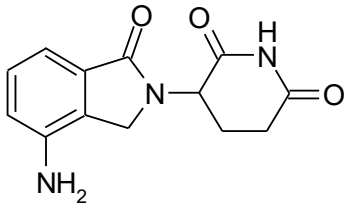
In clinical studies of newly diagnosed multiple myeloma, a higher number of second cancers were reported in patients treated with lenalidomide as induction therapy and/or bone marrow transplant followed by lenalidomide. This risk seems to be greatest when the duration of lenalidomide therapy exceeded 2 years.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

Lenalidomide Description

Lenalidomide, a thalidomide analogue, is an immunomodulatory agent with anti-angiogenic properties. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro -2*H*-isoindol-2-yl) piperidine-2,6-dione and it has the following chemical structure:

Chemical Structure of Lenalidomide



3-(4-amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl) piperidine-2,6-dione

The empirical formula for lenalidomide is $C_{13}H_{13}N_3O_3$, and the gram molecular weight is 259.3.

Lenalidomide is an off-white to pale-yellow solid powder. It is soluble in organic solvent/water mixtures, and buffered aqueous solvents. Lenalidomide is more soluble in organic solvents and low pH solutions. Solubility was significantly lower in less acidic buffers, ranging from about 0.4 to 0.5 mg/ml. Lenalidomide has an asymmetric carbon atom and can exist as the optically active forms S(-) and R(+), and is produced as a racemic mixture with a net optical rotation of zero.

Clinical Pharmacology

Mechanism of Action:

The mechanism of action of lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and antiangiogenic properties. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibited cell proliferation with varying effectiveness (IC50s) in some but not all cell lines. Of cell lines tested, lenalidomide was effective in inhibiting growth of Namalwa cells (a human B cell lymphoma cell line with a deletion of one chromosome 5) but was much less effective in inhibiting growth

of KG-1 cells (human myeloblastic cell line, also with a deletion of one chromosome 5) and other cell lines without chromosome 5 deletions. Lenalidomide inhibited the expression of cyclooxygenase-2 (COX-2) but not COX-1 in vitro.

Pharmacokinetics and Drug Metabolism

Absorption:

Lenalidomide, in healthy volunteers, is rapidly absorbed following oral administration with maximum plasma concentrations occurring between 0.625 and 1.5 hours post-dose. Co-administration with food does not alter the extent of absorption (AUC) but does reduce the maximal plasma concentration (Cmax) by 36%. The pharmacokinetic disposition of lenalidomide is linear. Cmax and AUC increase proportionately with increases in dose. Multiple dosing at the recommended dose-regimen does not result in drug accumulation.

Pharmacokinetic analyses were performed on 15 multiple myeloma patients treated in the phase I studies. Absorption was found to be rapid on both Day 1 and Day 28 with time to maximum blood levels ranging from 0.7 to 2.0 hours at all dose levels (5mg, 10mg, 25mg, and 50mg). No plasma accumulation was observed with multiple daily dosing. Plasma lenalidomide declined in a monophasic manner with elimination half-life ranging from 2.8 to 6.1 hours on both Day 1 and 28 at all 4 doses. Peak and overall plasma concentrations were dose proportional over the dosing range of 5mg to 50mg. Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers.

Pharmacokinetic Parameters

Distribution

In vitro (^{14}C)-lenalidomide binding to plasma proteins is approximately 30%.

Metabolism and Excretion

The metabolic profile of lenalidomide in humans has not been studied. In healthy volunteers, approximately two-thirds of lenalidomide is eliminated unchanged through urinary excretion. The process exceeds the glomerular filtration rate and therefore is partially or entirely active. Half-life of elimination is approximately 3 hours.

Supplier

Celgene Corporation will supply lenalidomide (Revlimid®) to study participants at no charge through Celgene's Revlimid Risk Evaluation and Mitigation Strategy™ (REMS) (formerly known as RevAssist® Program).

2.3 Rationale

There have been a number of case reports indicating responses with thalidomide and to a lesser extent lenalidomide in LCH. However, these agents have not been systematically studied and the mechanism of action remains unclear

2.4 Correlative Studies Background

Preclinical data suggest that polymorphisms of the TNF-α promoter occur in LCH patients and that TNF-α levels are elevated in patients with LCH. We will therefore measure baseline TNF-α levels and obtain serial measurements every 2 months for one year or until the patient is removed from protocol (whichever is sooner). This assay will be performed by a reference laboratory (Mayo Clinic).

V600E mutations have been described in up to 50% of patients with ECD and LCH. A commercially available cell free DNA urine assay now exists. In a study of ECD patients with available archival tissue, the result of BRAF mutation analysis was concordant with plasma and urine cfDNA results in all 3 patients (100% agreement, kappa 1.00). In all 6 patients studied on this protocol, BRAF mutation analysis of plasma and urine cfDNA was concordant in 5 of 6 patients (83% agreement, kappa 0.67). We therefore will check baseline urine cell free DNA for BRAF V600E mutations and perform tissue testing for the V600E mutation whenever feasible (either by immunohistochemistry or molecular analysis depending on tissue availability). In addition, we will perform droplet digital PCR assays for BRAF mutations through the blood biomarker lab of the Belfer Institute. The Belfer group has developed a novel assay utilizing droplet digital PCR (ddPCR) technology to perform noninvasive and quantitative genotyping on cfDNA collected from plasma samples. This assay utilizes ddPCR to emulsify collected cfDNA from patient plasma into approximately 20 000 droplets that undergo individualized PCR reactions and analysis allowing for the quantification of mutant versus wildtype copy number [Figure 1]. Previously published work by this group (Oxnard et al, CCR, 2014) has demonstrated the technical feasibility and accuracy of this plasma genotyping assay for detecting BRAF mutations in NSCLC patients. This assay is both highly sensitive and quantitative which creates unique advantages over previous plasma genotyping methods. In addition, the quantitative nature of this assay combined with quality control methods can minimize the false positive results that have plagued earlier assays. The quantitative nature of this assay may also be exploited to allow prediction of early treatment failure as well as the development of treatment resistance.

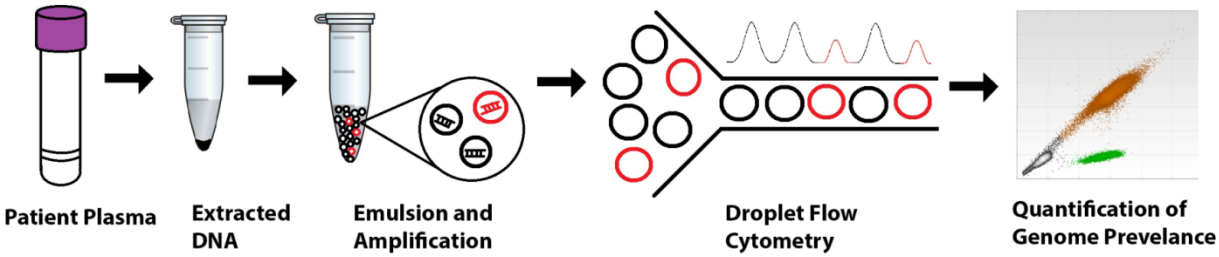


Figure 1: Plasma genotyping using droplet digital PCR (ddPCR). Cell free DNA (cfDNA) is extracted from a plasma specimen and emulsified with oil into ~20,000 droplets, each containing approximately 0-1 molecules of target DNA. PCR is performed to endpoint in each droplet. These droplets are run through a flow cytometer, where droplets containing mutant and wildtype DNA emit different colored signals. The count of these signals allows quantification of allelic prevalence.

Patients who have a detectable BRAF V600E mutation in the urine and/or plasma at baseline will have serial measurements every 2 months for 12 months or until protocol discontinuation (whichever occurs sooner). Note, if patients have a positive urine or serum BRAF assay both tests will repeated at all of the aforementioned timepoints. The results of urine and ddPCR-based plasma genotyping will be compared to tissue genotyping for BRAF V600E.

In a descriptive fashion we will investigate whether serum TNF-α levels, ddPCR for BRAF and/or urine cell free DNA harboring the BRAF V600E serves as a biomarker for response.

3. PARTICIPANT SELECTION

Patients aged 18 or older with histologically confirmed Langerhans cell histiocytosis, Erdheim-Chester disease, or histiocytic sarcoma will be eligible. Confirmation of pathology at Brigham and Women's Hospital is preferred but patients may be enrolled based upon outside review of pathology if in the opinion of the principle investigator the pathology is consistent with LCH, ECD, or HS and if a delay in treatment due to review of pathology at BWH would be detrimental to the patient. Tissue will be requested for Oncopanel testing on all patients but this is not an eligibility criteria. Patients will be consented to DFCI protocol 11-104 for Oncopanel testing. Patients who decline participation in this companion protocol will not have material sent for Oncopanel.

Patients with LCH must require systemic treatment according to the Histiocyte Society LCH Evaluation and Treatment Guidelines (2009). There is no limit on the number of prior therapies received so long as the patient otherwise meets eligibility criteria. Patients with HS that cannot be surgically excised and/or encompassed in a radiation field will be eligible even if they have not received prior systemic therapy.

Laboratory tests required for eligibility must be completed within 14 days of registration and radiographic tests must be completed within 30 days of registration.

Since the response criteria differ for histiocyte disorders than for other malignancies, radiographically detectable disease is required but does not need to be bidimensionally measurable as in Cheson criteria.

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed LCH, ECD or HS. Confirmation of outside pathology at BWH will be performed but is not mandatory prior to study enrollment (see section 3).

3.1.2 Detectable disease by at least one of the following modalities: CT, PET, bone scan, or MRI.

3.1.3 Patients with LCH must require systemic therapy according to the Histiocyte Society LCH Evaluation and Treatment Guidelines (HS 2009)

Or

Patients with HS requiring systemic treatment as defined by disease that cannot be surgically resected and/or encompassed in a single radiation field.

3.1.4 Age 18 years or older.

3.1.5 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A)

3.1.6 Participants must have normal organ and marrow function as defined below:

– absolute neutrophil count	≥1,000/mcL
– platelets	≥100,000/mcL
– total bilirubin	within 1.5 times normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤3 × institutional upper limit of normal
– creatinine	within 2 times normal institutional limits
	OR
– creatinine clearance	≥30 mL/min/1.73 m^2. Note, dose adjustments are required for CrCl ≥ 30 mL/min but ≤ 60 ml/min.

3.1.7 Able to take aspirin 81 mg daily as prophylactic anticoagulation if not on warfarin, low molecular weight heparin or oral factor Xa inhibitor. Patients intolerant to ASA may use warfarin or low molecular weight heparin at doses designed to treat deep venous thrombosis.

3.1.8 All study participants must be registered into the mandatory Revlimid REMS® program, and be willing and able to comply with the requirements of the REMS® program.

3.1.9 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

3.2.0 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior chemotherapy or radiation within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.

3.2.2 Participants who are receiving any other investigational agents.

3.2.3 Prior treatment with lenalidomide. Patients previously treated with thalidomide who discontinued treatment for reasons aside from an adverse reaction to thalidomide are permitted.

3.2.4 History of another invasive malignancy unless treated with curative intent 5 years or more prior to study entry. Patients with localized carcinoma of the cervix, non-melanoma skin cancer, or early stage prostate cancer requiring observation only are eligible regardless of timing of diagnosis.

3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because lenalidomide has known teratogenic effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with lenalidomide, breastfeeding should be discontinued if the mother is treated with lenalidomide.

3.2.7 Known active hepatitis B (HBV) or hepatitis C (HCV) infection. Patients who are positive only for HBV surface antibody as a result of prior vaccination are eligible. Patients with a positive HBV core antibody but undetectable HBV viral load are eligible.

3.2.8 HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with lenalidomide. In addition, these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.9 Concomitant corticosteroids unless patient has been on a stable dose of prednisone (or equivalent) of $\leq$ 10 mg daily for at least 2 weeks prior to first dose of study drug.

3.2.10 Inability to swallow pills.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the QACT protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin protocol therapy during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

- Obtain written informed consent from the participant prior to the performance of any protocol specific procedures or assessments.

- Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical record and/or research chart. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**

  Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for a treatment protocol. Registration to both treatment and ancillary protocols will not be completed if eligibility requirements are not met for all studies.

- Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295. For Phase I protocols, attach participant dose level assignment confirmation from the sponsor.

- The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.

- An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for lenalidomide are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

5.1 Treatment Regimen

For cycle 1, lenalidomide will be administered at a dose of 10 mg daily on days 1-21 of a 28 day cycle. Patients will be assessed for toxicity between days 22 and 28. If no grade 3 or greater toxicity has occurred the dose will be escalated to 25 mg daily on days 1-21 of a day 28 cycle (patients with a baseline CrCl of 30 to $\leq$ 60 will stay at the 10 mg dose). Patients with a baseline CrCl of 30 to $\leq$ 60 can be dose escalated to 25 mg if there is no grade 3 or greater toxicity at week 8 visit if CrCl has improved to >60. No dose escalation is permitted past week 8. Once a dose reduction has occurred the dose will not be re-escalated unless it is a toxicity due to neutropenia in which case the dose can be escalated if there is no recurrent neutropenia with growth factor support for two continuous cycles. Treatment will be administered on an outpatient basis. The maximum number of cycles is 12 (inclusive of cycle 1 and/or 2 dosing at 10 mg). Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Lenalidomide dosing					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Lenalidomide	None	10 mg tablet (cycle 1)*	PO at approximately the same time each day(+/- 2 hours)	Days 1-21	28 days
Lenalidomide	None	25 mg tablet (cycle 2 and subsequent)*	PO at approximately the same time each day	Days 1-21	
*Reference treatment plan and schema for dose instructions for patients with baseline CrCl of 30 to ≤ 60					

The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Participants must meet all of the inclusion criteria, have none of the exclusion criteria, and must be registered to the protocol prior to initiating therapy.

The following studies and evaluations should be carefully reviewed to determine the patient's suitability to initiate study treatment.

1. Diagnosis of LCH, ECD, or HS is established by disease evaluations and laboratory studies and confirmed histologically
2. Complete physical examination.
3. Medical history: Detailed documentation of disease and treatment history with outcomes.
4. Performance status ≥60%
5. Concurrent medical conditions.
6. Hematology: CBC with automated differential.

7. Serum chemistries: Albumin, alkaline phosphates, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
8. Baseline adverse event assessment from time of signing informed consent.

5.2.2 Subsequent Cycles

A new course of treatment may begin on the scheduled Day 1 of a new cycle if:

- The ANC is ≥ 1000/mm³;
- The platelet count is ≥75,000 /mm³
- Any drug-related rash that may have occurred has resolved to ≤ grade 1 severity
- Any other drug-related adverse events that may have occurred have resolved to ≤ grade 2 severity.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of treatment will not be initiated until the toxicity has resolved as described above. If a given toxicity has not resolved to an acceptable level within 28 days of the planned initiation of a cycle, the participant will be permanently removed from treatment.

5.3 Agent Administration

In general, there is a +/- 3 day window for lenalidomide administration to allow for scheduling related issues.

Lenalidomide
Celgene Corporation will supply lenalidomide to study participants at no charge through Celgene's Revlimid Risk Evaluation and Mitigation Strategy™ (REMS) (formerly known as RevAssist® Program). Lenalidomide will be supplied as capsules for oral administration and will be taken on days 1-21 of a 28 day cycle. The first cycle will be dosed at 10 mg with a dose escalation to 25 mg at cycle 2 in the absence of toxicity. If the creatinine clearance is 30 to ≤ 60 then the starting dose will be still be 10 mg at cycle 2. In the absence of grade 3 or greater toxicity during cycle 1 the dose will stay at 10 mg during cycle 2 in this patient population. In the absence of grade 3 or greater toxicity at the week 8 assessment the dose can be escalated to 25 mg daily on days 1-21 of a 28 day cycle in patients whose baseline CrCl was 30 to ≤ 60 and in whom CrCl subsequently improves to >60. No dose escalation if permitted after week 8. The maximum number of cycles is 12 inclusive of the dose escalation cycle(s). Lenalidomide will be taken at approximately the same time every day (+/- 2 hours) and there are no dietary considerations. Vomited doses will not be made-up. Missed or forgotten doses can be made up if taken within twelve hours of the scheduled administration on the same calendar day. For example, if the patient is scheduled to take lenalidomide at 8 AM a missed dose can be made up prior to 8 PM on the day of treatment. However, if the patient was scheduled to take a dose at 4 PM and that dose is missed, the dose must be taken before midnight of the same calendar day. If

these conditions cannot be fulfilled the dose should be skipped.

Packaging
Lenalidomide will be shipped directly to patients from a specialty pharmacy. Bottles will contain a sufficient number of capsules for one cycle of dosing**.** Lenalidomide supplies are dispensed in individual bottles of capsules. Each bottle will identify the contents as study medication. In addition, the label will bear Celgene's name, quantity contained and the standard caution statement as follows: "Caution: New drug - Limited by Federal law to investigational use." The study drug label must be clearly visible. Additional labels must not cover the Celgene label.

Return
Patients should return all unused study medication to the study site at each applicable study visit.

AccountabilityThe disposition of all Lenalidomide study drug should be documented from the time of receipt at the site through patient dispensing and return.

The Investigator and/or the responsible site personnel must maintain accurate records of the receipt of all study drug shipped by Celgene Corporation or its designee. Study drug accountability records must also be maintained that include the patient number to whom the study drug was dispensed, the date and quantity of the study drug dispensed.

The study drug supply should be retrieved from patients at the end of each dosing cycle. The quantity of study drug and the date returned by the patient should be recorded in the study drug accountability records.

Remaining unused study drug supply will be destroyed at the clinical site, standard institutional policy should be followed. Records documenting the date of study drug destruction, relevant lot numbers, and destroyed should be maintained,

Storage

Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

Unused study drug supplies

Patients will be instructed to return empty bottles or unused capsules to the clinic site. Celgene will instruct the Investigator (or designee) on the return or destruction of unused study drug. If any study drug is lost or damaged, its disposition should be documented in the source documents. Study drug supplies will be retained at the clinical site pending instructions for disposition by Celgene.

Lenalidomide should not be handled by females of child-bearing potential unless wearing gloves.

5.3.1 CTEP and/or CIP IND Agent(s), or other IND agent
N/A

5.3.2 Other Agent(s): N/A

5.3.3 Other Modality(ies) or Procedures

N/A

5.4 General Concomitant Medication and Supportive Care Guidelines

Anti-emetics will be prescribed per physician and patient preference with the exception that steroids should not be utilized as an anti-emetic. If patients require glucocorticoids for any reason during treatment they should be removed from the protocol unless the expected duration of treatment is less than 7 days and the glucocorticoids will not be given on a recurring basis. Erythroid growth factors are not permitted on protocol due to concerns about augmenting clotting risk. Filgrastim and pegfilgrastim are not permitted during cycle 1 but are permitted during the second and subsequent cycles as outlined in section 6 on dose modification (note, filgrastim and pegfilgrastim are not allowed during the first 8 weeks in patients with a CrCl of 30 to $\leq$ 60).

Because there is a potential for interaction of lenalidomide with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

Lenalidomide is known to increase the risk of arterial and venous thrombosis. Patients not already on systemic anticoagulation (warfarin, low molecular weight heparin, or equivalent) for other medical reasons are required to take aspirin 81 mg daily for the duration of the study.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for 12 cycles or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements

- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Recurrent toxicity (see section 6)
- Patient becomes pregnant or cannot comply with birth control requirements.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

A QACT Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the QACT website or obtained from the QACT registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Eric Jacobsen, MD at 617-632-6633 or 617-632-3000 (pager ID 41475*)*.

5.6 Duration of Follow Up

Participants will be followed for 3 years from the initiation of treatment, until subsequent therapy or until removal from study or death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Initiation of subsequent therapy
- Death
- Completion of all study procedures

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A QACT Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the QACT website or obtained from the QACT registration staff.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for dose delays and dose modifications and version 3.0 should be used for tumor flare. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Note that tumor flare reaction is managed differently from other AEs as outlined in Table 4. Lenalidomide should be held for any grade 3 or higher toxicity except in the incidence of a blistering skin rash, in which case lenalidomide should be permanently discontinued though patients will be followed for resolution of toxicity and for survival. In the event of a blistering skin rash the patient should have an urgent dermatology referral if grade 1 or 2 and should be hospitalized if grade 3 or 4. If a patient develops venous thromboembolism and/or pulmonary embolism while on therapeutic anticoagulation lenalidomide treatment should be permanently discontinued regardless of grade unless anticoagulation dosing was low (e.g., INR or anti-factor Xa was not in the generally accepted therapeutic range). Patients experiencing a venous thromboembolic event while on aspirin alone may stay on protocol at the discretion of the treating physician as long as the patient is subsequently on systemic anticoagulation with warfarin, low molecular weight heparin or equivalent for the duration of the study and as clinically indicated thereafter by the nature and extent of the thrombotic event. Patients experiencing an arterial thromboembolic event will be removed from study regardless of the anticoagulation status at the time of the event.

Per table 2, participants may be dose reduced if toxicities occur during cycle 1 at the starting dose of 10 mg daily. No more than 2 dose reductions will be permitted. If the participant cannot tolerate therapy after 2 dose reductions they should be permanently removed from the protocol.

Dose reductions for cycle 2 and subsequent cycles that involve a dose of 25 mg daily are outlined in table 1. If a participant is receiving the 25 mg dose, a total of 3 dose reductions will be allowed. If the participant cannot tolerate treatment despite 3 dose reductions then the participant should be permanently removed from the protocol. Once a dose reduction occurs patients will not be dose escalated unless the dose reduction is a result of neutropenia and no recurrent neutropenia occurs with filgrastim or pegfilgrastim support for 2 continuous cycles.

Regardless of dosing level, held doses will not be made-up. Missed doses or forgotten doses will not be made up unless lenalidomide is taken within 12 hours of the scheduled time of administration on the same calendar day of the missed or forgotten dose. Vomited doses will not be made up.

Dose Reduction Steps for toxicity aside from TFR.

Table 1: LENALIDOMIDE Dose Reduction Steps when dose is 25 mg	
Dose at Prior Cycle	**25 mg daily on Days 1-21 every 28 days**
Dose Level – 1	20 mg daily on Days 1-21 every 28 days
Dose Level – 2	15 mg daily on Days 1-21 every 28 days
Dose Level – 3	10 mg daily on Days 1-21 every 28 days

Table 2: LENALIDOMIDE Dose Reduction Steps when dose is 10 mg	
Dose at Prior Cycle	**10 mg daily on Days 1-21 every 28 days**
Dose Level – 1	5 mg daily on Days 1-21 every 28 days
Dose Level – 2	2.5 mg daily on Days 1-21 every 28 days

There are no dose reductions below dose level -2 if dose reductions are necessary at the 10 mg starting dose or dose level -3 once the patient has been dose escalated to 25 mg. Patients who cannot tolerate lenalidomide at these dose reductions will discontinue treatment.

Instructions for dose modifications or interruption during a cycle

Table 3: Dose Modifications	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Grade 3 neutropenia associated with fever (temperature ≥ 38.5° C) or Grade 4 neutropenia	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If neutropenia has resolved to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide at next lower dose level and continue through the scheduled Day 21 of the current cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. If neutropenia is the only toxicity for which a dose reduction is required, G-CSF may be used and the lenalidomide dose maintained.
Thrombocytopenia ≥Grade 3 (platelet count < 50,000/mm³)	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If thrombocytopenia resolves to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide at next lower dose level and continue through the scheduled Day 21 of the current cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.
Platelet count < 50,000/mm³	• Hold prophylactic anti-coagulation, if applicable. • Restart prophylactic anti-coagulation when platelet count is ≥ 50,000/mm³.
Non-blistering rash **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21 of the current cycle, restart lenalidomide at next lower dose level and continue through the scheduled Day 21 of the current cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide. Remove patient from study.

Table 3: Dose Modifications	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Desquamating (blistering) rash- any Grade	• Discontinue lenalidomide. Remove patient from study. Immediate referral to dermatology and/or hospitalization if deemed clinically appropriate.
Neuropathy **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21 of the current cycle, restart lenalidomide at next lower dose level and continue through the scheduled Day 21 of the current cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide. Remove patient from study.
Venous thrombosis/embolism ≥ Grade 3	• Hold (interrupt) lenalidomide and start therapeutic anticoagulation, if appropriate. If VTE occurs while patient is on therapeutic anti-coagulation (not counting aspirin) permanently discontinue treatment. • Restart lenalidomide at investigator's discretion (maintain dose level). • See Anticoagulation Consideration (Section 5.6.1.2)
Hyperthyroidism or hypothyroidism	• Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. • See Instructions for Initiation of a New Cycle and reduce the dose of lenalidomide by 1 dose level.
Other non-hematologic toxicity ≥ Grade 3	• Hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide and continue through the scheduled Day 21 of the current cycle. Otherwise, omit for remainder of cycle. Omitted doses are not made up. For toxicity attributed to lenalidomide, reduce the lenalidomide dose by 1 dose level when restarting lenalidomide.

Table 4. Tumor Flare Reaction Treatment (TFR)

Treatment of TFR is up to the discretion of the investigator, depending upon the severity and clinical situation. It is suggested that Grades 1 and 2 TFR be treated with NSAIDs (i.e., ibuprofen 400 to 600 mg orally every 4 to 6 hours as needed), corticosteroids, and/or narcotic analgesics for pain management.

In mild to moderate (Grades 1 and 2) cases, it is suggested that study drug be continued along with symptomatic treatment as above. In more severe cases, study drug should be interrupted.

Tumor Flare Reaction (TFR)[a] Grades 1-2	• Continue lenalidomide, maintain dose level • At the investigator's discretion may initiate therapy with NSAIDs, limited duration corticosteroids, and/or narcotics
Grades 3-4	• Hold (interrupt dose) and initiate therapy with NSAIDs, corticosteroids, and/or narcotics • When symptoms resolve to ≤ Grade 1, restart at same dose level for the rest of the Cycle

Treatment of Overdose
In the case of overdose, clinic staff should be notified immediately and supportive care is to be given as indicated. Patients should be informed to contact their doctor immediately if they have taken an overdose and should stop taking lenalidomide. There is no known antidote for lenalidomide.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Event List(s) for lenalidomide

The most frequently reported adverse events reported during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, dehydration, rash, itching, infections, sepsis, pneumonia, UTI, Upper respiratory infection, atrial fibrillation, congestive heart failure, myocardial infarction,

chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, CVA, convulsions, dizziness, spinal cord compression, syncope, disease progression, death not specified and fractures.

Second New Cancers

In clinical studies of newly diagnosed multiple myeloma, a higher number of second cancers were reported in patients treated with lenalidomide as induction therapy (treatment for several cycles to reduce number of cancer cells) and/or bone marrow transplant followed by lenalidomide for a long period of time compared to patients treated with induction therapy and/or bone marrow transplant then placebo (a capsule containing no lenalidomide). Patients should make their doctors aware of their medical history and any concerns they may have regarding their own increased risk of other cancers.

Tumor Flare

Tumor flare assessments are conducted in Cycle 1 on Days 1, 8, and 15 (±1 day), and when clinically indicated thereafter. Tumor flare reaction (TFR) is defined in the NCI-CTCAE version 3 as a constellation of signs and symptoms of tumor pain, inflammation of visible tumor, hypercalcemia, diffuse bone pain, and other electrolyte disturbances in direct relation to initiation of therapy (Cancer therapy evaluation program, 2003).

Tumor flare reaction is an adverse effect of lenalidomide previously reported in subjects with CLL (27). In clinical studies of lenalidomide in subjects with non-Hodgkin lymphoma (NHL) (28), TFR has also been reported at a lower rate in NHL patients than in CLL patients (29,30). The onset of TFR has been as early as within a few hours after the first dose of lenalidomide and occurs within the first 2 to 3 weeks of the first Cycle in the vast majority of TFR cases. TFR subsides over time and usually resolves in 1 to 2 weeks with or without intervention (28).

Pregnancy

Lenalidomide is known to be teratogenic thus women of child-bearing potential and men must agree to use adequate contraception as outlined in the REMS® program. Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on lenalidomide, or within 28 days of the subject's last dose of lenalidomide, are considered immediately reportable events. Lenalidomide is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by facsimile or email using the Pregnancy Initial Report Form. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling. The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form. If the outcome of the

pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

Expedited Reporting by Investigator to Celgene

Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required. The Celgene tracking number (RV-CL-OTHER-PI-004617) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

Celgene Drug Safety Contact Information:
Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr. Suite 6000
Berkeley Heights, NJ 07922
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4 Expedited Reporting to Hospital Risk Management.

Participating investigators will report to the Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the

toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

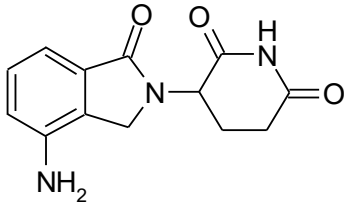
A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 Lenalidomide

8.1.1 Description

REVLIMID® (lenalidomide), a thalidomide analogue, is an immunomodulatory agent with anti-angiogenic properties. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro -2*H*-isoindol-2-yl) piperidine-2,6-dione and it has the following chemical structure:

1.1.1.1.1.1.1 Chemical Structure of Lenalidomide



3-(4-amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl) piperidine-2,6-dione

The empirical formula for lenalidomide is $C_{13}H_{13}N_3O_3$, and the gram molecular weight is 259.3.

8.1.2 Form

Lenalidomide will be supplied as off-white capsules for oral administration. Capsule strengths are 2.5 mg, 5 mg, 10 mg, 15 mg, 20 mg and 25 mg. Lenalidomide will be shipped directly to patients or to the clinic site. Bottles will contain a sufficient number of capsules for one cycle of dosing**.**

8.1.3 Storage and Stability

Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

8.1.4 **Compatibility**

Not applicable

8.1.5 **Handling**

- Females of childbearing potential should not handle or administer lenalidomide unless they are wearing gloves. Females of reproductive potential must adhere to the scheduled pregnancy testing as required in the Revlimid REMS® program.

8.1.6 **Availability**

Celgene Corporation will supply Revlimid® (lenalidomide) to study participants at no charge through Celgene's Revlimid Risk Evaluation and Mitigation Strategy™ (REMS) (formerly known as RevAssist® Program). Lenalidomide will be provided in accordance with the Celgene Corporation's Revlimid REMS® program. Per standard Revlimid REMS® program requirements, all physicians who prescribe lenalidomide for research subjects enrolled into this trial, and all research subjects enrolled into this trial, must be registered in, and must comply with, all requirements of the Revlimid REMS® program.

8.1.7 **Preparation**

Lenalidomide will come pre-prepared by the manufacturer.

8.1.8 **Administration**

The subject will self-administer lenalidomide at home.

8.1.9 **Ordering**

Lenalidomide will be shipped on a per patient basis by the contract pharmacy to the patient. Only enough lenalidomide for one cycle of therapy will be supplied to the patient. Lenalidomide supplies are dispensed in individual bottles of capsules. Each bottle will identify the contents as study medication. In addition, the label will bear Celgene's name, quantity contained and the standard caution statement as follows: "Caution: New drug - Limited by Federal law to investigational use." Lenalidomide should not be handled by FCBP unless wearing gloves.

The study drug label must be clearly visible. Additional labels must not cover the Celgene label.

8.1.10 **Accountability**

The Investigator or designee is responsible for taking an inventory of each shipment of study drug received by the patient, and comparing it with the patient's drug diary. The Investigator or designee will verify the accuracy of the information on the drug diary, sign and date it, and retain a copy in the study file, and return a copy to Celgene or its representative upon request.

8.1.11 **Destruction and Return**

Patients will be instructed to return empty bottles or unused capsules to the clinic site. Celgene will instruct the Investigator on the return or destruction of unused study drug. If any study drug is lost or damaged, its disposition should be documented in the source documents. Study drug supplies will be retained at the clinical site pending instructions for disposition by Celgene.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

BRAF is a human gene encoding a cellular growth signalling protein, which has been shown to be mutated in certain cancers including up to 50% of cases of LCH. The frequency of BRAF mutation in HS is unknown but appears to be minimal. The Trovagene cell free DNA (cfDNA) urine assay uses a proprietary method for extracting cell-free transrenal DNA from large volume urine samples and a PCR assay to amplify small DNA fragments while enriching for mutant alleles. These are then detected by droplet digital PCR (ddPCR).

This test was developed using urine samples from patients with stage IV / metastatic cancer. This test confirms the presence of a BRAF V600E mutation in urine DNA at levels of ≥0.03% in comparison to the wild-type BRAF genes.

This is a laboratory developed test (LDT). This test was developed and its performance characteristics determined by Trovagene. It has not been cleared or approved by the FDA as clearance or approval of LDTs is not currently required.

Trovagene's laboratory is certified by the State of California LICENSE CLF 00338242 in compliance with CLIA (Clinical Laboratory Improvement Amendments) LICENSE 05D1094618, and is accredited by the College of American Pathologists LICENSE LAP 7190902**.**

- Optimal testing requires a 90-110ml urine sample
- No refrigeration necessary - collect and ship at room temperature
- Collection kits will be provided by the manufacturer.

Plasma genotyping of cfDNA will be performed by the Belfer Institute blood biomarker lab using a validated ddPCR-based assay for the detection of BRAF V600E. Samples from venous blood draws will undergo centrifugation within 1 hour of sample collection and standard plasma collection (**Appendix C - SOP**). The plasma will then be transported directly to the Belfer Institute. Immediate extraction of cfDNA will then be performed on 2 mL of plasma using the QIAmp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's protocol. DNA will then be eluted in 100 uL of AVE buffer and frozen at -80C until genotyping is performed. DNA quantification will be be performed using a fluorescence absorbence PicoGreen assay (Invitrogen).

Genotyping will subsequently be performed by ddPCR using standard reagents ordered from Bio-Rad. Allele specific probes for BRAF V600E will be used in participants. The design and optimization of these primers and the associated methodology has previously been described by our group (Oxnard et al., CCR, 2014).

Patients will undergo urine and plasma cfDNA testing at study entry. The results of this test will be compared with standard BRAF testing via immunohistochemistry and/or PCR on available tissue samples. If patients have a detectable BRAF mutation burden in cfDNA in the urine at study entry the test will be repeated at various timepoints as outlined in the study calendar (section 10). If the test is negative at baseline it will not be repeated. Results will be recorded in a quantitative fashion. This test may be performed in clinic (preferred) or taken home and shipped in a prepaid, addressed overnight mailer provided with the test kit.

9.2 Laboratory Correlative Studies

9.2.1 Serum TNF-alpha

9.2.1.1.1. Specimens will be collected in a DFCI clinical space (lab or infusion floor). One red top tube will be collected at specified timepoints. The test can be ordered via Lab Order Entry (LOE) in which a standard collection and processing workflow already exists.

9.2.1.1.2. The specimen will be frozen per standard DFCI lab procedure

9.2.1.1.3. Specimens are delivered via courier from DFCI lab control to the Mayo Clinic Laboratory three times daily

9.2.1.1.4. Collection of the specimen will occur at DFCI. The assay will be run at the Mayo Clinic Laboratory per current standard procedure.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Pre-Study	C1 D1	C1 D15	C2 D1	C2 D15	C3 D1	C3 D15	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9 D1	C10 & 11 D1	C12 D1	Off Treatment[l]	Follow Up[m]
Lenalidomide		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Anticoagulation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[h]	
Informed consent	X																
Demographics	X																
Medical history	X																
Concurrent meds		X----------															----------X
Physical exam	X	X	X	X		X		X		X		X	X		X	X	X
Vital signs[a]	X	X	X	X		X		X		X		X	X		X	X	X
Height	X	X	X	X		X		X		X		X	X		X		X
Weight	X	X	X	X		X		X		X		X	X		X	X	X
Performance status	X	X		X		X		X		X		X	X		X	X	X
CBC w/diff, plts	X	X[j]	X	X[j]	X[k]	X	X[k]	X		X		X	X		X	X	X
Serum chemistry[b]	X	X	X	X	X[k]	X	X[k]	X		X		X	X		X	X	X
EKG	X																
Bone marrow biopsy	X[i]																X[i]
Adverse event evaluation		X----------															----------X
Tumor measurements[c]	X					X				X			X		X	X	X
Radiologic evaluation[d]	X					X				X			X		X	X	X
B-HCG[e]	X[b]	X		X		X		X		X		X	X		X		X
Urine/ Plasma Assay for BRAF mutation[f]	X[e]					X				X					X	X	X
Serum TNF-alpha[g]	X					X				X					X	X	X

a: Temperature, BP, HR and O2 saturation by pulse oximetry at rest.

b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

c: Tumor measurements are repeated at C3D1, C6D1, C9D1 and C12D1 and then every 3 months thereafter until month 24. After month 24 tumor assessments will occur every 6 months (+/- 2 weeks) until month 36 from treatment initiation. Documentation (preferably radiologic) must be provided for participants removed from study for progressive disease. A window of +/- 1 week is allowed prior to month 24 but tumor assessments must occur prior to dosing on the relevant cycle when active treatment is being administered

d: MRI of the brain is required for all patients but only needs to be repeated at follow-up if abnormal at baseline; PET/CT from scalp to toes is preferred for all patients but if PET/CT is not covered by insurance CT of chest, abdomen and pelvis as well as bone scan is an acceptable alternative; the same imaging modality should be used at each response time point. Radiologic measurements should be performed on C3D1, C6D1, C9D1, and C12D1 and then every 3 months thereafter until month 24. After month 24 tumor assessments will occur every 6 months (+/- 2 weeks) until month 36 from treatment initiation. A window of +/- 1 week is allowed prior to month 24 but tumor assessments must occur prior to dosing on the relevant cycle when active treatment is being administered.

e: Serum pregnancy test [women of childbearing potential defined as a sexually mature female who 1) has not undergone a hysterectomy or bilateral oopherectomy; or 2) has not been naturally menopausal for at least 24 consecutive months]. WCBP must have a pregnancy test prior to each cycle. This will be performed in clinic when there is an evaluation on day 1 of any cycle scheduled. Pregnancy testing will occur per the REMS program when a patient is not scheduled for clinic evaluation on day 1 of any cycle.

f: Urine/serum assay will only be repeated at the time points indicated if abnormal at baseline. Urine assay for BRAF mutation has a +/- 7 day window. Serum assay has a +/- 3 day window.

g: Samples can be drawn pre or post dose at the time points indicated, they are not dependent on dosing.

h: If patient did not experience a venous thromboembolic event and there is no other clinical indication for anticoagulation then per protocol anticoagulation can be discontinued at this juncture. If there is separate clinical indication for anticoagulation, including a thromboembolic event while on protocol, the nature and duration of anticoagulation should be determined as clinically indicated by standard of care and the discretion of the treating physician.

i: Only required if at least one unexplained cytopenia of grade 2 or greater is present at baseline or if PET/CT suggests bone marrow involvement; only needs to be repeated if abnormal at baseline.

j: CBC with automated differential will be checked every 7 days (+/- 1 day) during cycles 1 and 2; local labs are acceptable.
k: Labs only necessary if dose escalation occurs. Note, these labs can be obtained at a local laboratory. Window for labs is +/- 3 days.
l: Window for end of treatment visit is +/- 7 days from the date of last dose.
m: The first follow up visit will occur 3 months from the date of last dose and then every 3 months thereafter. Window for follow up visits is +/- 2 weeks.

11. MEASUREMENT OF EFFECT

11.1 Definitions

Evaluable for toxicity: All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response: Any patient who receives at least one dose of lenalidomide will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. Patients not evaluable for response will not be replaced by another protocol participant.

11.1.1 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules) and ≥5 mm in diameter as assessed using calipers. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended. Skin lesions are considered non-target lesions for response assessment and will be described qualitatively as resolved, improved, stable, or progressive.

PET/CT: this is the preferred method of evaluation for all patients. Because of the propensity of histiocyte disorders to involve the skin and the bones of the skull, the PET/CT should encompass the area from the scalp to the toes. If PET/CT is not covered by insurance, a CT of the chest, abdomen and pelvis in combination with a bone scan is an acceptable alternative.

MRI: MRI is required for all patients at baseline and must be repeated at specified timepoints only if abnormal at baseline or if normal at baseline but there is subsequent clinical suspicion of a CNS recurrence.

11.1.2 Response Criteria

In contrast to lymphoma or other malignancies the terms "remission" or "relapse" should be avoided. In accordance with the nature of LCH the following definitions should be applied to judge the effect of treatment. The response criteria stated below has been designed by the Histiocyte Society to be applied in therapeutic studies of LCH and should be used for therapeutic decisions:

Table 3 Definition of disease state by organ system

NON ACTIVE DISEASE (NAD)	no evidence of disease	resolution of all signs or symptoms
	regressive disease	regression of signs or symptoms, no new lesions
ACTIVE DISEASE (AD)	stable disease	persistence of signs of symptoms, no new lesions
	progressive disease	progression of signs or symptoms and/or appearance of new lesions

Definition of response criteria
Table 4 Three categories of overall response

BETTER	complete resolution	NAD
	regression	AD better
INTERMEDIATE		mixed new lesions in one site, regression in another site
	Stable	Unchanged
WORSE	progression*	

* In isolated bone disease progression is defined as appearance of new bone lesions or lesions in other organs.

11.1.2.1 **Evaluation of Target Lesions**

Unfortunately the Histiocyte Society does not specify what constitutes radiographic regression or progression of disease. Since histiocyte disorders are hematologic malignancies we will use modified Cheson criteria to assign response as outlined below.

Response	Definition	Nodal Masses	Spleen and Liver	Bone Marrow
CR	Disappearance of all evidence of disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measurable disease and no new sites	≥50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT	**Any but PD below**	**Any but PD below**
PD	Any new lesion or increase by _ 50% of previously involved sites from nadir	Appearance of a new lesion(s) > 1.5 cm in any axis, ≥ 50% increase in SPD of more than one node, or ≥50% increase in longest diameter of a previously identified node > 1 cm in short axis Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy	>50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

Deauville Criteria for PET scan interpretation in lymphoma will be used in this study as outlined below:

Five-point scale:
1. No Uptake
2. Uptake ≤ mediastinum
3. Uptake >mediastinum but ≤ liver
4. Uptake moderately increased compared to liver at any site
5. Uptake markedly increased compared to the liver at any site or/and new sites of disease

A score of 1-3 is PET NEGATIVE.
A score of 4-5 is PET POSITIVE**.**

11.1.2.2 **Evaluation of skin lesions**

Skin lesions are not always detectable by PET/CT or other radiographic techniques. Skin lesions will be assessed by direct measurement. Photographs are encouraged but not required. Responses in skin are outlined below:

Complete resolution: no detectable skin abnormality
Regression: 50% or greater reduction in the sum of the cumulative area of skin lesions
Stable: not fulfilling criteria for resolution, regression, or progression
Progression: new skin lesions (biopsy confirmation preferred but not mandatory) or 50% increase in area of at least one skin lesion

11.1.2.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. Responses should be maintained for at least 8 weeks. If a patient has multiple sites of disease and any of these sites are active the patient is considered to have a best overall response of active disease.

11.1.3 Duration of Response

Duration of overall response: The duration of overall response will be measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR will measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease will be measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.4 Progression-Free and Overall Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

11.1.5 Response Review

Radiology will be centrally reviewed by the DF/HCC Tumor Imaging Metrics Core (TIMC) and the treating clinician will add clinical data (e.g., status of skin lesions not assessable by radiology) as appropriate.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the QACT according to the schedule set by the QACT.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date

participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

N/a

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The primary endpoint of this study is to evaluate the overall response rate of lenalidomide in patients with LCH. This is a pilot study with a planned accrual of 12 patients. A small sample size was selected due to the rarity of the disease and the difficulty in accruing a larger number of patients at a single center.

Study treatment will be considered worthy of further evaluation if at least three (25%) of 12 patients experience a response. The 90% exact binomial confidence limits for three responses in 12 patients is 7-53%. We will consider a response rate of 5% unworthy of further study for this patient population, and note that two responses in 12 patients is the maximum number of responses which still includes the unworthy response rate of 5% in its confidence limits.

The limited sample size may cause us to miss a small but potentially clinically meaningful response rate or to overestimate the response rate. We are willing to accept this in order to complete the trial in a reasonable time period while recognizing that the results of this trial will be used to determine whether a larger phase II trial of lenalidomide is warranted in which case such a trial would be proposed to the Histiocytosis Society.

Secondary endpoints will include:

- Duration of response
- Progression free survival

- Overall survival
- Description of toxicity
- Quantitative serial measurements of urine and plasma cell free DNA for BRAF mutation as a biomarker of response
- Quantitative serial measurements of serum TNF-alpha levels as a biomarker of response

13.2 Sample Size, Accrual Rate and Study Duration

The planned accrual is 12 patients. The estimated accrual is 5 patients per year so we anticipate the accrual will occur over a 2 to 2.5 year period. Up to an additional 6 months of follow-up will be required on the last participant accrued to assess response. Thus the total study duration is anticipated to be 2.5 to 3 years.

13.3 Stratification Factors

n/a

13.4 Interim Monitoring Plan

The study will be referred to the DSMC for review of dosing and toxicity at the first occurrence of a toxic death.

13.5 Analysis of Primary Endpoints

The primary endpoint will be the proportion of responders. A responder is defined as an eligible, evaluable participant who achieves either complete resolution or regression of disease, per section 7, as the best overall response at any point during study therapy.

13.6 Analysis of Secondary Endpoints

Secondary endpoints are

a) Duration of response defined as the time elapsing between the documentation of the onset of remission until clinical and/or radiographic evidence of progression.
b) Progression-free survival (PFS), where time to event for PFS is the time from study enrollment until the time of first occurrence of new lesions, progressive disease, or death from any cause, or until last contact if no event occurs;

c) Overall survival (OS), calculated as the time from enrollment until death or last contact; and,

d) The proportion of participants reporting grades 3-4 toxicity, for each CTC v4 toxicity code

e) Serial quantification of BRAF mutation in urine and plasma cell free DNA in patients with an identifiable BRAF mutation at baseline.

f) Serial quantification of serum TNF-alpha levels in all patients, even if not elevated at baseline.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Toxicity

All patients receiving at least one dose of lenalidomide will be evaluable for toxicity

13.7.2 Evaluation of the Primary Efficacy Endpoint

All participants included in the study who receive at least one dose of lenalidomide will be assessed for response to treatment, even if there are major protocol treatment deviations.

14. PUBLICATION PLAN

The results will be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

REFERENCES

1. Venkatramani R, Rosenberg S, Indramohan G, Jeng M, Jubran R, An exploratory epidemiological study of Langerhans cell histiocytosis, Pediatr Blood Cancer 2012; 59: 1324-1326.
2. Badalian-Very G, Vergilio JA, Degar BA, et al, Recurrent BRAF mutations in Langerhans cell histiocytosis, Blood 2010; 116: 1919-1923.
3. Gadner H, Grois N, Pötschger U, et al, Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification, Blood 2008; 111: 2556-2562.
4. Derenzine E, Fina MP, Stefoni V, et al, MACOP-B regimen in the treatment of adult Langerhans cell histiocytosis: experience on seven patients, Ann Oncol 2010; 21: 1173-1178.
5. Vassallo R, Ryu JH, Schroeder DR, Decker PA, Limper AH, Clinical outcomes of pulmonary Langerhan's cell histiocytosis in adults, New Engl J Med 2002; 346: 484-490.
6. Simko SJ, McClain KL, Allen CE, Up-front therapy for LCH: Is it time to test an alternative to vinblastine/prednisone?, Br J Haematol 2014; epub.
7. Giona F, Caruso R, Testi AM, et al, Langerhans' cell histiocytosis in adults: a clinical and therapeutic analysis of 11 patients from a single center, Cancer 1997; 80: 1786-1791.
8. Howarth DM, Gilchrist GS, Mullan BP, et al, Langerhans cell histiocytosis: diagnosis, management, natural history, and outcome, Cancer 1999; 85: 2278-2290.
9. Apollonsky N, Lipton JM, Treatment of refractory Langerhans cell histiocytosis (LCH) with a combination of 2-chlorodeoxyadenosine and cytosine arabinoside, J Pediatr Hematol Oncol 2009; 31:53-6.
10. Schini M, Makras P, Kanakis G, Voulgarelis M, Kaltsas G, Cladribine therapy in adults with advanced Langerhans cell histiocytosis, Leuk Lymphoma 2013; 54:1541-3.
11. Gadner H, Minkov M, Grois N, et al, Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis, Blood. 2013;121: 5006-5014.
12. Abraham A, Alsultan A, Jeng M, Rodriguez-Galindo C, Campbell PK, Clofarabine salvage therapy for refractory high-risk langerhans cell histiocytosisPediatr Blood Cancer. 2013;60:E19-
13. Haroche J, Cohen-Aubart F, Emile JF, et al, Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation, Blood 2013;121:1495-500.
14. Kobayashi M, Tojo A, The BRAF-V600E mutation in circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis, Blood 2014;124:2610-2611.
15. Dalia S, Jaglal M, Chervenick P, Cualing H, Sokol L, Clinicopathologic Characteristics and Outcomes of Histiocytic and Dendritic Cell Neoplasms: The Moffitt Cancer Center Experience Over the Last Twenty Five Years, Cancers (Basel). 2014; 6: 2275-2295.
16. Arnaud L, Hervier B, Neel A, et al, CNS involvement and treatment with interferon-α are independent prognostic factors in Erdheim-Chester disease: a multicenter survival analysis of 53 patients, Blood 2011; 117(10):2778-82.
17. Haroche J, Cohen-Aubert F, Emile JF, et al, Reproducible and Sustained Efficacy of Targeted Therapy With Vemurafenib in Patients With BRAFV600E-Mutated Erdheim-Chester Disease, J Clin Oncol 2015; 33(5):411-8.
18. Ferrero E, Belloni D, Corti A, Doglioni C, Dagna L, Ferrarini M, et al, TNF_α in Erdheim-Chester disease pericardial effusion promotes endothelial leakage in vitro and is neutralized by infliximab, Rheumatology 2014; 53(1):198-200.

19.McClain KL, Kozinetz CA, A phase II trial using thalidomide for Langerhans cell histiocytosis, Pediatr Blood Cancer 2007;48:44-49.
20. Szturz P, Adam Z, Rehak Z,et al, Salvage lenalidomide in four rare oncological diseases, Tumori. 2013; 99:e251-6.
21. Szturz P, Adam Z, Rehák Z, et al, Lenalidomide proved effective in multisystem Langerhans cell histiocytosis, Acta Oncol. 2012; 51:412-425.
22. Emile JF, Diamond EL, Hélias-Rodzewicz Z, et al, Recurrent RAS and PIK3CA mutations in Erdheim-Chester disease, Blood 2014; 124: 3016-3019.
23. Arceci RJ, Allen CE, Dunkel I, et al, Evaluation Of Afuresertib, An Oral Pan-AKT Inhibitor, In Patients With Langerhans Cell Histiocytosis, Am Soc Hematol, Ann Meeting 2013; abstract 2907.
24.Dredge K, Horsfall R, Robinson S, et al. Orally administered lenalidomide (lenalidomide) is anti-angiogenic in vivo and inhibits endothelial cell migration and Akt phosphorylation in vitro. Microvascular Research 2005; 69: 56-63.
25. Corral LF, Haslett PAJ, Muller FW, et al, Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha, J Immunol 1999;163:380-386.
26. Schafer PH, Gandhi AK, Loveland MA, et al, Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs, J of Pharmacology and Exp Therapeutics, 2003; 305:1222-1232.
27. Chanan-Khan AA, Cheson BD. Lenalidomide for the treatment of B-cell malignancies. J Clin Oncol 2008a; 26(9):1544-52.
28. Witzig TE, Vose JM, Luigi Zinzani PL, Reeder CB, Buckstein R, Polikoff JA, et al. An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Ann Oncol. 2011;22(7):1622-7.
29. Corazzelli G, De Filippi R, Capobianco G, Frigeri F, DeRosa V, Laccarino G, et al. Tumor flare reactions and response to lenalidomide in patients with refractory classic Hodgkin lymphoma. Am J Hematol. 2010;85(1):87-90.
30. Eve HE, Rule SA. Lenalidomide-induced tumour flare reaction in mantle cell lymphoma. Br J Haematol. 2010;151(4):410-2.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

NCI CTC Version 4.03

Toxicity will be scored using NCI CTC Version 4.03 for toxicity and adverse event reporting. A copy of the NCI CTC Version 4.03 can be downloaded from the CTEP homepage: (http://ctep.info.nih.gov). All appropriate treatment areas have access to a copy of the CTC Version.

APPENDIX C

SOP plasma preparation for EDTA & Streck tubes

NOTE: Time period from draw to freezing of plasma must be less than 3 hours.

1. Draw venous blood into one (1) 10 mL tubes (either EDTA or Streck) labeled CFDNA and immediately gently invert the tubes 8-10 times. Write the patient and draw date number on the tube.

2. Immediately centrifuge for 10 minutes at 1500 (+\- 150) x g. NOTE: Brake switch must be off so the cell/plasma interface is not disturbed.

3. Pipette the plasma layer into a 15 mL tube labeled “CFDNA/with patient #”. Do not ship. NOTE: Do not dip the tip of the pipette into the plasma/cell interface. Leave a thin plasma layer intact over the interface.

4. Centrifuge the 15 mL tube containing the plasma only for 10 minutes at 3000 (+/- 150) x g.

5. Transfer using a fresh pipette, the supernatant into a second 15 mL tube labeled “CFDNA super.do not ship”. NOTE: Leave about 0.3 mL of supernatant in the centrifuged 15 mL tube. This leftover 0.3 mL contains cellular debris.

6. Using a fresh pipette, transfer 1 mL of plasma from the “super.do not ship” tube into max four (4) 2 mL cryovials labeled CFDNA.ship.patient#/

7. Freeze immediately upright at -70ºC or colder until shipping.