

**A Study of Immune-adjuvant Effect of Lenalidomide in Patients with Chronic
Lymphocytic Leukemia and Hypogammaglobulinemia and Impaired Response to
Vaccinations – RV-CL-CLL-PI-002544**

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1.0 Objectives

- To evaluate the ability of lenalidomide to improve the Hypogammaglobulinemia (achieve target IgG response) in patients with CLL.
- To evaluate the ability of lenalidomide to improve the protective antibody titers and serological responses specific to influenza and pneumococcal vaccination (seroconversion response).
- To evaluate the ability of lenalidomide to improve non IgG immune dysfunction such as T cells, cytokines and immunoglobulin subclasses.

2.0 Background

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. It arises from clonal and functionally incompetent B cells. Patients with CLL suffer from immunosuppression, predominantly due to hypogammaglobulinemia (IgG3 and IgG4), and infections are a common cause of morbidity and mortality among these patients [1]. Immunodeficiency in patients with CLL is complex and multifactorial involving defects in immunoglobulin secretion, T cell functions, complement cascade and neutrophil functions. Very few clinical studies have focused on strategies to overcome the humoral immunodeficiency associated with CLL [2]. Prophylactic antimicrobial therapy and/or intravenous immunoglobulins are often administered to patients with CLL who develop repeated infections. In patients requiring treatment, chemoimmunotherapy further compromises the immune system by decreasing T cell counts and worsening hypogammaglobulinemia. Thus there is a need for strategies which could correct the hypogammaglobulinemia and improve immune reconstitution in patients with CLL.

Streptococcus pneumoniae, *Haemophilus influenzae* type b (Hib) and influenza viruses are common causes of infections in patients with CLL [3]. Vaccinations for influenza and pneumococcus are recommended, but their effectiveness due to decreased antibody response to immunizations is questionable [4]. A prior study from our center [5] has shown that a 23-valent polysaccharide vaccine against pneumococcus does not mount an effective antibody response in 75% of patients with CLL even when multiple doses of GM-CSF are used concomitantly. Hypogammaglobulinemia is likely to further contribute to impaired vaccination responses in these patients [6]. Conjugation with immune adjuvants (like ranitidine) and conjugate vaccines with high valency were found helpful in mounting an effective antibody response to *Haemophilus influenzae* type B, but did not have an effect in the response to Influenza immunization [7],[8]. Thus immune strategies which could circumvent these issues are needed.

Lenalidomide is an immunomodulatory agent related to thalidomide. This oral agent induces clinical responses in patients with CLL. Its mode of action is pleiotropic, affecting the bone marrow and tumor microenvironments, angiogenesis, T cell immune synapse dysfunction, antibody production, regulatory T cells numbers and function and other unknown mechanisms. When patients with CLL received lenalidomide as initial treatment of their disease, improvement in serum levels of immunoglobulins (IgG, IgA and IgM) was observed in a subset of patients [9]. We demonstrated that out of 16 patients who had low IgG level (<700 mg/dL) before therapy, 8 patients (50%) showed a normalization of IgG after 15 cycles and another 3 (19%) showed a rise in IgG of >50% from that of baseline. [9]. In a parallel study from the same patient cohort, we showed that treatment with lenalidomide was also followed by normalization of T cell number

and function [10]. Lapalombella and colleagues have also shown that lenalidomide can enhance CD154 expression on CLL cells and enhance antibody producing ability of B cells in vitro and ex vivo further supporting our hypothesis that lenalidomide could have a positive immunomodulatory effect in patients with CLL [11].

Therefore, we propose to conduct a single arm prospective clinical trial to assess the efficacy of lenalidomide in improving immunoglobulin levels and to potentiate antibody responses to immunizations with influenza and pneumococcal vaccination in patients with CLL.

A low dose of lenalidomide, 5 mg, will be administered three times a week with the plan to escalate to daily dosing in the case of no response. The rationale for the low dose is the potential to obtain an immunoresponse while avoiding the risk for myelosuppression. In our previous experience increase in immunoglobulin levels were seen in patients receiving lenalidomide, 5 mg every-other-day.

3.0 Treatment Plan

We will administer lenalidomide at the dose of 5 mg/day on Monday, Wednesday and Friday for 3 months. If IgG levels improve by at least 25% of baseline, we will continue lenalidomide administration 3 months on and 3 months off for 2 years. If IgG levels do not improve we will increase the frequency of lenalidomide to 5 mg/day for additional 3 months and if response is achieved, lenalidomide will be continued at 5mg/day 3 month on/3 month off for a total of 2 years. Seasonal influenza vaccination (*Trivalent Influenza Vaccine, Fluarix, quadrivalent*) will be administered yearly, during the fall/winter season and pneumococcal immunization (Pneumococcal Polysaccharide Vaccine, Pneumovax)

will be administered once between month 6 and month 21. Patients, who have received the Pneumovax vaccine within last 5 years, will not receive Pneumovax vaccination.

Serum levels of all the immunoglobulin subclasses will be measured at baseline and every 3 months (+/- 3 weeks) during therapy. Serum levels of antipneumococcal capsular polysaccharide antibodies will be measured before, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after pneumococcal vaccination and at end of the study (to assess sustainable antibody responses) by ELISA. Haemagglutination inhibition antibody titres (HAI) will be assessed by WHO influenza reagent kit, before vaccination, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after influenza vaccination [12]. Influenza specific quantitative and qualitative CD8+ and CD4+ T cell responses will be determined by multiparameter flow cytometry technique from stored PBMC's from the patients before vaccination, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after influenza vaccination and at the end of the study [13, 14].

Dosage form

Lenalidomide will be supplied by Celgene Corporation as 2.5mg and 5mg capsules for oral administration.

Prescribing Information

Lenalidomide (Revlimid®) will be provided to research subjects for the duration of their participation in this trial at no charge to them or their insurance providers. 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.

Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle.

Pregnancy Testing

Must follow pregnancy testing requirements as outlined in the Revlimid REMS® prDose modification for lenalidomide:

Table 1: Dose Modification Guidelines for Lenalidomide

NCI CTC Toxicity Grade	Action
Grade 3 neutropenia associated with fever (temperature >38.5o C) or Grade 4 neutropenia (ANC <500/mm ³ , or ≤75% of baseline)*.	1) Hold (interrupt dose). 2) Follow CBC weekly until resolution or stabilization 3) If neutropenia has resolved to ≤ grade 2, implement dose reduction and continue therapy.
Thrombocytopenia Grade 4 (platelet count ≤ 25,000/mm ³) or ≤ 75% of baseline.*	1) Hold (interrupt dose). 2) Follow CBC weekly until resolution or stabilization 3) If thrombocytopenia resolves to ≤ grade 2, implement dose reduction and continue therapy.
Non-blistering rash Grade 3	1) If grade 3, hold (interrupt) dose. Follow weekly until resolution or stabilization. 2) If the toxicity resolves to ≤ grade 1, implement dose reduction and continue therapy.
Grade 4	1) Discontinue lenalidomide study drug.
Desquamating (blistering) rash-any Grade	Discontinue lenalidomide study drug.
Erythema multiforme - Grade 3	Discontinue lenalidomide study drug.
Neuropathy Grade 3	1) If grade 3, hold (interrupt) dose. Follow weekly until resolution or stabilization. 2) If the toxicity resolves to ≤ grade 2, implement dose reduction and continue therapy.
Grade 4	Discontinue lenalidomide study drug.
Sinus bradycardia/other cardiac arrhythmia Grade 2	1) Hold (interrupt dose). Follow at least weekly until resolution or stabilization. 2) If the toxicity resolves to <grade 1, implement dose reduction and continue therapy.
≥ Grade 3	Discontinue lenalidomide study drug.

<p>Allergic reaction or hypersensitivity Grade 3</p> <p>Grade 4</p>	<p>1) Hold (interrupt dose). Follow at least weekly until resolution or stabilization 2) If the toxicity resolves to <grade 1, implement dose reduction and continue therapy.</p> <p>Discontinue lenalidomide study drug.</p>
<p>Venous thrombosis/embolism ≥Grade 3</p>	<p>Hold (interrupt) dose and start anticoagulation; restart at investigator's discretion (maintain dose level).</p>
<p>Hepatic or other non-hematologic toxicity assessed as lenalidomide-related ≥ Grade 3</p>	<p>1) Hold (interrupt) dose. Follow at least weekly until resolution or stabilization. 2) If the toxicity resolves to <grade 2, implement dose reduction and continue therapy.</p>
<p>Tumor flare refractory to oral pain meds</p>	<p>Hold dose and differentiate tumor flare from progression. Restart therapy at the investigator's discretion.</p>

*The use of cytokine support and/or transfusions to maintain adequate blood neutrophil and platelet counts may be considered at the investigator's discretion.

Dose reduction consists of 2.5 mg/daily.

Anticoagulation Consideration

Lenalidomide increases the risk of thrombotic events in patients who are at high risk or who have a history of thrombosis in particular when combined with other drugs known to cause thrombosis. When lenalidomide is combined with other agents such as steroids, e.g. dexamethasone and prednisone, and Adriamycin or daunorubicin the risk of thrombosis is increased. Treating physicians may consider the use of aspirin or low molecular weight heparin in patients at high risk for thrombotic events.

Recommended Concomitant Therapy

Subjects should receive full supportive care, including transfusions of blood and blood products, antibiotics, and antiemetics when appropriate. Use of filgrastim (G-CSF), to

treat for neutropenia is permitted while on study. Use of erythropoiesis stimulating agent is strongly discouraged.

4.0 Patient Eligibility

Inclusion criteria:

1. Chronic lymphocytic leukemia (CLL) patients with IgG less than 500 mg/dl with/without symptoms who are either untreated or previously treated, regardless of response, at least 6 months from prior therapy (including mAb).
2. ECOG/WHO performance status of 0-2.
3. Adequate renal functions as indicated by serum creatinine ≤ 2 mg/dl.
4. Adequate hepatic function indicated as total bilirubin ≤ 2 mg/dl and ALT \leq two times the upper limit of normal.
5. Disease free of prior malignancies for 3 years with exception of currently treated basal cell, squamous cell carcinoma of the skin, or carcinoma "in situ" of the cervix or breast. Patients with malignancies with indolent behavior such as prostate cancer treated with radiation or surgery can be enrolled in the study as long as they have a reasonable expectation to have been cured with the treatment modality received.
6. Females of childbearing potential (FCBP). A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has not had menses at any time in the preceding 24 consecutive months).

7. FCBP must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL within 10 – 14 days prior to and again within 24 hours of starting lenalidomide and must either commit to continued abstinence from heterosexual intercourse or begin **TWO** acceptable methods of birth control, one highly effective method and one additional effective method **AT THE SAME TIME**, at least 28 days before she starts taking lenalidomide.
8. FCBP must also agree to ongoing pregnancy testing (weekly for the first four weeks and then every 28 days while on therapy and at discontinuation of treatment).
9. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy. All patients must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure.
10. Patients must be 18 years of age or older.
11. All study participants must be registered into the mandatory Revlimid® program, and be willing and able to comply with the requirements of Revlimid®.
12. Females of reproductive potential must adhere to the scheduled pregnancy testing as required in the Revlimid REMS® program. Able to take aspirin (81 or 325 mg) daily as prophylactic anticoagulation (patients intolerant to ASA may use warfarin or low molecular weight heparin).
- 13.

Exclusion criteria

1. Known sensitivity to lenalidomide or other thalidomide derivatives.

2. History of Guillain-Barre within 6 weeks of previous influenza vaccination
3. Patient on steroids therapy.
4. Documented polymphocytic leukemia (polymphocytes more than 55% in the blood) or Richter's transformation.
5. Known positivity for HIV or active hepatitis (B or C).
6. Pregnant or breast feeding females.
7. History of tuberculosis treated within the last five years or recent exposure to tuberculosis.
8. Any serious medical condition, laboratory abnormality, or psychiatric illness that places the subject at unacceptable risk if he/she were to participate in the study.
9. Patients with a recent history of deep vein thrombosis (DVT) or pulmonary embolus (PE), in six months prior to enrollment are not eligible for this study.
10. Subjects who have currently active hepatic or biliary disease (with exception of patients with Gilbert's syndrome).
11. Patients with severe allergic reaction (e.g., anaphylaxis) after previous dose of any influenza vaccine or to a vaccine component, including egg protein.
12. Moderate or severe acute illness with or without fever.
13. Use of any other experimental drug or therapy within 28 days of baseline.
14. Concurrent use of other anti-cancer agents or treatments.

5.0 Pretreatment evaluation

Within 7 days (+/- 4 days) prior to start of treatment, patients should have a history and physical examination. Electrocardiogram (ECG), CBC with differential and serum chemistry tests (sodium, potassium, calcium, magnesium, phosphorus, BUN, creatinine,

glucose, and albumin, total proteins, alkaline phosphatase, total bilirubin, ALT, uric acid). Serum beta-2-microglobulin and IgA, IgG, IgM levels. Serum levels of all the immunoglobulin subclasses will be measured at the baseline (+/- 4 days). T-cell lymphocyte number and T-cell subsets, CD4+, CD8+ will be measured baseline. Serum samples for cytokine levels (properdin, IFN- γ , IL-10, TNF- α , BAFF, IL-8, CCL3, and CCL4) will be stored at baseline. Pregnancy tests for females of childbearing potential and Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods will be discussed.. .

6.0 Evaluation during the Study

Complete blood counts (white blood cell, hemoglobin, platelets and differential), serum chemistry, basic metabolic profile, (sodium, potassium, chloride, CO₂, BUN, creatinine and glucose) will be monitored once every two-four weeks (+/- 4 days) while on lenalidomide.

At 3 months (+/- 3 weeks), patients will have a physical examination, CBC with differential and chemical survey, serum beta-2-microglobulin and IgA, IgG, IgM levels, flow cytometry (CD4+, CD8+). Pregnancy tests for females of childbearing potential and Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods, will be discussed with the patients.

Pneumococcal vaccination: Serum levels of antipneumococcal capsular polysaccharide antibodies will be measured before, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after vaccination and at the end of the study (to assess sustainable antibody responses) by ELISA. Influenza vaccination: Haemagglutination inhibition antibody titres (HAI) will be assessed by WHO influenza reagent kit before, 4 weeks (+/- 2 week) and 3 months

(+/- 3 weeks) after vaccination and at end of the study (to assess sustainable antibody responses) [12]. Influenza specific quantitative and qualitative CD8+ and CD4+ T cell responses will be determined by multiparameter flow cytometry techniques from stored PBMC's from the patients before vaccination, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after vaccination [13, 14]. Serum samples for cytokine levels (properdin, IFN- γ , IL-10, TNF- α , BAFF, IL-8, CCL3, and CCL4) will be stored before influenza vaccination at 4 weeks (+/- 2 week), 3 months (+/- 3 weeks) and at the end of the study). PBMC's will be stored at baseline, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after influenza vaccination to determine by multiparameter flow cytometry technique changes in influenza specific quantitative (using HLA-A2 restricted FluMP pentamer) and qualitative (using CD107a, IFN-gamma, IL-2 and TNF-alpha by intracellular staining) CD8+ and CD4+ T cell responses, as described [15].

Criteria for study discontinuation:

Treatment with study drug is discontinued and the patient will be removed from study when any of the following occurs:

1. Lack of response
2. Adverse event(s) that in the judgment of the investigator or the patient may cause severe or permanent harm or which rule out continuation of the study drug
3. Withdrawal of consent
4. Lost to follow-up
5. Suspected pregnancy

7.0 Evaluation of Toxicity

Adverse events are reported as per UTMDACC and Leukemia Phase 2-3 studies (Appendix I and Appendix D). Please refer to lenalidomide package insert for a complete list of side effects.

Myelosuppression and associated complications are expected events during leukemia therapy and are part of the treatment success (marrow emptying of leukemic cells). Therefore, myelosuppression and associated complications such as fever, infections, bleeding and related hospitalizations will not be reported as individual ADRs, but will be summarized in the updated and final reports. Only prolonged myelosuppression, as defined by the new NCI criteria specific for leukemia, i.e., marrow cellularity <5% on day 42 or later (6 weeks) from start of therapy without evidence of leukemia, will be reported as an ADR and considered in defining the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of particular agents or regimens.

M. D. Anderson (Sponsor) Reporting Requirements for Serious Adverse Events (SAE) and Dose Limiting Toxicities:

Serious Adverse Event (SAE) Definition

A serious adverse event is one that at any dose (including overdose):

- Results in death
- Is life-threatening¹
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity²
- Is a congenital anomaly or birth defect

- Is an important medical event³
- Suspected positive Pregnancy

¹ “Life-threatening” means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

² “Persistent or significant disability or incapacity” means that there is a substantial disruption of a person’s ability to carry out normal life functions.

³ Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

Adverse Drug Reaction Reporting

As Per UTMDACC and Leukemia Phase II-III Studies (Appendix I and D), toxicity will be scored using CTC Version 4.0 for toxicity and adverse event reporting according to the M.D. Anderson guidelines. Hematologic toxicity will be assessed, graded, and summarized according to the 2008 IWCLL Guidelines (See Table IWCLL Guidelines). Adverse events will be documented in the medical record and entered into the case report form according to the Leukemia-Specific Adverse Event Recording and Reporting Guidelines (Appendix D). The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

2008 IWCLL Grading for Hematological Toxicity Grade	Decrease in PLT* or HGB** (nadir) from pretreatment value, %	Absolute neutrophil count (ANC)/l*** (nadir)
0	10%	2000
1	11 – 24%	1500 – < 2000
2	25 – 49%	1000 – < 1500
3	50 – 74%	500 – < 1000
4	75%	< 500

Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

* PLT counts must be below normal levels for grades 1-4. If, at any level of decrease, the PLT count is < 20K/l, this will be considered grade 4 toxicity, unless there was severe or life-threatening low initial PLT count (< 20K/l) pretreatment, in which case the patient is not evaluable for toxicity referable to PLT count.

** HGB levels must be below normal levels for grades 1-4. Baseline and subsequent HGB determinations must be performed before any given transfusions.

*** If the ANC reaches <1000/l, it should be judged to be grade 3 toxicity. If the ANC was <1000/l before therapy, the patient is not evaluable for toxicity referable to the ANC.

If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Events Reporting: The principal investigator has the obligation to report all serious adverse events to the University of Texas M. D. Anderson Cancer Center (MDACC) IRB via the Office of Protocol Research within 24 hours.

All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 24 hours of knowledge regardless of the attribution. SAEs beyond 4 weeks after the end of study drug administration will be reported if thought to be drug related.

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-XX-PI-###) 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.

Pregnancies

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.

Male Subjects

If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking lenalidomide should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

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

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), if available. 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-CLL-PI-002544**) 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
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8.0 Criteria for Response

Response criteria -

Ig response – Only IgG levels will be considered for response. IgG levels will be measured as mg/dL by nephelometry (normal range 700-1,600 mg/dL). Response will consist of normalization or improvement by 25% of baseline levels at 6 months during therapy.

Response criteria for the immunization –

Quadrivalent Influenza Vaccine (Fluvarix). HI assay was performed as previously described. Samples were assayed at baseline, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) post-vaccination. Response or protection to influenza vaccination was defined as > 40 HU or a fourfold increase in HI titer at 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) post vaccination, against any of the three strains.

Assessment of the functionality of antigen-specific CD8+ and CD4+ T cells will be performed by intracellular cytokine staining for interferon (INF)-g and tumor necrosis factor (TNF)-a and evaluation of CD154 expression and CD107a cytotoxicity. In brief, PBMCs will be thawed and stimulated for 24 hours with or without the seasonal

influenza vaccine [A/Brisbane/59/2007(H1N1)-, A/Brisbane/10/2007 (H3N2)- and B/Florida/30/2008-like strain; CSL Biotherapies, Hattersheim, Germany]. A response will be considered positive if the % of antigen-specific IFN- γ -, TNF- α -, or CD107a-expressing T cells is ≥ 2 -fold higher than background (unstimulated PBMCs) and if there is a minimum of 0.05% antigen-specific T cells (after subtracting the background or FMO fluorescence minus one).

Pneumococcal Polysaccharide Vaccine - Seroconversion will be defined as at least a two fold increase in levels of antipneumococcal capsular polysaccharide (PCP) IgG antibody or achievement of concentration of $>0.5\mu\text{g/ml}$ in anti-pneumococcal capsular polysaccharide antibody titer (against 6 serotypes). They will be measured before, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) post vaccination using ELISA and at the end of the study.

Exploratory Endpoints:

1. Infection rates of influenza or Pneumococcal pneumonia will be recorded during the study.
2. Sustained immunoglobulin responses will be recorded along with serological responses to both the vaccines.
3. Sera will be analysed for cytokine levels and properdin levels.

Correlative studies –

Cytokine levels from stored serum samples (before, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) and at the end of the study) – properdin, IFN- γ , IL-10, TNF- α , BAFF, IL-8, CCL3, and CCL4. Influenza specific quantitative (using HLA-A2

restricted FluMP pentamer) and qualitative (using CD107a, IFN-gamma, IL-2 and TNF-alpha by intracellular staining) CD8+ and CD4+ T cell responses will be determined by multiparameter flow cytometry technique from stored PBMC's before, 4 weeks (+/- 2 week) and 3 months (+/- 3 weeks) after influenza vaccination as described [15].

9.0 Statistical design with exploratory endpoints –

The primary efficacy endpoint is IgG response, defined as having improvement in IgG level by at least 25% at 6 months, compared to baseline. We are targeting an IgG response rate of 50% and we would not be interested in pursuing further if the IgG response rate is 20% or lower. Using Simon's optimal two-stage design, we will enroll 12 patients in the first stage and the trial will be stopped if there are 3 or less IgG responders among the first 12 patients. If there are 4 or more responders, we will continue the enrollment until 31 patients are reached. The effect of lenalidomide is demonstrated and worth pursuing further if among the total 31 patients, at least 10 patients achieve IgG response. This design is based on a type I error rate of 0.025 and a 90% power. Under the null hypothesis, the probability of early termination is 79% and the expected sample size is 16 patients. Patient enrollment will be suspended if there are not at least 3 IgG responders in the first 12 patients and not all 12 patients have been evaluated at 6 months. To account for a potential 10% early drop-out rate, we will enroll 35 patients so as to have 31 patients with both baseline and 6-month evaluations on IgG. Potential reasons for early dropouts are patients' choice, inability to return to our center or changes in CLL status that may require a different intervention. Sensitivity

analysis will be performed to assess the impact of early dropouts. For example, we will analyze the data counting early dropouts either as "failures" (the most conservative approach) or as "missing" (thus, excluded from the analysis) and evaluate the response rate in both scenarios. Also, since the treatment requires at least 3 months of therapy before seeing an effect on immunoglobulin levels, we will also perform a secondary analysis by including only those patients who have been treated for at least 3 months, while counting those early dropouts between 3 and 6 months as "failures".

Lenalidomide-related toxicity (defined as any grade 3 or greater non-hematological toxicity) will be monitored using the method of Thall, Simon and Estey [16] and the study will be terminated early if toxicity occurs in more than 40% of patients. Specifically, denoting the probability of toxicity by p , the study will be stopped early if $\text{Prob}(p > 0.40 \text{ data}) > .95$. The above decision criterion will be applied in cohort size of 5. Assuming a beta (.4, .6) priori for p , the above decision criterion implies that we will stop the trial according to the table below. For example, the trial will be terminated if 4 or more patients experienced toxicities among the first 5 patients.

Number of patients	Number of patients with toxicities is at least
5	4
10	7
15	10
20	12
25	15
30	17

The operating characteristics of this study design based on 10,000 simulations are illustrated in Table 2.

Table 2. Operating Characteristics for Toxicity Monitoring Rule.

True toxicity rate	Prob (stop early)	Sample Size 25 th , 50 th and 75 th percentiles
0.20	0.006	35, 35, 35
0.30	0.039	35, 35, 35
0.40	0.152	35, 35, 35
0.50	0.447	10, 35, 35
0.60	0.808	5, 15, 30

For the primary analysis, we will estimate the rate of IgG response along with the 95% confidence interval. As secondary analyses, we will also estimate the rates of Pneumococcus and Influenza seroconversion response, as defined in the response criteria section, and the corresponding 95% confidence intervals. The study will be considered positive in regard to the secondary endpoints if seroconversion is observed in 60% or more of the subjects for at least one of the vaccinations given. Similar method will be used to estimate the rate of influenza or Pneumococcal pneumonia. The duration of IgG response will be estimated using the Kaplan-Meier method. The cytokine levels and serum properdin levels will be summarized using descriptive statistics and will be compared between IgG responders and non-responders using two-sample t-test or Wilcoxon rank-sum test, as appropriate.

Amendments, Deviations, Regulatory

Protocol Amendments

Any amendment to this protocol must be agreed to by the Principal Investigator and reviewed by Celgene. Amendments should only be submitted to IRB after consideration of Celgene review. Written verification of IRB approval will be obtained before any amendment is implemented.

Protocol Deviations

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify the IRB in writing of such deviation from protocol.

Investigator Responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

The Investigator will permit study-related monitoring visits and audits by MDACC, Celgene or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, and TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each visit and be made available to MDACC and the Celgene representative so that the accuracy and completeness may be checked.

Institutional Review Board/Ethics Committee Approval

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study. The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any amendments to the protocol after receipt of IRB approval must be submitted by the Investigator to the IRB for approval. The Investigator is also responsible for notifying the IRB of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB prior to use.

Informed consent

The Investigator must obtain informed consent of a subject or his/her designee prior to any study related procedures as per GCPs as set forth in the CFR and ICH guidelines.

Documentation that informed consent occurred prior to the subject's entry into the study and the informed consent process should be recorded in the subject's source documents.

Study records requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.]) be retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

Premature Discontinuation of Study

Single Center

The responsible local clinical Investigator as well as Celgene has the right to discontinue this study at any time for reasonable medical or administrative reasons in any single center. Possible reasons for termination of the study could be but are not limited to:

1. Unsatisfactory enrollment with respect to quantity or quality.
2. Inaccurate or incomplete data collection.
3. Falsification of records.
4. Failure to adhere to the study protocol.

10.0 References

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