

Abbreviated Title: Anti-KIR (IPH2101) in SMM

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A Phase II Trial of IPH2101 (Anti-KIR) in Smoldering Multiple Myeloma (SMM)

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Drug Name:	IPH2101, anti-KIR
IND Number:	109654—IND withdrawn 01-03-2014
Sponsor:	Ola Landgren, MD

Commercial Agents: None

PRÉCIS

Background:

- Multiple myeloma (MM) is an incurable plasma cell neoplasm with a median survival of 3-4 years.
- Smoldering multiple myeloma (SMM) is a premalignant plasma cell disorder characterized by monoclonal protein ≥ 3 g/dL or bone marrow plasma cells $\geq 10\%$ in the absence of myeloma-related tissue impairment with 51% progression to MM at 5 years.
- Current recommendations do not endorse treatment of SMM with chemotherapy.
- Transplanted Natural Killer (NK) Cells have anti-myeloma activity.
- Anti-KIR (IPH2101) is a monoclonal antibody that facilitates NK cell mediated killing of myeloma cells by blocking inhibitory receptors (KIR) on NK cells.

Objectives:

- To assess the response rate of anti-KIR(IPH2101) in patients with SMM
- To evaluate the toxicity of anti-KIR(IPH2101) in patients with SMM
- To evaluate the pharmacokinetic parameters and biological activity of anti-KIR (IPH2101)

Eligibility:

- A confirmed diagnosis of SMM
- Age greater than or equal to 18 years
- ECOG performance status in the range of 0-1.
- Without serious co-morbidity that would interfere with receipt of anti-KIR(IPH2101)

Design:

- Single-arm Phase II trial of anti-KIR(IPH2101) for patients with SMM.
- All patients will have initial evaluation and confirmation of diagnosis.
- Patients will receive anti-KIR(IPH2101) (1mg/kg) every other month for 6 cycles.
- Patients will have routine blood work with SPEP and immunofixation monthly.
- Pre- and post-treatment bone marrow biopsies will be obtained for confirmation of diagnosis and correlative studies.
- Patients may donate cellular products or tissues as appropriate for research purposes.
- Optimal two-stage phase II design will be employed, initially enrolling 9 patients. If 3 or more have a positive outcome, then a total of 21 patients will be enrolled in this study.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- The primary objective of the study is to assess the response rate¹ of anti-KIR(IPH2101) in patients with SMM.

1.1.2 Secondary Objectives

- To evaluate the toxicity of anti-KIR(IPH2101) in patients with SMM
- To estimate time to progression (refer to Section 3.7.2.1 for definition of progression), time to development of MM, and duration of response to anti-KIR(IPH2101)
- To evaluate pharmacokinetic parameters of the alternate month dosing schedule of anti-KIR(IPH2101)
- To evaluate the biological activity of anti-KIR(IPH2101) on KIR occupancy, NK cell function, and NK cell phenotype.

1.2 BACKGROUND AND RATIONALE

Multiple myeloma (MM) is a neoplasm characterized by the proliferation and accumulation of malignant plasma cells in the bone marrow that lead to the overproduction of monoclonal proteins in the serum or urine, affecting nearly 20,000 people annually.² End-organ damage resulting from this disorder includes hypercalcemia, renal insufficiency, anemia, and lytic bone lesions.³ Myeloma remains incurable, with a median survival of 3-4 years in the United States, although newer therapies appear to be improving survival.⁴⁻⁶ Importantly, two recent studies have proven that all cases of MM are preceded by a premalignant state, monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM), although at this time the biological mechanism of this progression is not understood.^{7,8} Currently, clinicians do not have access to any established markers that reliably predict progression to myeloma in patients with MGUS or SMM.

MGUS is a premalignant plasma cell proliferative disorder that is characterized by elevated monoclonal immunoglobulin (M-protein) < 3 g/dL and bone marrow plasma cells < 10% in the absence of any other plasma cell disorder.³ Epidemiological studies have estimated the prevalence of MGUS as 3.2% in patients older than 50 years; these patients have a 1% annual risk of progression to MM.^{9,10} However, risk factors of M-protein ≥ 1.5 g/dL, non-IgG M-protein, and abnormal serum free-light chain ratio are known to confer a higher rate of progression (58% at 20 years).¹¹ Similar to MGUS, SMM is a precursor condition to MM defined by the clinical parameters of M-protein ≥ 3.0 g/dL or bone marrow plasma cells ≥ 10%. Its risk of progression is higher than that of MGUS; 51% of patients progress to MM within 5 years of diagnosis.¹²

The current standard of care for SMM is close follow-up without treatment until symptomatic MM develops.¹³ Using standard chemotherapy in SMM, early treatment has not been found to delay of progression to active disease and overall survival¹⁴. The first randomized phase III study using novel drugs (lenalidomide/dexamethasone vs. surveillance) in SMM was presented by the Spanish study group at ASH 2009¹⁵. After a median of about 1.5 years of follow-up, their interim

analyses show the following: in the surveillance arm, the progression rate was similar to historical controls, while in the treatment arm, only 2 patients had progressed to MM.

Interestingly, there is emerging evidence to suggest that the innate immune system (natural killer (NK) cells) have anti-MM activity^{16 17 18}. In vitro studies demonstrated that allogeneic (allo) and auto-NK cells have the ability to kill CD138-purified primary MM cells¹⁹. Also in a previous study focusing on allogeneic stem cell transplantation in patients with high-risk hematologic malignancy, KIR-ligand mismatch between donor KIR and patient HLA class I resulted in markedly reduced relapse rates, indicating that KIR may exert a significant control over clinical NK cell-mediated responses²⁰. A more recent study using infused, haplo-identical, T-cell depleted, KIR-ligand mismatched NK cells in conditioned patients with relapsed or refractory MM found evidence of either complete response (absence of M-protein by SPEP and IFE with <5% bone marrow plasma cells) or very good partial response (absence of M-protein by SPEP) in 5 out of 10 patients treated, indicating a promising role for KIR-mediated NK cell activity in clinical responses for patients with myeloma.²¹ In Phase I and II trials, IPH2101 has so far shown to be tolerable and manageable in subjects with AML or MM. Based on the Investigator's Brochure, 4th Edition (February 25, 2010), adverse events evaluated as related to the trial product and reported for more than one subject were: fever/pyrexia, asthenia/fatigue, headache, chills, rash and pruritus. There are no evident signs of IPH2101 inducing AEs related to auto-reactivity (such as skin rash and gastrointestinal symptoms), infusion (such as rash, pruritus, erythema, fatigue, headache, pyrexia) or cytokine release (such as pyrexia, fatigue, malaise, headache) to a degree that has raised any safety concerns. As stated in the Investigator's Brochure, 62 of the 298 AEs reported (20.8%) were evaluated as possibly, probably or definitely related to the trial product. No increase in frequency of AEs after multiple dosing (trials 102 and 103) when compared to that after single dosing (trial 101) is evident. One subject in the 103 trial received 1 dose of 0.075 mg/kg trial product and presented with the SAE acute renal failure nine days later, which led to withdrawal from the trial. As this was judged to be a dose-limiting toxicity, three additional subjects were added at the 0.075 mg/kg dose level, in accordance with the protocol. The acute renal failure and the SAEs related to this event (hyperkalemia, hyperuricaemia and increased blood creatinine at two occasions) were initially evaluated as "definitely" related to the trial product, but later re-evaluated as "probably" related as the subject seemed to be in disease progression. The disease progression could potentially have caused the kidney failure, as this is a common co-morbidity in patients with MM. No further signs of any dose-limiting toxicity have been observed, and higher dose levels are currently being explored in the ongoing trials.

Based on these facts, we are planning a SMM treatment study based on anti-KIR(IPH2101), a fully human IgG4 monoclonal antibody (mAb) that facilitates natural killer (NK) cell-mediated killing of myeloma cells by blocking the interaction of inhibitory killer cell immunoglobulin (Ig)-like receptors (KIR) on NK cells with their human leukocyte antigen-C (HLA-C) ligands on target cells. Anti-KIR(IPH2101) binds specifically, and with high affinity, to specific members of the KIR family of NK cell receptors, namely KIR2DL1, -2 and -3 and 2DS1 and -2. The mAb prevents interactions between KIR and HLA-C and, in a dose-dependent fashion, augments NK cell-mediated killing of human tumor cells that express HLA-C. Knowing that effective activation of NK cells is the net result not only of the blockade of inhibitory signals but also of the binding of activating receptors by their ligands which are over-expressed by some MM tumor cells - but not, or less, by healthy cells - IPH2101 has the potential to enhance an elective cytotoxicity of NK cells against MM tumor cells without affecting healthy tissue. *In vivo* efficacy was demonstrated in a non-obese diabetic-severe combined immunodeficiency (NOD-

SCID) mouse model of NK cell-mediated tumor rejection.²² Similarly, tumor killing was observed in normal mice treated with the murine Anti-Ly49(5E6F(ab')₂) mAb, which is functionally homologous to Anti-KIR and was also used for the mechanism-related GLP-toxicology study. No killing of healthy cells was observed with either antibody.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Diagnosis of SMM will be made in accordance with the clinical diagnostic criteria set forth by the International Myeloma Working Group.³ These criteria include:

- Serum M-protein ≥ 3 g/dl and/or bone marrow plasma cells $\geq 10\%$
- Absence of anemia: Hemoglobin ≥ 10 g/dl
- Absence of renal failure: calculated creatinine clearance (according to MDRD) ≥ 40 ml/min (or alternatively based on standard creatinine level criteria of ≤ 2 mg/dl)
- Absence of hypercalcemia: Calcium ≤ 10.5 mg/dl
- Absence of lytic bone lesion (skeletal survey)
- The diagnoses will be confirmed by serum/urine protein electrophoresis, immunofixation and light-chain assays; as well as immunohistochemical analyses of the bone marrow biopsy.

2.1.1.2 Age greater than or equal to 18 years.

2.1.1.3 ECOG performance status of 0-1.

2.1.1.4 Male or female patient who accepts and is able to use recognized effective contraception (oral contraceptives, IUCD, barrier method of contraception in conjunction with spermicidal jelly) through the study and for four months following the final dose of study drug when relevant.

2.1.1.5 The patient must be competent to sign an informed consent form.

2.1.2 Exclusion Criteria

2.1.2.1 Patients with a diagnosis of MM or a clinical suspicion of an ongoing progression into full-blown MM

2.1.2.2 Patients without measurable disease defined as serum M-protein < 1 g/dL.

2.1.2.3 Previous treatment having a proven or potential impact on myeloma cell proliferation or survival (including conventional chemotherapies, immunomodulatory drugs (IMiDs), or proteasome inhibitors).

2.1.2.4 Use of any investigational agent within the last 3 months.

2.1.2.5 Clinical laboratory values at screening

- Platelet levels $< 75 \times 10^9$ /L
- ANC levels $< 1 \times 10^9$ /L
- Bilirubin levels > 1.5 ULN ; ALT and AST > 3.0 ULN (grade 1 NCI)

2.1.2.6 Primary or associated amyloidosis

2.1.2.7 Known abnormal cardiac status with any of the following:

- NYHA stage III or IV congestive heart failure
- Myocardial infarction within the previous 6 months
- Symptomatic and/or treatment-refractory cardiac arrhythmia. Patients with controlled or asymptomatic arrhythmia are not excluded from this study.

2.1.2.8 Current active infectious disease or positive serology for:

- Human Immunodeficiency Virus (HIV)
- Hepatitis C Virus (HCV)
- Hepatitis B Surface Antigen

2.1.2.9 Severe type of autoimmune disease defined as:

- One which currently requires or previously required long-term systemic immunosuppressive or immunomodulatory therapy (including corticosteroids, administered by systemic route)
- And/or it has a substantial probability to cause an irreversible injury to any tissue (e.g. Hashimoto thyroiditis).
- And/or it is recent or unstable, or has a substantial risk to progress and cause severe complications (e.g., Graves disease)
- Enrollment of other non-severe types of auto-immunes disease requiring topical therapy, or NSAIDS can be considered on a case by case basis by the Principal Investigator.

2.1.2.10 History of a lymphoproliferative malignancy.³

2.1.2.11 History of other malignancy (apart from basal cell carcinoma of the skin or in situ cervical carcinoma) except if the patient has been free of symptoms and without active therapy during at least the previous 5 years.

2.1.2.12 Serious concurrent uncontrolled medical disorder.

2.1.2.13 History of allograft or solid organ transplantation.

2.1.2.14 Any psychological or familial condition potentially interfering with compliance with the study protocol and follow-up schedule.

2.1.2.15 Pregnant or lactating women.

2.2 SCREENING EVALUATION

2.2.1 Clinical Evaluation

2.2.1.1 A complete history and physical examination with documentation of measurable disease (if any) and assessment of performance status using the ECOG scale must be performed prior to study entry.

2.2.1.2 The following laboratory tests will be completed prior to study entry:

- CBC with differential and reticulocyte count
 - If Hgb <10 g/dL, will follow with serum folate, vitamin B12, and iron studies.

- Chem 14 (metabolic panel that includes Calcium and Creatinine)
- Lactate Dehydrogenase level
- Uric Acid
- Quantitative immunoglobulin levels
- C-Reactive Peptide levels
- Viral serologies
 - HIV enzyme-linked immuno-assay (ELISA)
 - If positive, will follow with Western Blot
 - Hepatitis B surface antigen
 - Anti-Hepatitis C (HCV) antibody
 - If positive, will follow with HCV RNA PCR
- Review of bone marrow core biopsy and aspirate by NCI Department of Pathology
- Urine pregnancy test in women of child-bearing potential.

2.2.1.3 A skeletal survey of the axial and appendicular skeleton will be performed. Exception may be made if skeletal survey has been performed within the past 6 months and was found to be negative. In this case, films will be forwarded to the Clinical Center for an additional reading by the Department of Radiology.

2.2.2 Laboratory Tests

2.2.2.1 The following laboratory tests will be performed prior to start on study:

- Serum protein electrophoresis (SPEP) and immunofixation to assess for presence and quantity of monoclonal protein (M-protein)
- Urine protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria)
- Serum free light-chain studies, determined using the Freelite™ assay system
- Prior to treatment, patients will undergo high resolution HLA typing including the HLA Cw loci to define their KIR-L expression (i.e. to define the presence or absence of group I (binds KIR2DL2/3) and group II (binds KIR2DL1) KIR ligands).

2.2.2.2 The following laboratory tests will be performed within one month of study entry:

- Immunophenotyping of aberrant plasma cells by flow cytometry currently involves, but is not limited to, the use of the following reagents: CD138, CD19, CD45, CD38, and CD56. Characteristic changes in immunophenotypically abnormal plasma cells (CD138 positive) include but are not limited to absent CD19 and CD45, decreased CD38, and increased CD56.^{23,24} These studies will be performed under the direction of Maryalice Stetler-Stevenson of the flow cytometry unit in the NCI Laboratory of Pathology.
- Interphase FISH cytogenetics will be performed on patients enrolled in this protocol under the direction of Ana Roschke of the Molecular Cytogenetics Cor Laboratory of the NCI Genetics Branch.
- Samples for gene expression profiling (GEP) and array comparative genomic hybridization (aCGH) of CH138 positive plasma cells will be collected, batched, and entered into a biobank.
- Blood samples will be collected at baseline and after IPH2101 treatment for flow cytometric based functional assays to calculate the percentage change in NK cell

cytotoxic granule release and NK cell intracellular cytokine secretion against KIR-Ligand matched myeloma cells. See section **6.1.4**.

- Bone marrow aspirates will be analyzed by multiparameter flow cytometry pre- and anti-KIR (IPH2101) for the impact on cells of the bone marrow microenvironment including but not limited to dendritic cell subsets, macrophages and stromal cells. Expression of NK cell markers will also be analyzed pre- and post-therapy.

2.2.3 Novel Imaging Studies

2.2.3.1 There are no planned novel imaging studies for this protocol.

2.2.3.2 Imaging studies such as PET-CT or MRI will be used as clinically indicated.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

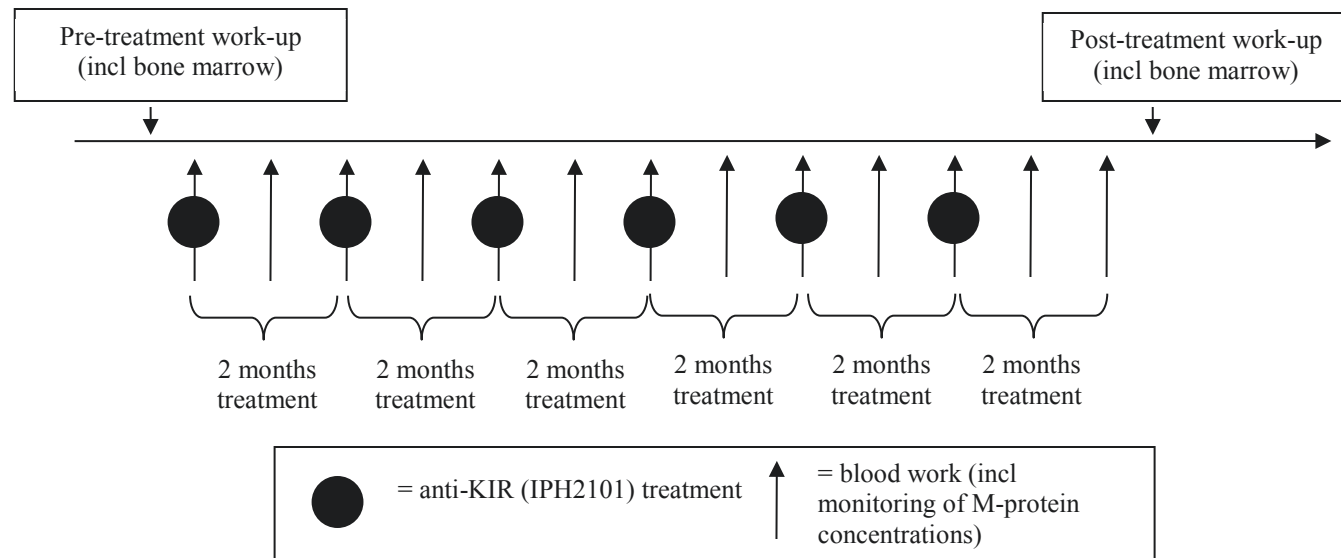
3.1 STUDY DESIGN

Patients with SMM will enroll in the study for the purpose of determining if the use of the monoclonal anti-KIR (IPH2101) will result in a sufficiently large fraction of patients with SMM who will experience a decline of 50% or more in their monoclonal immunoglobulin levels as measured at up to 12 months after their initial baseline M-protein level determination.

This study will be conducted as a single-arm phase II trial. Patients will receive one infusion of anti-KIR (IPH2101) 1mg/kg by intravenous route over 1 hour every other month for 6 cycles. For the purposes of evaluation, a cycle will be defined as 2 months. Patients will have monthly evaluation of M-protein by SPEP and immunofixation. Additionally, bone marrow biopsy will be performed prior to treatment and after treatment is completed. **Figure 1** shows the study design schematically.

If a patient has more than 25% reduction in M-spike (minimal response criteria for multiple myeloma) in combination with concomitant decreased disease burden in bone marrow biopsy after 6 cycles, the patient may receive a second year of treatment at the discretion of the investigator. The same dosing schedule would be implemented (6 cycles of anti-KIR (IPH2101) (1mg/kg) every other month) with the same monitoring including a bone marrow biopsy after treatment is completed.

Figure 1:



Remark: If a patient has more than 25% reduction in M-spike (minimal response criteria for multiple myeloma) in combination with concomitant decreased disease burden in bone marrow biopsy after 6 cycles, the patient may receive a second year of treatment with the same dosing schedule at the discretion of the investigator (See Section 3.1 above).

3.2 CLINICAL STUDIES

3.2.1 Routine Evaluations

3.2.1.1 Studies that will be performed at each visit, which will occur at monthly intervals following enrollment or as clinically indicated. These include:

- History and Physical Examination
- Evaluation of Performance Status using the ECOG scale
- Routine laboratory studies including:
 - CBC with differential of WBCs and platelet count
 - Reticulocyte count
 - Chem 14 Metabolic Panel (Na, K, Cl, CO₂, Cr, glucose, BUN, albumin, Ca, Alk Phos, ALT/AST, total and direct bilirubin, total protein)
 - Lactate Dehydrogenase level
 - Uric Acid
 - SPEP with immunofixation
 - Urine protein electrophoresis (UPEP)
 - Serum free light-chains with quantitative serum immunoglobulins
 - Research samples, as described below in section 3.2.2.

Review of available medical records will take place at the pre-study visit.

3.2.2 Research Evaluations

Research samples may be obtained for correlative research studies and will be obtained in strict compliance with clinical center guidelines. Samples may be obtained at the time of diagnostic

procedures. Procedures may also be performed for research studies only, as long as they constitute minimal risk to the patient.

3.2.2.1 Venipuncture:

Up to 100cc of peripheral blood will be collected into heparinized tubes. The amount of blood collected will be dictated by the number of experiments to be performed, and by the patient's peripheral blood count.

3.2.2.2 Urine Sample Collection:

Approximately 45 mL of urine will be collected into a standard urine collection cup for further analysis. The amount of urine collected will be dictated by the number of experiments to be performed.

3.2.2.3 Bone Marrow Aspiration and Biopsy:

For the purpose of analyzing CD138 positive and CD138 negative cells from the bone marrow, two bone marrow aspirates and core biopsies will be collected, one prior to treatment and one subsequent to treatment. 3 to 5cc of marrow per aspirate will be collected into sterile, heparinized tubes. Core biopsies will be collected into a core cylinder. Core biopsies and one fraction of the aspirate samples will be fixed and paraffin-embedded for histological/immunohistochemical analysis and long-term storage. One fraction of marrow aspirates will be stored as air-dried aspirate smears and the rest will be frozen. CD138 positive plasma cells will be isolated from a subset of these samples (see [APPENDIX A](#)).

3.3 DRUG ADMINISTRATION

3.3.1 Anti-KIR (IPH2101): One infusion of IPH2101 (Innate Pharma) 1mg/kg by intravenous route over 1 hour every other month for 6 cycles. The chosen dose of 1 mg/ kg is slightly above the dose saturating the receptors for a period of at least 1 month. For the purposes of evaluation, a cycle will be defined as 2 months. For patients with more than 25% reduction in M-spike (minimal response criteria for multiple myeloma) in combination with concomitant decreased disease burden in bone marrow biopsy after 6 cycles, the patient may receive a second year of treatment with the same dosing schedule at the discretion of the investigator (6 cycles of anti-KIR (IPH2101) (1 mg/kg) every other month).

3.3.2 Patients will be treated at the Clinical Center as inpatient for the first dose (monitoring and PK blood draws). Subsequent doses may be administered in the Outpatient Day Hospital if the first dose is tolerated without toxicity. Toxicity monitoring for the first dose is as follows: Vital signs: heart rate, blood pressure (diastolic and systolic) and body temperature prior to infusion and every hour until stable, then at 3 and 6 hours post infusion, or more frequently as clinically indicated. Toxicity monitoring for subsequent doses is as follows: Vital signs: heart rate, blood pressure (diastolic and systolic) and body temperature prior to infusion and every hour until stable, then at 3 and 4 hours post infusion, or more frequently as clinically indicated.

3.3.3 An ECG should be performed within 2 hours after the end of infusion.

3.3.4 Concomitant Treatment Permitted:

Patients should receive all necessary supportive care in the form of treatment or prophylaxis as clinically indicated (e.g. transfusion of blood products, antibiotics, anti-histaminic drugs, or analgesics).

Any blood product and/or concomitant medication administered should be recorded in the medical record and clinical research database (C3D), including dose, start, and stop dates.

Prophylactic oral bisphosphonates for non-myeloma indications (such as osteoporosis) are allowed if they were already administered before enrollment and if their dosage is not increased during the study.

3.3.5 Concomitant Treatment NOT Permitted (minimal time interval for stopping concomitant treatments prior to start of treatment on this study is 6 months)

The following concomitant treatments are not permitted during this study and must be stopped before the start of treatment accordingly with their pharmacological effects:

- Any chemotherapy
- Corticosteroids IV or PO administered with the intent of treating plasma cell myeloma, with the exception of substitutive doses of hydrocortisone
- Any other investigational drug(s)
- Any other anti-myeloma treatment including thalidomide, lenalidomide or other immunomodulatory drugs; proteasome inhibitors; IV bisphosphonates
- Cytokines

3.4 DOSE MODIFICATIONS

No individual dose reduction is planned for IPH2101.

Dose reduction of IPH2101 has not yet been explored in previous or still ongoing clinical studies. Lower doses (< 1 mg/kg) may still fully occupy and block the mAb target receptors, KIRs, although more transiently (e.g. slightly less than 1 week at 0.015 mg/kg). Such a dose reduction could likely not prevent, if any, acute toxicity observed within the first 24 hours (or even 48 hours) after IPH2101 administration.

Very low doses, leading to incomplete KIR occupancy, would likely be ineffective, a complete blockade of KIR being required to achieve enhancement of NK cytotoxicity, the expected biological effect of IPH2101.

Treatment delay is to be made according to the body system showing the greatest degree of toxicity. Toxicities will be graded according to the CTCAE version 4.0.

In case of CTCAE grade ≥ 2 toxicity: the first day of a cycle, administration will be delayed by one week and up to two weeks until the toxicity has resolved to CTCAE grade ≤ 1 . Any patient who requires a delay of more than 1 month whatever the reason, will be taken off study.

3.5 STUDY CALENDAR

Study ^a	Pre- Study Visit	Wk 1 Day 1	Wk 1 Day 2	Wk 2, 3, 4	Wk 5, 13, 21, 29, 37, 45	Wk 9, 17, 25, 33, 41	Wk 49 Day 1	Wks 53, 61, 69, 77, 85, 93	Wks 57, 65, 73, 81, 89	Follow Up Visits ^c
History and Physical	X	X			X	X	X	X	X	X
Review of Medical Record	X						X			
Informed Consent	X						X			
Routine Labs (Chem14 metabolic panel, LDH, Uric Acid, CBC/diff) ^b	X	X	X	X	X	X	X	X	X	X
Serum Protein Analysis (SPEP, Immunofixation, Free-Light Chains, Quantitative Immunoglobulins)	X	X			X	X	X	X	X	X
C-Reactive Protein	X						X			
Urine Pregnancy Test	X						X			
Viral Serology (HIV ELISA, anti-HCV, HBV surface antigen)	X									
Urine Protein Analysis (UPEP)	X	X			X	X	X	X	X	X
Research Blood Collection		X	X	X	X	X	X	X	X	X
Research Urine Collection		X	X	X	X	X	X	X	X	X
Bone Marrow Biopsy and Aspirate (with Flow Cytometry)		X ^b								X
Skeletal Survey	X									
Administer Anti-KIR (IPH2101)		X				X	X		X	

- Any of the above studies, excluding administration of anti-KIR (IPH2101) may be performed if clinically indicated.
- May be performed after pre-study visit but before treatment week 1.
- All follow-up visits will be completed by May 1, 2014.

3.6 POST-TREATMENT EVALUATION

Patients who complete treatment will proceed to follow up. The first follow-up visit will be held 2 months after the last dose of anti-KIR and will consist of a physical examination, routine blood tests, collection of research samples and a second bone marrow aspiration and biopsy.

After the first follow-up visit, patients will be followed every 3 to 6 months for two years after the completion of therapy. Thereafter, patients will be followed annually for a total of five years after completion of treatment, or until May 1, 2014, whichever occurs first. At these follow up visits, patients will undergo a physical examination and laboratory testing but will not undergo a bone marrow biopsy unless this is clinically indicated. Adverse events that occur during the follow-up period that are unrelated to study treatment will not be reported.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.7.1 Criteria for removal from protocol therapy

- 3.7.1.1 Patients with grade 3 or 4 adverse events related to anti-KIR (IPH2101) may be taken off therapy at the discretion of the principal investigator or lead associate investigator, but will be followed until treatment related AE resolves or returns to baseline.
- 3.7.1.2 Patient completes protocol therapy as outlined in Section 3.3.
- 3.7.1.3 Worsening of smoldering myeloma as defined by an increase in M-protein of 25% from baseline measurement. The absolute increase in M protein concentration must be ≥ 0.75 gm/dl AND the measurement must be repeated at 2 time points a minimum of 4 weeks apart to document the increase.

3.7.2 Criteria for removal from Study

- 3.7.2.1 Progression of disease to MM or a related lymphoproliferative malignancy requiring treatment. Treatment is indicated when myeloma related end-organ or tissue impairment is clinically evident. Indicators of myeloma related end-organ or tissue impairment are listed in published guidelines³ and comprise:
 - Hypercalcemia defined as serum calcium at least 1 mg/dL above the upper limit of normal with no alternate etiology
 - Renal failure defined calculated creatinine clearance (according to MDRD) <40 ml/min (or alternatively based on standard creatinine level of >1.95 mg/dl) in the absence of another cause of acute kidney injury or chronic kidney disease
 - Anemia, defined as hemoglobin <10 g/dL with iron studies within normal limits and decreased reticulocyte count.
 - Bone lesions defined as lytic lesions noted on skeletal survey.
- 3.7.2.2 Patient non-compliance.
- 3.7.2.3 Patient voluntary withdrawal.
- 3.7.2.4 The principal investigator may take any patient off study if it is determined that this would be in the best interest of the patient.
- 3.7.2.5 Treatment delay: any patient who requires a delay of more than one month, whatever the reason, will be taken off study.
- 3.7.2.6 All patients will be removed from the study as of May 1, 2014.

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and faxed to 301-480-0757.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 SUPPORTIVE CARE

Patients enrolled on this protocol with ECOG performance status 0-1 are not expected to require substantial supportive care. Supportive care will be offered as clinically indicated.

4.2 CONCURRENT THERAPIES

Patients on this protocol are not to receive any concurrent therapies for their diagnosis of Smoldering Myeloma.

4.3 RADIATION THERAPY GUIDELINES

Patients on this protocol are not to receive radiation therapy while on study.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

5.1.1 Data Collection

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

Data will be prospectively collected and entered into the NCI C3D clinical trials database. Adverse events related to either anti-KIR (IPH2101) or research procedures will be collected into the NCI C3D database.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

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

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

5.1.2 Record Keeping

Complete records must be maintained on each patient; these records will consist of the hospital chart as well as any other outside information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses are done. The primary source documentation will include patient eligibility data, patient history, flow sheets (including specialty forms for pathology, radiology, or surgery), an off-study summary sheet, and a final assessment by the treating physician.

5.1.3 Forwarding of Patient Data from Other Institutions

Either due to extenuating medical circumstances or for convenience, some patients may elect to have certain routine laboratory studies or protein marker analyses performed at an outside institution between scheduled interval visits to the CRC for this protocol. These results will be forwarded to the research nurse on the Myeloma Team who will enter the data into the study database. Additional blood or tissue samples drawn on patients enrolled in this protocol between scheduled visits may be forwarded and entered into the database as well.

5.2 RESPONSE CRITERIA

Response criteria for MM have been defined by the International Myeloma Working Group.²⁵ As data is limited and current recommendations are to not treat SMM outside of clinical trials, there are no formal guidelines to define response to therapy in SMM. Similar to the ongoing Spanish Multicenter SMM treatment study¹⁵, we define response criteria based on change in monoclonal antibody levels:

- Stringent Complete Response: absence of M-protein by SPEP and immunofixation as well as <5% bone marrow plasma cells and serum free light-chain ratio within normal limits
- Complete Response: absence of M-protein by SPEP and immunofixation
- Very Good Partial Response: absence of M-protein by SPEP but presence by immunofixation
- Partial Response: 50% reduction in M-protein
- Minor Response: 25% reduction in M-protein.

Progression of smoldering myeloma is defined in Section [3.7.1.3](#).

5.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. Adverse events occurring during the study will be graded according to

the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0)
http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_v40.

6 BIOSPECIMEN COLLECTION

6.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

6.1.1 Biomarker Studies

Peripheral blood and/or urine samples from patients will be analyzed for potential serum or urine biomarkers for disease progression and correlated to clinical outcomes. Such biomarkers may include but are not limited to: monoclonal protein, free immunoglobulin light-chains, circulating proteosomes, and total immunoglobulin levels. Biomarkers will be chosen, in part, in accord with their utility as markers of progression in other, ongoing studies.

6.1.2 Immunophenotyping by Flow Cytometry

Erythrocyte lysed bone marrow aspirate samples from patients will be analyzed by multiparameter flow cytometry to determine the percentage of aberrant (myelomatous) plasma cells. Previously reported aberrant phenotypes are shown in **Table 1** DNA index will also be assessed by flow cytometry of these same samples using a double-staining procedure using propidium iodide (to stain nuclear DNA) and anti-CD38 plus anti-CD138 antibodies.

Table 1: FLOW CYTOMETRY BASED IMMUNOPHENOTYPES OF ABERRANT PLASMA CELLS IN MGUS AND SMM.²⁴

CD45	CD19	CD56	%
-	-	++	50
-	-	-	24
-/↓	-	+	11
-	+	++	8
↓	-	-	5
-	+	-	1
+	↓	++	1

Perez-Persona et. al. *Blood* 2007.²⁴

Instructions for collecting bone marrow specimens for immunophenotyping by flow cytometry are described below in section **6.1.3** and can be found at:

<http://home.ccr.cancer.gov/lop/Clinical/specimencollections.asp#Bone%20Marrow>

6.1.3 Collection of Bone Marrow

Bone marrow samples will be collected as bone marrow core biopsies and aspirates for analyses. Bone marrow core biopsies and one fraction of marrow aspirates will be fixed and paraffin-embedded for histological/immunohistochemical analysis and long-term storage. One fraction of marrow aspirates will be stored as air-dried aspirate smears and the rest will be frozen. CD138 positive plasma cells will be isolated from a subset of these samples. The procedure for sample acquisition is as follows:

- Notify the CCR Hematology lab that flow immunophenotyping is being performed (301-496-4473). The hematology BM collection tech will bring a BM transport media tube to

the specimen collection site and prepare an extra smear for the Flow Cytometry Laboratory.

- Get sterile heparin suitable for injection from the nurse's station.
- Rinse syringe and needle with sterile heparin, leaving no more than 0.2-0.5 mL in syringe.
- After aspirate for morphology, reposition needle and, for cellular specimens, slowly aspirate 2-3 mL of bone marrow for flow cytometry. Aspiration of greater than 3mL results in significant dilution of the specimen in peripheral blood. For specimens with low cellularity, reposition and aspirate 3-5mL of bone marrow a second time. Note this on the requisition or the specimen will be rejected.
- Immediately discharge syringe into transport media tube, cap tube tightly and mix by gentle inversion 5-6 times. Label tube with patient name, unique identifier number and date.
- Deliver immediately to the Flow Cytometry Laboratory B1B58 (specimens containing hematopoietic neoplasms have a tendency to clot and must be processed immediately). Call for STAT Escort pickup and delivery if you cannot deliver the specimen yourself (301-496-9295).
- The Active CRIS order should include the fellow's name and pager number, the patient history and clinical question. This information is vital as to the set-up strategy used. If rapid diagnosis and notification of result is required to begin treatment, indicate STAT nature, explain reason and leave name as well as pager number of fellow on evening call.

6.1.4 Functional Assays for Natural Killer Cells

Blood samples will be collected to calculate the percentage change in NK cell cytotoxic granule release and NK cell intracellular cytokine secretion against KIR-L matched myeloma cells. Prior to treatment, patients will undergo high resolution HLA typing including the HLA Cw loci to define their KIR-L expression (i.e. to define the presence or absence of group I (binds KIR2DL2/3) and group II (binds KIR2DL1) KIR ligands). Blood will be collected into ACD tubes at baseline, 24 hours following IPH2101 treatment, then weekly for the first 4 weeks following IPH2101 treatment. Thereafter blood will be collected monthly until the completion of treatment or until the time of withdrawal from protocol study. PBMCs will be isolated from these blood samples by ficolling then will be frozen viably for subsequent NK cell cytotoxicity, NK cell intracellular cytokine-based flow studies, and FACS analysis. To assess for changes in NK cell cytotoxicity, PBMC will be thawed and NK cell cytotoxic granule release following an 8 hour co-culture with a KIR-ligand matched myeloma tumor cell line will be assessed by flow cytometry, gating on CD3-/ CD56+ and/or CD16+ NK cells that become CD107a positive vs NK cell controls that are not cultured with myeloma cells. To assess for changes in NK cell cytokine release associated with IPH2101 treatment, PBMC will also be thawed and co-cultured for 6 hours with a KIR-ligand matched myeloma tumor cell line. Flow cytometry will be performed 6 hours later, gating on CD3-/ CD56+ and/or CD16+ NK cells to quantitate the percentage of NK cells that become intracellular cytokine positive for either IFN-g, IL-2, or TNF-alpha vs NK cell controls that are not cultured with myeloma cells. The myeloma tumor targets used in these experiments will have group I and/or group II KIR-ligands that are matched to the patient (i.e. if the patient is homozygous for group I KIR-ligands, the myeloma line used in the cytotoxicity assays will also be homozygous for group I KIR-ligands, if the patient is heterozygous for both group I and group II KIR-ligands, the myeloma line used in the cytotoxicity assays will also be

heterozygous for group I and group II KIR-ligands). Using this technique, the percentage change in NK cell cytotoxic granule release and NK cell intracellular cytokine secretion following treatment with IPH2101 at multiple time-points compared to baseline prior to antibody treatment will be determined.

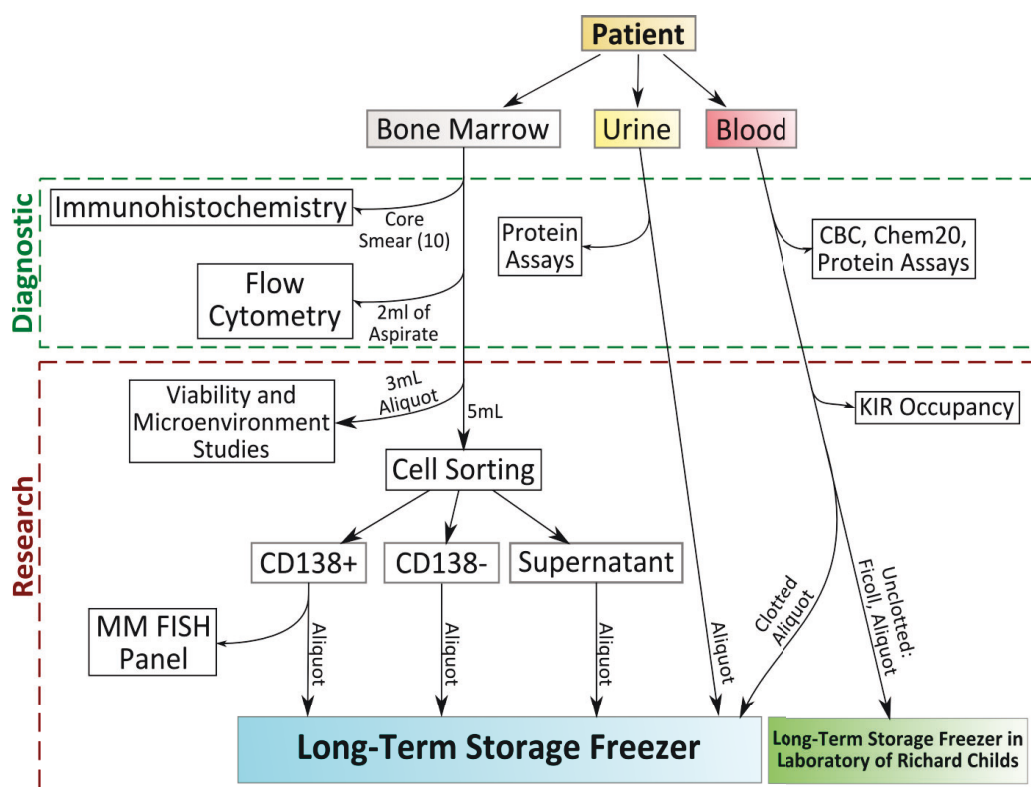
6.1.5 Analysis of Microenvironmental Interactions

The extrinsic interactions of bone marrow stromal cells, angiogenesis, and immunologic factors with CD138 positive plasma cells have been implicated in both the pathogenesis of MM and the mechanism of action of anti-KIR(IPH2101). CD138 negative bone marrow aspirate cells are components of the bone marrow microenvironment. Analysis of the microenvironmental changes in the marrow by methods such as determination of cytokine profiles and immunohistochemistry of matrix proteins, osteoblasts, reticular cells, osteoclasts, and endothelial cells will be performed. The plasma from aspirate and peripheral blood plasma will also be used to determine matrix components of the myeloma microenvironment.

6.1.6 Pharmacodynamic Studies

KIR occupancy will be analyzed on peripheral blood samples by the Trepel Lab at baseline, before each dose and 2 months after the last dose. Testing will be performed using the KIR occupancy flow cytometric assay protocol measuring the binding of a labeled immunofluorescent anti-KIR relative to standard fluorescent beads, developed and provided by Innate Pharma.

Figure 2: Schematic for handling of patient samples for diagnostic and research studies.



6.2 SAMPLE STORAGE, TRACKING, AND DISPOSITION

6.2.1 General

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting patients will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the IRB as soon as he is made aware of such loss.

If the patient withdraws consent his/her data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (e.g., broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6.2.2 Transplantation Immunotherapy Laboratory (Childs Lab)

All BMPC (unclothed) blood samples obtained for this protocol will be stored in the Section of Transplantation Immunotherapy in the Hematology Branch of the NHLBI. PBMCs obtained from peripheral blood samples will be ficoll, then cryopreserved viably in 1 mL aliquots in freezing media containing 10% DMSO by technicians working in the Section of Transplantation Immunotherapy Laboratory headed by Richard Childs, M.D. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:

- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
 - The database resides on a dedicated program server that is kept in a central, locked computer facility.
 - The facility is supported by IT specialists who maintain up to date security features including virus and firewall protection.
 - Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
 - The database sample entry program itself is accessed through a password protected entry screen.

- The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique laboratory accession ID number. All products generated by the laboratory will be stored in the laboratory liquid nitrogen freezers and are identified by this accession ID.
- Inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers.
- Access to stored clinical samples is restricted. Investigators establish sample collections under “Source Codes” and the investigator responsible for the collections, typically the protocol Principal Investigator or Lead Associate Investigator, specifies who has access to the collection. Specific permissions will be required to view, input or withdraw samples from a collection. Prior to that date sample input was not restricted and restrictions were limited to specimen withdrawal.
- Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. The repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (generally the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

6.2.3 Clinical Pharmacology Program (Figg Lab)

Aliquots of clotted blood samples and all urine samples obtained for this protocol will be stored in the Clinical Pharmacology Program (CPP), which will process and cryopreserve samples in support of this study under the direction of William D Figg, PharmD.

For sample pickup, page 102-11964. For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number). For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

6.2.3.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) of the Clinical Pharmacology Program under the direction of Dr. Figg will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password

restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

6.2.3.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in locked freezers at appropriate temperatures (e.g., -20°C to -80°C) according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6.2.4 Procedures for Collecting, Processing, and Storage of Bone Marrow Biopsies

- Orders for bone marrow biopsies should be placed in the Clinical Research Information System (Clinical Research Center, NIH, Bethesda, MD).
- Bone marrow biopsies will be submitted in native condition to the NCI Department of Pathology and handled according to routine procedures for diagnosis. Bone marrow core biopsies will be fixed and paraffin-embedded for histological and immunohistochemical analysis and long-term storage. Bone marrow aspirates will be prepared according to routine procedures. Five to ten air-dried aspirate smears will be stored long-term.
- Materials for research studies will be documented on form NIH 2803-1.
- Initial processing of bone marrow aspirates for research will depend on the size of the aspirate. CD138 positive plasma cells will be isolated from a subset of these samples.
- For the purposes of storage, all research bone marrow samples will be assigned a unique number and cataloged. Viable frozen cells will be stored in a temperature controlled, alarm secured nitrogen tank in the NCI Department of Hematopathology. Frozen bone marrow biopsies and processed biologic material (such as RNA and protein) will be stored at -80°C in a temperature controlled, alarm secured freezer.
- For research purposes, one aliquot of aspirate will be sent to the laboratory of Jane Trepel of the Developmental Therapeutics Branch, phone number (301) 496-1547, prior to sorting. Biospecimens in the Trepel lab will be processed using validated SOPs that will

ensure both specimen quality and patient confidentiality. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. Specimen labels will indicate: protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

- All other research samples except stored serum will be stored in the laboratory of the Lymphoid Malignancies Branch. For information regarding these samples, please contact Mark Roschewski, MD at 301-451-9021.
- Processed biologic material (such as DNA, RNA, and protein) from the bone marrow aspirates will be stored at -80°C in a temperature controlled, alarm secured freezer. All processed bone marrow aspirates will be stored in the laboratory of the Lymphoid Malignancies Branch. For information regarding these samples, please contact Mark Roschewski, MD at 301-451-9021.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in

the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH definition)

Any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
 - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

(b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that research places subjects or others at a greater *risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NIH INTRAMURAL IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the to the NIH Intramural IRB and NCI CD:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NIH Intramural IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
 - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
 - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
 - All Grade 5 events regardless of attribution;
 - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

7.3 FDA REPORTING CRITERIA

NOTE: This section retained for historical purposes; however, note that the IND for this study was withdrawn in 2014.

7.3.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

7.3.1.1 Expedited reporting to the FDA

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The study Sponsor will also report expeditiously as above:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinical important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendar days after receiving the request.

7.3.1.2 Exclusions to expedited reporting to the FDA

The following events will not be reported in an expedited manner but will be included in the annual report:

- pain due to disease;
- thrombus due to vascular access devices;
- events associated with indwelling stents placed for disease management and not part of the protocol;
- events associated with anesthesia that resolve as anticipated.

7.3.2 FDA Annual Reports (Refer to [21 CFR 312.33](#))

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

7.3.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer

During the duration of this trial, the Principal Investigator will assume responsibility for reporting any serious and unexpected adverse events that occur in subjects prior to their off-study date, to Innate Pharmaceuticals Inc. by fax within 24 hours of receipt of the SAE notification to the following contact:

Primary contact: Fax: +33.4.30.30.30.10
Christine D'ARNOUX Email : Christine.D-Arnoux@innate-pharma.fr
Phone: +33.4.30.30.30.89

Secondary contact: Fax: +33.4.30.30.30.10
Renaud BUFFET Email : renaudbuffet@wanadoo.fr
Phone: +33. 6.20.76.74.74

7.4 DATA SAFETY AND MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS (if applicable) and to the IND manufacturer.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by a CCR Contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' will be randomly selected and monitored at least biannually or as needed, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

8.1 SAMPLE SIZE DETERMINATION

The primary objective of this study is to determine if the use of a monoclonal antibody will result in a sufficiently large fraction of patients with smoldering multiple myeloma who will experience a decline of 50% or more in their antibody levels as measured at up to 12 months after their initial baseline antibody level determination.

The study will be conducted as an optimal two-stage phase II trial ²⁶, in order to rule out an unacceptably low 25% of patients who experience at least a 50% decline in their antibody levels from baseline to up to 12 months ($p_0=0.25$) in favor of a modestly high 50% fraction who have this magnitude of decline ($p_1=0.50$). Declines of 50% at either time point will be considered a 'positive outcome'. With $\alpha=0.15$ (probability of accepting a poor treatment=0.15) and $\beta=0.15$ (probability of rejecting a good treatment=0.15), the study will initially enroll 9 evaluable patients and if 0-2 of the 9 have a positive outcome, then no further patients will be accrued. If 3 or more of the first 9 have a positive outcome, then accrual would continue until a total of 19 patients have enrolled. As it may take up to maximum 12 months to determine if a patient has experienced a positive outcome as defined, a temporary pause in the accrual to the trial may be

necessary to ensure that enrollment to the second stage is warranted. If there are 3-6 patients with a positive outcome in 19 patients, this would be an uninterestingly low response rate, while if there were 7 or more patients with a positive outcome in 19 patients, then this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (25% positive outcome rate), the probability of early termination is 60%.

Secondary endpoints include evaluation of toxicity of anti-KIR(IPH2101), estimated time to progression, duration of response, pharmacokinetic analysis and evaluation of biological activity; and are intended to be exploratory in this initial trial. Data collected on these endpoints will be analyzed and reported descriptively.

8.2 ACCRUAL RATE

It is anticipated that up to 2 patients per month may be enrolled onto this protocol. To allow for the possibility of a small number of inevaluable patients (10% drop out rate), the accrual ceiling for the trial will be set at 21. Thus, approximately one year may be required to enroll 19 evaluable patients. In order to allow for the possibility of a small number of non-evaluable patients, the accrual ceiling will be set at 21.

8.3 ANALYSIS OF SECONDARY ENDPOINTS

The time to event secondary endpoints are defined as follows: time to progression (time from registration to progression, censored at date last known progression-free for those who have not progressed), and duration of response (time from response to disease progression or death, or date last known progression-free and alive for those who have not progressed or died). Time to progression to symptomatic MM to be documented in study, and in a post study follow up (See Section 3.5 Study Calendar). Time to progression and the duration of response will be estimated using the Kaplan-Meier and log-rank methods.

Toxicity, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries or changes in standard care will be taken into account in any decision to terminate the study.

9 COLLABORATIVE AGREEMENT

9.1 CLINICAL TRIAL AGREEMENT (CTA)

Anti-KIR (IPH2101) is provided to the investigator by Innate Pharmaceuticals, Inc. under a CTA (00843-10).

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

MM is an almost always incurable plasma cell neoplasm that comprises approximately 10% of all hematologic malignancies.² It has recently been shown that, prior to diagnosis of MM, a precursor state (MGUS or SMM) can be detected in all patients who ultimately progress to MM.^{7,8} MGUS, SMM, and MM increase in incidence with age.^{10,27} MGUS affects approximately 3.2% of Caucasians over the age of 50 and 5.9% of African-Americans.^{12,28,29} SMM affects all races and genders.¹² As such, we expect that the majority of patients enrolled in this trial will be older adults of either gender or race. SMM patients enrolled on this study will consist of patients referred to and screened at the NIH Clinical Center. There will be no subject

selection bias with regard to gender, ethnicity, or race. This protocol will be reviewed by the IRB. This protocol excludes lactating and pregnant women from receiving this investigational drug to avoid any possible risks to the fetus or newborn. A potential toxicity of IPH2101 for embryo-foetogenesis is not expected but has not been explored. Additionally, the median age of diagnosis for SMM is in the range of 50 to 60 years, thus few patients with SMM are expected to be pregnant or lactating.

10.2 PARTICIPATION OF CHILDREN

Pediatric patients with SMM are extremely rare. Patients under the age of 18 are excluded from this study because inclusion of a rare younger patient will not provide adequate generalizable information to justify their inclusion in this study. Also, there is currently no Phase I data on this use of anti-KIR (IPH2101) in the pediatric population.

10.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

NK cells are large granular lymphocytes that comprise approximately 10-15% of peripherally circulating lymphocytes and play a crucial role in cellular innate immunity. NK cells lyse tumor cell targets through the balance of activating and inhibitory signals received through receptor families such as KIR for inhibitory signals.

IPH2101 (anti-KIR) has features that should allow prolonged pharmacological blockade of KIR in patients that prevent the inhibitory signals induced by all HLA-C allotypes of NK cells. NK cells have therefore the potential to kill tumor or infected cells without affecting healthy tissues.

Three phase I studies have been conducted with IPH2101.

A single-dose escalation phase 1 trial (IPH2101-101) has been performed in patients with AML in complete remission followed by an extension protocol (IPH2101-102) allowing patients treated in the IPH2101-101 study to receive repeated administrations once the dose level they had been assigned to was considered safe by the safety committee.

A phase 1 dose-escalation repeated dosing trial in patients with relapsed or refractory MM (IPH2101-103) has been performed in the US.

For both trials IPH2101-101 and IPH2101-103 the escalation phase, i.e. 7 dose levels ranging from 0.0003mg/kg to 3mg/kg has been successfully completed, with good tolerance and safety up to the maximum dose tested.

A first phase II study (IPH2101-201) is ongoing in France, evaluating the product in monotherapy, in patients with MM in stable plateau phase (maintenance setting) after first-line of treatment.

Overall, tolerance was good for all dose levels tested, without serious unexpected serious adverse reactions (SUSAR) reported except for the following two:

- One SUSAR was reported in a patient in trial IPH2101-103 after he received one dose of 0.075 mg/kg and considered as “probably related” to IPH2101. This was a grade 3 acute renal failure in a patient with progressing MM. It was decided to hydrate the subjects with 250 mg/hr one hour before and after dosing to prevent acute renal failure due to dehydration.
- Another SUSAR occurred in a patient in trial IPH2101-101 after he received one dose of 3 mg/kg IPH2101; this was a grade 2 bradycardia possibly related to IPH2101 treatment.

A cardiologist believed it to probably be of vagal origin in a setting of grade 2 fever, chills, and hypotension.

- No other clinically relevant events were reported in the other patients recently treated at the same 3 mg/kg dose.
- The most frequently reported adverse events were:
 - Fever
 - Fatigue
 - Headache
 - Chills
 - Rash
 - Itching (pruritis)
 - Bradycardia

There are no evident signs of IPH2101 inducing AEs related to auto-reactivity, infusion, or cytokine release to a degree that has raised any safety concerns.

IPH2101 has so far shown to be tolerable and manageable in subjects with AML or MM. It has to be emphasized that the maximal tolerated dose (MTD) has not been reached at the maximal tested dose of 3 mg/kg in any of the phase I trials.

10.4 RISKS/BENEFITS ANALYSIS

The safety profile of IPH2101 appears to be very satisfactory as documented in phase I and II studies up to 3 mg/kg at single and repeated dosing. Regarding the pharmacodynamic effect, the preliminary data show that all patient displayed KIR occupancy; the degree and duration is dose dependant. A dose of 1mg/kg allows a full saturation (90%) of KIR receptors over 4 weeks. Therefore, this dose every two months should lead to the maintenance of a complete saturation between doses.

Smoldering multiple myeloma (SMM) is an asymptomatic plasma cell proliferative disorder with a high risks of progression (cumulative risk 73% at 15 years) to symptomatic multiple myeloma (MM). MM is an incurable malignancy and any possible way to decrease or delay this risk of progression will bring a clear benefit to these patients with SMM. No therapy for SMM is currently available so investigational approaches should be considered and should be safe in asymptomatic patients.

10.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Informed consent will be obtained from all patients on this trial. There will be no minors enrolled < 18 years of age; therefore, assent is unnecessary. The informed consent contains all elements required for consent. In addition, the Principal Investigator or an associate investigator or member of the research team will discuss the protocol in detail with the patient and will be available to answer all patient questions to allow the patient to give informed consent.

11 PHARMACEUTICAL INFORMATION

11.1 IPH2101 (ANTI-KIR)

11.1.1 Source

Anti-KIR (IPH2101) is provided to the investigator by Innate Pharmaceuticals, Inc. under a Clinical Trials Agreement (CTA).

11.1.2 Toxicity

Table 2: ALL ADVERSE EVENTS POTENTIALLY RELATED TO ANTI-KIR(IPH2101) in Trials 101 and 102

Adverse Event (CTCAE 4.0 Term)	IPH2101-101	IPH2101-102
	N (%) E	N (%) E
Related Adverse Events	15 (65) 27	6 (67) 15
Subjects Exposed	23	9
BLOOD AND LYPHATIC SYSTEM DISORDERS		
Anemia	1 (4) 1	
Thrombocytopenia	1 (4) 1	
CARDIAC DISORDERS		
Bradycardia	1 (4) 1	
Sinus Bradycardia	1 (4) 1	
EAR AND LABYRINTH DISORDERS		
Vertigo	1 (4) 1	
EYE DISORDERS		
GASTROINTESTINAL DISORDERS		
Diarrhea	1 (4) 2	
Dry Mouth		1 (11) 1
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Fever	3 (13) 3	1 (11) 1
Chills	1 (4) 1	1 (11) 1
Weakness	1 (4) 1	
Fatigue		1 (11) 1
General physical health deterioration		1 (11) 1
Malaise		1 (11) 1
INVESTIGATIONS		
Lipase Increased	1 (4) 1	
METABOLISM AND NUTRITION DISORDERS		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
NERVOUS SYSTEM DISORDERS		
Dizziness	1 (4) 1	
Headache	1 (4) 1	1 (11) 1
Parosmia		1 (11) 1
Tremor		1 (11) 1
PSYCHIATRIC DISORDERS		
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS		
Dyspnea		1 (11) 1
REPRODUCTIVE SYSTEM AND BREAST DISORDERS		
Gynecomastia	1 (4) 1	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
Rash	4 (17) 4	1 (11) 1
Papular Rash		1 (11) 1
Pruritis	4 (17) 5	1 (11) 3
Erythema	1 (4) 1	
VASCULAR DISORDERS		

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Adverse Event (CTCAE 4.0 Term)	IPH2101-101	IPH2101-102
Hypotension	1 (4) 1	

N = number of subjects having the given event; E = the number of adverse events reported; % = the percentage of exposed subjects experiencing the event.

Table 3: ALL ADVERSE EVENTS RELATED TO IPH2101 IN TRIAL 103

Adverse Event (CTCAE 4.0 Term)	IPH2101-103
	N (%) E
Related Adverse Events	7 (28) 20
Subjects Exposed	25
BLOOD AND LYPHATIC SYSTEM DISORDERS	
CARDIAC DISORDERS	
Tachycardia	1 (4) 1
GASTROINTESTINAL DISORDERS	
Abdominal Pain	1 (4) 1
Nausea	1 (4) 2
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Fever	3 (12) 3
Chills	3 (12) 3
Fatigue	1 (4) 1
INVESTIGATIONS	
Serum creatinine increased	1 (4) 2
Neutrophil count decreased	1 (4) 1
METABOLISM AND NUTRITION DISORDERS	
Hyperkalemia	1 (4) 1
Hyperuricemia	1 (4) 1
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
NERVOUS SYSTEM DISORDERS	
Headache	2 (8) 2
RENAL AND URINARY DISORDERS	
Acute Renal Failure	1 (4) 1
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	
VASCULAR DISORDERS	
Flushing	1 (4) 1

N = number of subjects having the given event; E = the number of adverse events reported; % = the percentage of exposed subjects experiencing the event.

11.1.3 Formulation and Preparation

IPH2101 is a fully human IgG4 monoclonal antibody. It will be provided as 5 mL fill in a 6 mL vial of a liquid for infusion with a formulation strength of 10 mg/mL

11.1.4 Stability and Storage

11.1.4.1 The storage and shipping conditions for IPH2101 are:

- Temperature: +2°C to +8°C (35.6 to 46.4°F)

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11.1.4.2 Stability studies of IPH2101 (anti-KIR) are ongoing.

11.1.5 Packaging and labeling

IPH2101 will be provided in boxes of 4 vials. Labeling will be in accordance with local law and trial requirements

11.1.6 Administration Procedures

For all dose administration, the individual dose will be a single dose per kg of body weight. The dose calculation will be based on the body weight of the subject. IPH2101 will be administered undiluted over a **one hour IV infusion** by the use of a pro-programmed syringe driver. See Section **3.3**. Saline can be used to flush the line.

11.1.6.1 Agent Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from Innate Pharmaceuticals Inc. using the NCI Drug Accountability Record Form (DARF).

11.1.7 Incompatibilities

No incompatibility has been identified at this stage of development.

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13 APPENDICES

APPENDIX A: BONE MARROW SAMPLE PROCESSING

13.1.1 Procedure for separating specimen samples

Before performing the bone marrow aspirate procedure make sure that the syringe, used to draw the specimen, contains 1 ml of 7% EDTA solution to prevent coagulation.

1. Harvest 10 ml bone marrow aspirate in one or two tubes containing at least 1ml of 7% EDTA solution per tube.
2. Place immediately on ice.
3. Dilute sample to 32 ml with of PBS+2mM EDTA.
4. Separate mononuclear cells from red cells by Ficoll gradient.
 - a. Place 6 ml Ficoll into each of four 15 ml conical tubes.
 - b. Carefully layer 8 ml of diluted bone marrow aspirate sample over the ficoll in each tube, creating a sharp sample-ficoll interphase.
 - c. Centrifuge (at RT) at 1200xg for 30 min without brake.
 - d. Remove 1 ml of upper layer from each tube and place in 4 separate 1.5 ml cryo tubes. Freeze on dry ice and store at -80 C until shipment.
 - e. Remove and discard the top layer of clear supernatant to within 1 ml above the mononuclear cells layer, leaving it undisturbed at the interphase.
 - f. For all four tubes, collect the remainder of the top layer, the mononuclear cell layer, and about half of the ficoll below it; transfer it to a 50 ml tube.
 - g. Fill the 50 ml conical tube with PBS/EDTA, mix gently, and centrifuge for 10 minutes at RT at ~300g (1200 rpm).
 - h. Carefully remove the supernatant completely.
 - i. Wash the cells again with PBS/EDTA containing 0.5%BSA pH7.2
 - j. Resuspend cells in 40 ml buffer
 - k. Pass cell through a through a 35 μ m Nylon cell strainer cap (BD #35-2235) in a 50 ml tube, no bubbles.
 - l. Centrifuge for 5 minutes at 1200 RPM. Aspirate supernatant.
 - m. Resuspend cells in 1.6 ml of buffer used for magnetic separation (below).
 - n. Remove 10ul aliquot for cell count, which can be done later with other counts; should be a total of about 0.5-1.5X10E8 mononuclear cells.
 - o. Proceed to magnetic positive selection of CD138-positive cells.

13.1.2 CD-138 Magnetic Bead Isolation for fresh Bone Marrow Samples

(Protocol per Miltenyi-Biotech)

1. CD-138 Isolation procedure: Use cold (but not ice cold) degassed fresh separation buffer only. (PBS/EDTA/0.5% BSA pH 7.2). Work fast to keep cells cold.
2. Add 400ul beads to 1.6 ml of mononuclear cells. Mix by gently pipetting.
3. Incubate in the refrigerator for 15 minutes.
4. Prepare three MS columns for every sample. While incubating samples, place columns on magnets and wash with 0.5 ml buffer. Avoid introducing bubbles while pipetting.
5. After incubation, wash cells in 20 ml of separation buffer.
6. Spin at 1200 rpm for 10 min. Remove supernatant completely. Resuspend pellet in 0.5 ml separation buffer.

7. Place a clean 15ml tube under the first column to collect the negative fraction. Apply cell suspension to column, no bubbles. Allow unlabeled cells to pass through.
8. Wash columns 3x with 0.5 ml buffer.
9. Apply entire flow through of 2 ml to a second column, and repeat washes.
10. Collect total effluent of 3.5 ml in 15 ml centrifuge tube, and mix gently. Spin at 1200 rpm for 10 min, remove supernatant, and resuspend in 1 ml. This is the unlabeled cell fraction (negative fraction). Remove 10 microliters for a cell count, which can be done later.
11. Set aside this negative fraction for further processing (below)
12. Remove the two columns from magnet and place in a suitable tube, pipette 1ml separation buffer on top of each column and elute retained cells using the plunger supplied with the column.
13. Spin down eluted cells and resuspend pellet in 0.5 ml of buffer.
14. Apply eluted fraction to a new column.
15. Discard effluent and washes.
16. Same as 12 except with one column.
17. Spin down eluted cells and resuspend in 1 ml of buffer. This is the positively selected fraction. Remove 10 microliter aliquot for cell count

13.1.3 After CD 138 enrichment

NOTE: 10% of the cells will be used to harvest protein for Tissue Lysate Arrays; the remaining cells will be used to generate RNA for gene expression profiling and cDNA for *RAS* sequencing. Please separate the cells into duplicate samples for each, as described.

1. Remove 2 aliquots of 100ul each from the 1ml of CD138+ cells and the 1ml of flow-through CD138-negative cells into separate microcentrifuge tubes. These will be used for the Tissue Lysate Arrays.
2. Separate the remaining 800ul of each fraction into two separate tubes containing 400ul each.
3. Centrifuge all aliquots (ie, the positive and negative fractions) at 1000xg for 5 min.
4. Remove all supernatant.
5. Freeze pellets on dry ice and store at -80 deg until shipment.

13.1.4 Summary of samples from each bone marrow aspirate:

Supernatant from Ficoll = 4 cryotubes containing 1 ml each

Cell pellets = 8 tubes for each aspirate:

- 10% of the CD138+ fraction (for protein) in each of two tubes,
- 40% of the CD139+ fraction (for nucleic acids) in each of two tubes,
- 10% of the CD138 negative fraction (for protein) in each of two tubes,
- 40% of the CD138 negative fraction (for nucleic acids) in each of two tubes.

13.1.5 Reagents:

Miltenyi Biotec - 12740 Earhart Ave - Aubutn, CA 95602 - (530) 888-8925

Columns: Miltenyi MS MACS columns (cat # 130-042-201)

Magnets: MiniMACS high-energy permanent magnet, and MACS Multistand

Beads: Miltenyi CD 138 microbeads (cat# 130-051-301)

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Separation Buffer PBS + 2mM EDTA + 0.5% BSA, pH 7.2
(make fresh, use within 2 weeks)

For 50 ml: 5 ml 10x PBS
 0.2 ml 0.5 M EDTA
 0.25 g BSA
 qs to 50 ml with ultra H₂O, pH to 7.2, and sterile filter. Store 4oC.

0.5 M EDTA 18.61 g/ 100 ml H₂O, pH 8.0
 Sterile filter. Store at room temp.

APPENDIX B: 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.