EASTERN COOPERATIVE ONCOLOGY GROUP

Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

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STUDY PARTICIPANTS	**ACTIVATION DATE**
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Table of Contents

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Schema

Register

↓

Submission of required research samples

↓

Diagnostic review, if applicable

↓

Register to ECOG Treatment trial

1. Introduction

This protocol's purpose is to provide a mechanism for collection of samples obtained at pre-study evaluations from patients considering enrollment on an ECOG treatment trial for myeloma. Collection procedures can be invasive (e.g., bone marrow biopsies) and costly, thus patients often refuse to have collection procedures repeated after registration to a treatment trial. Institutions often collect and submit pre-study samples prior to enrollment on a treatment trial, but these samples lack any ECOG study identification and there is no mechanism prior to treatment trial registration to collect the needed consent information pertaining to sample use.

This protocol will also be used to establish a mechanism for submission of samples for diagnostic review to determine eligibility of patients for accrual to the Eastern Cooperative Oncology Group (ECOG) myeloma trials. Until now, the majority of patients accrued to ECOG's myeloma trials have not had their pathology formally reviewed or verified. Eligibility has been established based on percentages of plasma cells derived from outside reports. It is clearly desirable to have a mechanism to review samples from potential candidates in order to confirm eligibility and potentially assign patients to the appropriate treatment trial.

Moreover, there has been a phenomenal expansion of knowledge over the past ten years regarding the molecular interactions between myeloma cells and the bone marrow microenvironment. Myeloma cells are thought to specifically home to bone marrow where the cells proliferate, recruit new blood vessels, and interfere with the process of normal bone remoldeling[1] This understanding has led to the development of new therapies that target these interactions, such as thalidomide and bortezomib, which have activity even in relapsed and refractory patients[2, 3] . Furthermore, a host of other agents are being investigated which disrupt the autocrine paracrine relationship between stromal and myeloma cells, such as heat shock protein inhibitors[4], vascular endothelial growth factor receptor inhibitors, and farnesyl transferase inhibitors[5]. Clearly, access to bone marrow samples from a variety of patients with myeloma in its various stages will further these endeavors.

1.1 The majority of current ECOG myeloma trials do include collection of baseline specimens. However, the major problem impeding the collection of these diagnostic samples is the need for patients' consent on the treatment trial before the sample is collected. This raises practical issues in myeloma trials as opposed to sample collection for leukemia trials. Firstly, very few patients have evidence of circulating plasma cells in large numbers and not infrequently, the patient and occasionally the physician is not completely sure of the diagnosis at the time the diagnostic bone marrow biopsy is performed. Secondly, some of the current ECOG trials stipulate that patients must have certain minimum percentages of plasma cells in the marrow for patients to be eligible, and patients have biopsies done before enrollment in order to prove or disprove the extent of their disease, and thus eligibility. The difficulty then is not in patient unwillingness to consent to sample collection or banking but more a timing issue. Finally there is emerging evidence regarding the prognostic importance of certain cytogenetic features, such as deletion of part or all of chromosome 13, hypodiploidy and translocations of t (4:14) and t (14:16) [6,7]. Recently, ECOG completed trial E2A02, which specifically selected out high risk patients, defined as those with chromosome 13 abnormalities and/or high beta-2-microglobulin. Obviously the cytogenetic abnormality had to have been proven prior to enrollment, so that many of these patients did not have prestudy samples collected for ECOG. Future studies will undoubtedly focus on other such special populations, implying that at least one bone marrow sample must be done prior to enrollment to define the cytogenetic abnormality. There are likely numerous instances in which such patients could be unwilling to receive a second marrow for research purposes, or that such a test would not be covered by insurance.

A further interest of ECOG is to establish protocols that are targeted toward the large population of patients with monoclonal gammopathy of undetermined significance (MGUS), which is present anywhere from 1-11% of the population, depending on age[8, 9,10]. Not infrequently, MGUS patients are referred for bone marrow biopsy at some time and these samples are not currently systematically collected or studied by ECOG. With the recognition of the frequency of chromosomal and genetic abnormalities in plasma cells from MGUS patient samples, and newer prognostic information, the opportunity to collect such samples will be invaluable [11, 12].

ECOG's previous experience with protocols EST 1435 and more recently, E3903, which called for mandatory submission of samples for patients enrolled on leukemia and dysplasia protocols, shows that such a laboratory correlative study is feasible. In these studies, samples have been collected in more than 90% of patients enrolled on ECOG's leukemia trials. The goal of this new trial is to achieve that same accrual rate for patients with plasma cell dyscrasias.

1.2 Study Objectives

Primarily, the laboratory study consent will have a protocol dependent scientific section. This section will state that upon enrollment into the particular ECOG treatment trial the samples submitted may be used for the correlatives defined in that protocol. The various aspects of sample submission will be formulated in distinct questions so that patients can consent separately to an individual section.

Additionally, this study will serve as a mechanism to obtain the materials required for diagnostic review to assure eligibility for a specific trial. Preliminary reports may be issued confirming multiple myeloma, MGUS or amyloidosis, followed by more comprehensive results at a later time. We recognize that in some cases with multiple myeloma, patients will be entered on clinical trials based on the institutional diagnosis only, because of the requirement for immediate treatment. If such a case is subsequently found not to be eligible based on the ECOG review, the patient will be removed from the statistical analysis.

1.3 Logistical considerations

There are multiple implications of having patients enrolled on a laboratory protocol prior to going on the treatment study. Most importantly, it will solve the problem of not having patients' consents for laboratory testing at the time the diagnostic bone marrow aspiration is done.

1.4 Gender and Ethnicity Statement

Entry to this study is open to both men and women, and to persons of any national or ethnic group.

2. Objectives

2.1 To obtain baseline materials for correlative studies outlined in the ECOG treatment trial.

2.2 To provide a mechanism for sample collection and submission for diagnostic review to determine eligibility of patients for accrual to ECOG treatment trials for plasma cell disorders.

3. Selection Of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG Patient No. _____

Patient's Initials (L, F, M) _____

NOTE: All questions regarding eligibility should be directed to the ECOG Coordinating Center at (617) 632-3610.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

_____ 3.1 Patients must be considered for enrollment into one or more ECOG treatment trials for plasma cell disorders.

_____ 3.2 Any ECOG treatment trial that is being considered for the patient must be active and accruing. Please see Section 6.1 for a list of applicable active trials.

_____ 3.3 Patients must not yet have started treatment on their respective ECOG treatment trial.

4. Registration Procedures

Submitting Regulatory Documents

Before an ECOG Institution may enter patients, protocol specific regulatory documents must be submitted to the CTSU Regulatory Office at the following address:

CTSU Regulatory Office
Coalition of National Cancer Cooperative Groups
1818 Market Street, Suite 1100
Philadelphia, PA 19103
FAX: (215) 569-0206

Required Protocol Specific Regulatory Documents

1. **CTSU Regulatory Transmittal Form.**

2. **Copy of IRB Informed Consent Document.**

   NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

3. **A. CTSU IRB Certification Form.**

   Or

   B. HHS 310 Form.

   Or

   C. IRB Approval Letter

   NOTE: The above submissions must include the following details:

   - **Indicate all sites approved for the protocol under an assurance number.**
   - **OHRP assurance number of reviewing IRB**
   - **Full protocol title and number**
   - **Version Date**
   - **Type of review (full board vs. expedited)**
   - **Date of review.**
   - **Signature of IRB official**

The CTSU encourages you to link to the following RSS2.0 webpage so that more information on RSS2.0 as well as the submission forms can be accessed at http//www.ctsu.org/rss2_page.asp**. If you have questions regarding regulatory document submission, please telephone the CTSU Help Desk at 1-888-823-5923 or E-mail** CTSUContact@westat.com**. Monday through Friday, 9:00am - 6:00pm**.

Patients must not start parent protocol treatment prior to registration.

Institutions may register eligible patients to this study via the ECOG webpage 24 hours a day, 7 days a week, using the Web-based Patient Registration Program (https://webreg.ecog.org). If you need assistance or have questions, please telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022, Monday through Friday 9:00am – 5:00pm Eastern Time. Please note that a password is required to use this program.

The following information will be requested:

4.1 Protocol Number

4.2 Investigator Identification

4.2.1 Institution name and/or affiliate

4.2.2 Investigator's name

4.3 Patient Identification

4.3.1 Patient's initials and chart number

4.3.2 Patient's Social Security number

4.3.3 Patient Demographics

4.3.3.1 Sex

4.3.3.2 Birthdate (MM/YYYY)

4.3.3.3 Race

4.3.3.4 Ethnicity

4.3.3.5 Nine-digit zip code

4.3.3.6 Method of payment

4.3.4 ECOG treatment protocol number

4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3. An eligibility checklist has been appended to the protocol. A confirmation of registration will be forwarded by the ECOG Coordinating Center.

4.5 Additional Requirements

4.5.1 Patient must provide signed and dated written informed consent.

4.5.2 Peripheral blood, bone marrow aspirates, and slides must be submitted as per section 5.

4.5.3 Protocol number of potential therapeutic trial should be indicated along with the respective ECOG patient case number, if available.

NOTE: Registration of a patient to this Ancillary Laboratory Protocol is separate from participation in the correlative study requirements specific for the treatment protocol. Thus investigators should adhere to the correlative studies requirements and submissions outlined in the parent protocol and not specified by this laboratory protocol.

4.6 Instructions for Patients Who Do Not Register to a Parent ECOG Treatment Protocol

If a patient does not register to any ECOG treatment trial, document the reason for not registering to an ECOG treatment study on the E3A05 Laboratory Study Follow-up Form (#2711).

5. Sample Submission Guidelines

NOTE: An E3A05 Material Submission Form (#2632) must be submitted with the sample(s) at all required time points. All samples and collection time points are to be indicated on the form(s) at each submission. The original E3A05 Material Submission Form (#2632) should accompany the samples to the central laboratory(ies), and a copy is to be forwarded to the ECOG Coordinating Center (Attn: Translational Sciences) at the same time it is sent to the central laboratory.

5.1 Sample Submission Schedule

The following baseline (pre-treatment) materials are required:

- Peripheral blood, 7 mL ACD yellow top vacutainer
- Bone marrow aspirate, 5 mL ACD yellow top vacutainer
- Bone marrow aspirate, PCLI
  - Bone marrow biopsy may be substituted if the aspirate is a "dry tap"
- Bone marrow biopsy slide
- Copy of the pathology report, faxed as indicated in Section 5.2.5

Blood and aspirates are to be shipped the day of collection. Baseline samples for research are to be collected, if possible, at the same time as the diagnostic blood and bone marrow studies that are required to establish the diagnosis and determine eligibility.

To prepare and ship these samples use the **Myeloma Tumor Biology Kit.**

To obtain the Myeloma Tumor Biology Kit, contact:

Kim Henderson
Mayo Clinic Myeloma Reference Laboratory
Tel: (507) 284-3805

Affiliates who anticipate participating in this study should please call in advance for kits.

Any questions concerning these samples, please contact Kim Henderson at the Mayo Clinic Myeloma Reference Laboratory, Rochester, MN 55905 at (507) 284-3805.

5.2 Sample Preparation Guidelines

Samples are to be shipped the day they are obtained. The specimens are placed in separate, labeled tubes and bags, but are shipped in a single package to the Mayo Clinic Myeloma Reference Laboratory. **EXCEPTION:** Unstained core biopsy slides can be mailed later if they are not available at time of shipping of bone marrow aspirate and peripheral blood.

All specimens are to be clearly labeled with the protocol number E3A05, the patient's initials, sequence number, sample type and date of collection. **A current white blood count and differential** must be included with each submission.

5.2.1 Peripheral Blood

Draw 7 mL of peripheral blood into one ACD vacutainer tube from the Myeloma Tumor Biology Kit.

5.2.2 Bone Marrow Aspirate

This redirect bone marrow aspirate should preferably be done at the same time of the regular bone marrow aspiration/biopsy being done for clinical and diagnostic purposes. The bone marrow aspirate should be done through the same skin puncture site that the clinical sample was obtained.

Draw 5 mL of a "redirect" bone marrow aspirate sample into one ACD vacutainer tube.

5.2.3 Bone Marrow Aspirate Plasma Cell Labeling Index Studies

2-3 mL of redirect bone marrow is to be processed as follows:

1. Process the marrow specimen immediately after aspirating.
2. Mix 2-3 mL of bone marrow, from a "redirect" aspirate, into the tube labeled Plasma Cell Labeling Index (PCLI) and immediately mix gently but thoroughly.
3. Cap tube (loosely if using a CO_2 incubator) and incubate for 1 hour at 37°C.
4. Transfer the contents of the distilled water vial (provided) into the stopping Reagent vial. Mix until dissolved.
5. After the 1 hour incubation, transfer the stopping solution into the PCLI tube. Mix gently, but thoroughly.
6. Cap tightly. Be sure tube is labeled with patient initials (last name, first name), sequence number and date. Store at 4°C until shipped.

For patients who have an initial **dry tap,** try to repeat the bone marrow tap to see if a marrow aspirate can be obtained. If no aspirate can be obtained, a bone marrow biopsy is requested and should be processed as follows:

1. Add bone marrow core biopsy sample to a vial containing 2 mL of Normal Saline (PBS).
2. Vortex sample vigorously for 5 minutes.
3. Transfer contents of vial to the Pulsing Tube in the Labeling Index kit (included in the Myeloma Tumor Biology Kit), and continue to process as instructed above, beginning with step 3.

5.2.4 Bone Marrow Core Biopsy Slides

5 air-dried, unstained "charged" slides from the paraffin block of a bone marrow core biopsy are to be submitted at each time point.

Send the slides with the bone marrow aspirate and peripheral blood samples if possible, or separately, within one month of collection, if core biopsy slides cannot be prepared on same day.

5.2.5 Pathology Report

A copy of the pathology report must be faxed to 800-887-9554, the efax number for the ECOG repository at Mayo Clinic.

5.3 Sample Shipping Guidelines

Specimens should be mailed the day they are obtained and shipped overnight to arrive during normal working hours. Follow packing guidelines listed in the kit. If samples are sent late in the week and will arrive on the weekend, please note "Saturday Delivery" on the Federal Express form.

FRIDAY AND PRE-HOLIDAY SHIPMENTS SHOULD BE AVOIDED.

It is requested that the bone marrow core biopsy slides be sent with the other samples in the Myeloma Tumor Biology Kit. However, they may be shipped separately, within one month of collection, if they cannot be prepared on the same day as the other samples are collected.

Complete the following: specimen ID label on each tube, Patient Information Form and E3A05 ECOG Material Submission Forms (all samples are to be listed). Each tube must be clearly labeled. Each sample should be labeled with the Protocol number, Patient initials (last name, first name), ECOG patient ID number, and sample type (peripheral blood [PB] or bone marrow [BM]).

1. Place the slightly thawed Kool-PAK in bottom of Styrofoam container (Kool-PAK should be frozen at least 24 hours in advance. Allow the frozen ice pack to thaw at room temperature for 2-3 hours before preparing the specimen for shipment).
2. Place absorbent toweling on top of Kool-PAK.
3. Place specimens in their individual plastic bags provided, wrap in paper toweling and place them in the Styrofoam container and close the lid. Do not place the specimen(s) directly on the ice pack.
4. Place the Styrofoam container and the completed Patient Information Form and Materials Submission Form within the cardboard mailing sleeve.
5. Prepare the package for shipping, applying packing tape as needed. Complete the sender portion of the return FedEx Airbill and adhere to the exterior lid of the box. Ship specimens priority overnight delivery the same day collected.
6. Notify Federal Express for pick-up and/or leave package at the designated FedEx drop-off location.

Please call Kim Henderson at (507) 284-3805 to notify the Mayo Clinic Myeloma Reference Laboratory when specimen(s) are being shipped. The samples in prepared kits should be shipped to the following:

Kim Henderson
Mayo Clinic Myeloma Reference Laboratory
613 Stabile
200 First Street Southwest
Rochester, MN 55905

5.4 Cytogenetics Review

The cytogenetics review will be performed by the ECOG Cytogenetics Laboratory, Dr. Gordon Dewald, director.

The following materials must be submitted within 1 month after registration.

- Two original karyotypes per clone
- Institution's cytogenetics lab report
- The ECOG Cytogenetics Report Form (#2746)
- E3A05 Material Submission Form (#2632)

Submit the required materials to:

Gary Hicks
Mayo Clinic Cytogenetics Laboratory
970 Hilton
200 First Street, S.W.
Rochester, MN 55905
Tel: (507) 284-2950
Fax: (507) 284-0043

If requested in writing, the original karyotypes will be returned to the institution when the review and analysis are complete.

5.5 Sample Inventory Submission Guidelines

Inventories of all samples collected and the respective aliquots made and used on the above mentioned laboratory correlative study(ies) will be submitted to the ECOG Coordinating Center upon request. Electronic submissions will be submitted to 303.lab@jimmy.harvard.edu.

5.6 Banking

The residuals and/or derivatives of samples collected for this study will be retained at the Mayo Clinic Myeloma Reference Laboratory for possible use in future ECOG approved studies. Please see Appendix V, “Multiple Myeloma Tissue Bank” for details of sample handling, data storage and requests to use banked materials. If future use is denied or withdrawn by the patient, the samples will be removed for consideration for use in any future study.

6. Diagnostic Review Reports and Patient Participation in the ECOG Treatment Trial

ECOG requests every myeloma patient considered for a myeloma treatment trial should be enrolled in E3A05 and samples are to be submitted to the Mayo Clinic Myeloma Reference Laboratory.

If enrollment to the treatment trial requires central assessment of patient status, but the institution cannot wait for diagnostic confirmation from these laboratories, due to medical considerations, they may go ahead and enroll the patients on the ECOG treatment trial. However, if subsequent testing unequivocally reveals ineligibility, the patient will be removed from the statistical analysis of the treatment trial, whether or not the patient continues on the treatment that had been started.

6.1 Active Treatment Trials Linked to this Laboratory Protocol

- Baseline samples submitted on E3A05 will be used for the treatment trial 'on-study baseline' submission for correlative studies defined within the treatment trial.
- If the treatment-trial-defined correlative studies require additional samples, these samples are to be submitted as outlined in the treatment trial. Additional consent for submission and use of those samples will be required. The request for consent will be contained in the treatment trial's model consent.

Current Applicable Treatment Trials

E1A02 – Phase II Pilot Study of Rituximab + CHOP in patients with newly diagnosed Waldenstrom's Macroglobulinemia

E2A01 – A Phase I Study of Amifostine Followed By High-Dose Escalation of Melphalan With Stem Cell Reconstruction for Patients with Primary Systemic Amyloidosis

7. Statistical Considerations

The statistician on this protocol will ensure that the overall processes meet with the Statistical Center approval. Since analysis of laboratory data will be treatment protocol specific, the analysis will be performed in each treatment protocol in conjunction with the analysis of clinical data.

This protocol is open to all patients who are considered to be enrolled on an ECOG myeloma treatment protocol. Thus, there is no specific accrual goal in this protocol.

7.1 Lab Data Transfer Guidelines

The data collected on the above mentioned lab correlative studies will be submitted to the ECOG Coordinating Center by the Central Laboratories on a quarterly basis. The quarterly cut-off dates are March 31, June 30, September 30 and December 31. Data is due at the Coordinating Center 1 week after these cut-off dates. Electronic submissions should be submitted to 303.lab@jimmy.harvard.edu. All other correspondence should be addressed to the attention of the Correlative Science Team.

Data will be transferred by FTP.

8. Patient Consent and Authorization, Confidentiality, Data Acquisition and Security

8.1 Patient Consent and Authorization

8.1.1 A suggested study consent form is provided in Appendix I. This consent is separate from that of the treatment protocol. The study consent is in compliance with the Common Rule (45CFR46.106) and contains the required elements for authorization to use protected health care information in compliance with HIPAA (45CFR164).

8.1.2 Consent may request authorization for the submission of samples as well as for the banking of and future research on residual specimens in Mayo Clinic Myeloma Reference Laboratory.

8.1.3 This ancillary laboratory protocol and consent must be approved by every local IRB in addition to approval of the treatment protocol.

8.1.4 Consent may request authorization for the use of submitted specimens in scientific studies affiliated with the treatment protocol that the patient will eventually be enrolled in. This consent is different from that sought for banking for future, yet undefined research.

8.1.5 Consent may request authorization for ECOG affiliated physicians to contact the patient in the future, should the need arise, with respect to laboratory studies performed on the patients' specimens.

8.1.6 The situation may arise that specimens are received by Mayo Clinic Myeloma Reference Laboratory from patients who after consenting to laboratory specimen submission are ultimately found ineligible for the treatment trial. If patients have not registered to a treatment trial, the submitted samples may be retained for future use if patient has consented to allow banking for future research. If consent for banking is denied, samples will be discarded.

8.2 Patient Confidentiality, Data Acquisition, and Security

ECOG has obtained a Confidentiality Certificate from the Department of Health and Human Services (DHHS). This document pertains to the Group Chair's Office, the ECOG Coordinating Center, the ECOG Statistical Center, and the ECOG Core Laboratories, including The Mayo Clinic Myeloma Reference Laboratory.

Demographic information obtained during registration and throughout the course of the laboratory protocol is stored in a firewalled database at ECOG's Mayo Clinic Myeloma Reference Laboratory and at the ECOG Statistical Center located at the Dana Farber Cancer Institute (DFCI). Laboratory investigators have no direct access to this information. Test results are transferred to the Statistical Center and correlated with outcome data from the parent protocol by the ECOG statisticians.

Test results from investigators are transferred electronically to the ECOG Coordinating Center or to the ECOG Statistical Center and transferred to the DFCI database. Identifying information transferred with the data include the protocol number, the ECOG assigned sequence number, and the collection date of the sample.

At the ECOG Coordinating Center, case report forms and other paperwork containing patient information are filed into patient files and stored in a locked room with limited key card access. Pathology reports and other forms received by the Mayo Clinic Myeloma Reference Laboratory are kept in file cabinets with limited access. The laboratory's myeloma database is password protected and access is limited to laboratory personnel. Specimens stored in the tissue bank are stripped of any patient identifiers and labeled with a unique identifier number assigned by the laboratory. Sample identification distributed to approved investigators contains exclusively this unique laboratory identifier, the specimen date and protocol number (not the protocol sequence number). Only the ECOG personnel assigned to data analysis will be is the position to link the unique laboratory identifier with the parent protocol number.

9. Records To Be Kept

Please refer to the E3A05 Forms Packet for the forms submission schedule and copies of all forms. The E3A05 Forms Packet may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org). Forms must be submitted to the ECOG Coordinating Center, FSTRF, 900 Commonwealth Avenue, Boston, MA 02215 (ATTN: DATA).

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG Coordinating Center to CTEP by electronic means.

10. Patient Consent And Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

11. References

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6. Fassas AB, Tricot G. Chromosome 13 deletion/hypodiploidy and prognosis in multiple myeloma patients. Leukemia & Lymphoma 2004 Jun; 45(6):1083-91.

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Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

Appendix I

Informed Patient Consent

Version Date: 8/28/06

You are being asked to take part in this Laboratory Protocol because you and your physician are considering your participation in an Eastern Cooperative Oncology Group (ECOG) Treatment Protocol. In some treatment protocols, there may be additional laboratory tests that will be done to find out more about your possible blood disorder and how you or your disease responds to treatment.

This Laboratory Protocol will allow samples to be collected at the same time samples are collected for tests being done by your doctor. This may reduce the number of times blood or biopsies will be done if you participate in an ECOG Treatment Protocol. These samples are sent to the laboratories where they would be studied. The tests done by the central laboratory may depend on which Treatment Protocol you participate. This Laboratory Protocol also gives you the choice to contribute to future research by allowing your specimen(s) to be banked in a central laboratory.

You have the following consent options: 1. You may decide to participate in eligibility studies and in the additional research or banking studies (Eligibility studies are those that are necessary for your participation in a Treatment Protocol, the research and banking efforts are not). 2. You may agree to participate only in the eligibility studies but not in the research and banking efforts; this will not compromise your participation in the Treatment Protocol and your sample will be discarded after use. 3. You may refuse to participate in both the eligibility studies and the research and banking efforts in which case you will not be eligible for the Treatment Protocol.

Why Is This Study Being Done?

Your doctor suspects that you have a blood or bone marrow disorder. Treatment will depend on the type of blood/bone marrow cell that is affected by this disease. Some treatment strategies may require that diseased cells (such as plasma cells) have certain properties, for instance, express a certain chromosome abnormality that may affect treatment outcome.

This study is being done so that we can collect and study blood and bone marrow cells to either determine if one of ECOG's treatment trials is right for you, or to study the biology of blood or bone marrow disorders. This study may determine your eligibility for a Treatment Trial and it will prepare your specimen for use in research and banking studies.

If you consent, we will store your cells in a central laboratory. In the future, research investigators may apply to ECOG for the use of stored specimens in the study of cancer or other diseases. This may include studies to explain how differences in your research sample affect treatment outcome. Applications for the use of stored specimens are reviewed by ECOG and approved investigators have no access to information that can identify you (such as your name). Only the ECOG Coordinating Center and Statistical Center can link your specimen to you.

What Is Involved In The Study?

The ECOG study involves submission of bone marrow and blood to a central laboratory. If required by the treatment trial, bone marrow and/or blood may be collected at various time points. The central laboratory will process the samples and then forward the appropriate materials to investigators at other laboratories, if so required by the studies in the Treatment Protocol.

If you have consented the research and banking of your samples, leftover material from your specimens will be stored for future research.

Why are central laboratories being used?

ECOG's Multiple Myeloma Tumor Biology Laboratory is where the samples from all patients considered for ECOG plasma cell blood disorder treatment trials are studied either to confirm their eligibility for a specific treatment trial, or study the samples to learn new things about your disease. These samples may be used to determine your eligibility BEFORE you enter a Treatment Protocol. This makes sure that you are entered and treated on the correct Treatment Protocol. By performing these tests in one central laboratory, the studies are all performed in the same way and are quality controlled. Furthermore, it allows institutions that may not be able to perform these studies to put patients on treatment trials.

The results from these laboratory studies that concern your eligibility for a treatment trial will be sent to your doctor. Any test results from optional research studies or future studies, if you consented to the use of your specimens for future research, will be used by ECOG for assessing your disease but results will not be sent to you or your doctor.

How Long Will I Be In The Study?

You will remain a participant in this laboratory study as long as you are participating in the treatment trial.

Your samples will be tested and then stored for future studies, if you give consent to allow for future use. If you do not give ECOG permission to bank the samples that are left over, they will be destroyed.

What Are The Risks Of The Study?

Bone marrow cells and/or blood cells will be obtained by your physician and submitted to the central laboratory as part of your initial work-up for your disease and later on to evaluate your disease during treatment. Risks associated with bone marrow aspiration and blood drawing will be explained to you by your health care provider.

There are very few risks to you from using your bone marrow or blood for research. The greatest risk is the release of information from your health records. ECOG will protect your records so that the information will be kept private. The chance that this information will be given to someone else is very small. If your specimens are used for future research, they will be distributed only to investigators whose projects have been approved by ECOG. These investigators will have no access to your health records or any information that could lead them to your identity.

Are There Benefits To Taking Part In The Study?

The benefits of research using your blood and bone marrow include learning more about what causes your disease, how to prevent them, how to treat them, and how to cure them. We hope the information learned from this study will benefit other patients with blood disorders in the future.

If the Treatment Protocol you are considering employs the diagnostic part of this study, we can make sure that you have the correct diagnosis for the study. Having the correct diagnosis will make you "eligible" for that treatment protocol. Since the tests that are being performed determine the diagnosis, there will be a direct medical benefit to you.

Will My Medical Information Be Kept Private?

The Eastern Cooperative Oncology Group (ECOG) is conducting this study. ECOG is a cancer research group that conducts studies for the National Cancer Institute. Your doctor is a member of ECOG or another group that is participating in this study. To help protect your privacy, ECOG has obtained a Confidentiality Certificate from the Department of Health and Human Services (DHHS).

With this Certificate, ECOG cannot be forced (for example, by court subpoena) to disclose information that may identify you in any federal, state or local civil, criminal, administrative, legislative or other proceeding. Disclosure will be necessary, however, upon request of DHHS for audit or program evaluation purposes.

You should know that a Confidentiality Certificate does not prevent you or a member of your family from voluntarily releasing information about you or your involvement in this research. If an insurer or employer learns about your participation and obtains your consent to receive research information, then ECOG may not use the Certificate of Confidentiality to withhold this information. This means that you and your family must also actively protect your privacy.

You should also understand that your doctor and ECOG may take steps, including reporting to authorities, to prevent you from seriously harming yourself or others.

Finally, the Certificate does not prevent the review of your research records under some circumstances by certain organizations for an internal program audit or evaluation. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as:

- Eastern Cooperative Oncology Group (ECOG)
- National Cancer Institute (NCI)
- Food and Drug Administration (FDA)
- Other regulatory agencies and/or their designated representatives
- Drug manufacturers and/or their representatives
- Central laboratories, banks and/or reviewers

[Note to Local Investigators: The NCI has recommended that the local institution address HIPAA regulations. The regulations may or may not be included in the informed consent form depending on local institutional policy.]

Laboratory research investigators other than those involved directly in this laboratory protocol may obtain no personal information that would allow them to identify you. These investigators will not have direct access to your medical records or to the database where your patient information is stored.

What Are The Costs?

You and/or your insurance company will NOT be charged for submission or testing of the samples, even if these tests determine if you may participate in a Treatment Protocol. If you are found not to be eligible for an ECOG treatment trial, you and/or your insurance company will not be charged for the laboratory tests that were done as part of this laboratory protocol.

You will receive no payment for taking part in this study.

What Are My Rights As A Participant?

Taking part in this study is voluntary. You may choose not to take part in this study at all. However, if the tests to be done by the central laboratory determine your diagnosis and your eligibility for a particular Treatment Protocol, you cannot be considered for participation in that particular Treatment Protocol, since it is important that you are treated on a given Treatment Protocol only if you have the correct diagnosis for that treatment. Remember that the diagnostic tests done as part of this laboratory protocol may be the same tests that will be done by your own institution in order to diagnose your disease correctly and to be able to treat you appropriately. The only difference is that if you take part in this study, these tests will be done by the central laboratory.

If you decide to take part in the entire study, including the research part, you may change your mind at any time. The central laboratory where your specimens are stored will be promptly informed of your decision and any stored specimens will be destroyed. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your physician will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Will Any of the Samples Taken from Me Be Used for Other Research Studies?

As a part of this study, you are going to have some bone marrow and/or blood submitted to be analyzed by researchers located at an Eastern Cooperative Oncology Group (ECOG) central laboratory.

We would like to keep some of the bone marrow and/or blood that are left over for future research. If you agree, this bone marrow and blood will be kept and may be used in research to learn more about cancer and other diseases. This bone marrow and blood will be given only to researchers approved by ECOG. The researcher's Institutional Review Board also must approve any research done on the tissue.

The research that may be done with your bone marrow and/or blood will probably not help you. It might help people who have blood disorders, some types of cancer or other diseases in the future. Only by doing research can we learn more about these diseases and improve our chances to treat and eventually cure patients with these diseases.

Reports about the research done with your bone marrow and blood will not be given to you or to your doctor. These reports will not be put into your health record. The research will not have an effect on your care.

Things to Think About

The choice to let ECOG keep the leftover bone marrow and/or blood for future research is entirely up to you. No matter what you decide to do, it will not affect your care and you may still take part in the ECOG treatment protocol that you have been found to be eligible for.

If you decide now that your bone marrow and blood can be kept for research, you can change your mind at any time. Just contact your study doctor and let him or her know that you do not want us to use your bone marrow and blood. Then the bone marrow and blood will no longer be used for research.

In the future, people who do research may need to know more about your health. When ECOG gives them reports about your health, it will not give them your name.

Sometimes bone marrow and blood are used for genetic research (about disease that are passed on in families). Even if your bone marrow and/or blood are used for this kind of research, the results will not be put into your health records.

Your bone marrow and blood will be used only for research, and it will not be sold. You will not be paid for allowing your leftover bone marrow and blood to be used in research, even though the research done with your bone marrow and blood may help to develop new products in the future. Similarly, there will be no cost to you for any bone marrow and blood collected and stored by ECOG.

It is possible that, at some time in the future, as part of deciding on which therapy to give you, a new test might become available that could be done on some of the bone marrow and blood that is now thought of as "leftover." This situation is unusual, but it could happen. In order to see that not all of this leftover bone marrow and blood is used up, ECOG will take care to see that some of your diseased bone marrow and blood is stored for at least 10 years so it is available if you or your doctors should need it. Whether this is feasible will depend upon the amounts of bone marrow and/or blood that are left over from your

specimens that were submitted to the ECOG laboratory. Sometimes, the entire specimen is used up in the research tests required by the protocol and there is no left over material that can be stored.

Benefits

The benefits of research using bone marrow and blood include learning more about what causes cancer and other diseases, how to prevent them, how to treat them, and how to cure them.

Risks

There are very few risks to you. The greatest risk is the release of information from your health records. ECOG will protect your records so that your name will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice

By signing this consent you agree that blood and/or bone marrow may be collected and then submitted to a central laboratory for testing. The type of testing to be done on the blood and/or bone marrow will determine which Treatment Protocol you may participate in.

We also would like to use these samples for additional research. Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No." *No matter what you decide to do, it will not affect your care*. You can participate in the treatment part of the Treatment Protocol without participating in all or part of the optional bone marrow and blood research studies. If you have any questions, please talk to your doctor or nurse or call our Institutional Review Board at ***[ADD TELEPHONE NUMBER]***.

My bone marrow and blood may be used for the optional scientific studies defined in the Treatment Protocol in which I am considering participation. **Yes No**
My bone marrow and blood may be kept for use in future research to learn about, prevent, treat, or cure cancer. **Yes No**
My bone marrow and blood may be kept for use in future research about other health problems (for example, causes of diabetes, Alzheimer's disease, and heart disease). **Yes No**
My doctor (or someone from the Eastern Cooperative Oncology Group) may contact me in the future to ask me to take part in more research. **Yes No**

Whom Do I Call if I Have Questions or Problems?

For questions about the study or a research-related injury, contact your cancer doctor,

_____. at _____.

Name(s) Telephone Number(s)

For questions about your rights as a research participant, contact the ***[NAME OF CENTER]*** Institutional Review Board, which is a group of people who review the research to protect your rights) at _____.

Where Can I Get More Information?

You may call the **National Cancer Institute's (NCI's) Cancer Information Service**.

Voice: **1-800-4-CANCER (1-800-422-6237)**
TTY: **1-800-332-8615**

Visit the NCI's Web sites:

CancerTrials provides comprehensive clinical trial information
http://cancertrials.nci.nih.gov

CancerNet™ provides accurate cancer information, including PDQ.
http://cancernet.nci.nih.gov

You will get a copy of this form.

Signature

I agree to take part in this study.

Participant: _____ Date: _____

Study Plan

Register

↓

Submission of required research samples

↓

Diagnostic review, if required

↓

Register to ECOG Treatment trial

Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

Appendix II

Informed Consent Guidelines

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations (they also represent sound research practice):

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines Federal Register Volume 46, Number 17, Jan 27, 1981, Part 50) and the Office for Protection from Research Risk Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations. Consent for specimen submission will be obtained and documented with consent for the appropriate treatment protocol (or laboratory study where applicable).

Use of Specimens for Research

The patient is free at any time in the future to decide to withdraw his/her specimens from future scientific research. Such a decision will have no impact on his/her treatment or other aspects of participation in the study.

Institutional Review

The study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (see Federal Register Guidelines, Volume 46, Number 17, Jan 27, 1981, part 56) and the Office for Protection from Research Risk Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

Appendix III

Patient Thank You Letter

[PATIENT NAME] [DATE]
[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in our research and banking effort for myeloma. Programs like this offer a chance to get the best care while helping us make better care available for all patients. Many questions remain unanswered in myeloma. With the help of people like you who participate in these programs, we will achieve our goal of effectively treating and ultimately curing myeloma.

This small gesture is a part of a broader program being undertaken by ECOG and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

On behalf of [INSTITUTION] and the Eastern Cooperative Oncology Group, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

Appendix IV

Myeloma Tumor Biology Kit

Specimen Checklist and Shipping Instructions

**** PLEASE AVOID DRAWING OR SENDING SPECIMENS ON FRIDAYS AND HOLIDAYS****

Kit Contents:

- 5 lb Styrofoam box and cardboard mailing sleeve
- Patient Information Form
- Materials Submission Form
- FedEx Airbill with pre-printed return address
- 7ml ACD (yellow top) collection tubes
- PCLI Kit
- (3) zip lock specimen bags labeled for bone marrow and peripheral blood
- (1) ice pack. Place the ice pack in the freezer for at least 24 hours prior to specimen shipment. Allow the frozen ice pack to thaw at room temperature for 2-3 hours **before** preparing the specimen for shipment.

Packing and Shipping Instructions:

1. Collect the following specimens:
   1) Peripheral blood - Draw 7mls of peripheral blood in one ACD tube
   2) Bone marrow aspirate - Draw 5mls of a 'redirect' bone marrow aspirate in 1 ACD tube
   3) Bone marrow aspirate - Draw 2-3mls of a 'redirect' bone marrow aspirate and place in the Plasma Cell Labeling Index tube; process according to kit instructions
   4) Bone marrow core biopsy slides - 5 air-dried unstained biopsy slides (plus or charged slides). **Exception**: Unstained core biopsy slides can be mailed later if they are not available at the time of shipping the bone marrow aspirate and peripheral blood.
2. Place the slightly thawed Kool-PAK in bottom of Styrofoam container.
3. Place absorbent toweling on top of Kool-PAK.
4. Place specimens in their individual plastic bags provided, wrap in paper toweling and place them in the Styrofoam container and close the lid. Do not place the specimen(s) directly on the ice pack.
5. Place the Styrofoam container and the completed Patient Information Form and Materials Submission form within the cardboard mailing sleeve.
6. Prepare the package for shipping, applying packing tape as needed. Complete the sender portion of the return FedEx Airbill and adhere to the exterior lid of the box. Ship specimens via overnight delivery the same day collected.
7. Notify Federal Express for pick-up and/or leave package at the designated FedEx drop-off location.

Please call Kim Henderson at (507) 284-3805 to notify the Mayo Clinic Myeloma Reference Laboratory when specimen(s) are being shipped. The samples in prepared kits should be shipped to the following:

Kim Henderson
Mayo Clinic Myeloma Reference Laboratory
613 Stabile
200 First Street Southwest
Rochester, MN 55905

Patient Information Form

Specimen Date: / /

Institution/Affiliate: _____

Physician: _____

Patient Initials (last name, first name): _____

Hospital ID or Social Security #: _____

ECOG Protocol #: E3A05

ECOG Patient Sequence #: _____

Contact Person: _____

Institution: _____

Address: _____

City State Zip

Phone #: _____

FAX #: _____

PLEASE INCLUDE A CURRENT WHITE BLOOD COUNT AND DIFFERENTIAL

WBC _____

% of Lymphocytes: _____

% of Monocytes: _____

Please indicate which samples are being shipped at this time:

1. Pre-Study

Any questions concerning these samples or to obtain a Myeloma Tumor Biology kit, please contact:

Kim Henderson
Mayo Clinic Myeloma Reference Laboratory
(507) 284-3805

Affiliates who anticipate participating in this study should please call in advance for kits.

Ancillary Laboratory Protocol for Collecting Diagnostic Material on Patients Considered for Studies of Plasma Cell Disorders

Appendix V

Multiple Myeloma Tissue Bank

The Multiple Myeloma (MM) Tissue bank was established as part of the ECOG Laboratory Correlative Study E9487 in 1988 and has continued with each successor study. The Myeloma Tissue bank (MTB) is physically located at the Mayo Clinic in Rochester, Minnesota. It is a part of ECOG's Multiple Myeloma Tumor Biology Labororatories with principle investigators Philip R. Greipp and Catherine Leith. The MTB processes samples to generate a multi-use resource tissue bank of serum, plasma, bone marrow and peripheral blood cells, DNA, RNA, cytospin and biopsy slides. The bank is organized such that it provides direct support to further scientific endeavors associated with ECOG MM clinical trials. These banked samples are available to investigators who secure extramural funding to investigate the biology of MM. The bank complies with the ECOG Policy for the Collection, Storage and Use of Biological Specimens, as well as state and federal guidelines.

A key strength of this tissue bank is its expertise in the collection, shipping and processing of the samples to obtain optimal biologic materials. To this effect the tissue bank has developed a standardized protocol to process samples, purify clonal plasma cells (PCs), and extract and store RNA, DNA, and protein. The MTB helps to store these samples under the support of the Mayo Clinic and is responsible for maintaining and validating quality assurance for these samples. Best Practices are used for standard operating procedures, continuous self-assessment, quality assurance and product offerings.

Database access is password-protected and limited to the MTB staff. Quarterly, the tissue bank directly transfers the verified data generated by the research efforts to the ECOG Coordinating and Statistical Centers and coordinates security procedures and documentation for this transfer. A limited data set is sent to insure privacy and confidentiality as outlined in our institutional Compliance and Privacy policies. This is compliant with standards to protect patient confidentiality, withdrawal of consent, inventory, and sample tracking procedures, consistent with ECOG's Policy on Confidentiality of Patient Information.

The principle of the MTB's Standard Operating Procedure is that quality assurance is a process of surveillance of significant aspects of the laboratory and its personnel to assure the quality of work done and reported by the laboratory.

We have the established the following guidelines for **general specimen integrity**.

1. Mayo provides kits to participating institutions containing the appropriate collection tubes, Federal Express shipping form, Patient Information Form, Instructions and Contact Person information.
2. All specimens are shipped by Federal Express Overnight Priority according to the specifications of each protocol.
3. Patient identification of specimens is assured by the requirement of labels on each collection tube including patient initials, sequence number (if available), date, type of sample and completion of the Patient Information Form and Materials Submission Form specific for each protocol.

Specimen collection occurs at every referring ECOG institution according to the guidelines of each clinical trial. Submission of both bone marrow (BM) and peripheral blood (PBL) is encouraged with ACD A or B as the preferred anticoagulant. Peripheral blood is accepted if BM is unavailable.

Patients who register to E3A05 (or another ECOG protocol) are assigned a protocol sequence number and their specimens are entered into our tissue bank. To facilitate the tracking of the multiple samples required the MTB utilize a Check-List as a part of the Patient Information Form and Shipping Instructions included in each kit. Various aspects of sample collection can affect the integrity of the specimen. Although collection procedures cannot be directly controlled by the MTB, multiple efforts have been made to train research nurses and clinical research assistants at referring ECOG institutions. Particular focus is given to sterility of specimens, use of anti-coagulants and mixing of specimens to avoid clotting, temperature of storage and transport of specimens (the WTB proposes transient storage of 2-8^{o}C and the use of ice-packs during transport) and the time-lapse between drawing and shipment (the WTB emphasizes that specimens should

be shipped the same day they are drawn). Clients are asked to avoid drawing specimens on Fridays and holidays.

Upon receipt in the laboratory, the following procedure is done:

1. Specimens are checked to verify samples were collected and shipped properly. Any problems (clotted sample, received frozen or ambient, expired tubes, missing samples, etc.) are noted on the Patient Information Form for reference when reviewing data and the Materials Submission Form.
2. Specimen labels are checked to verify patient initials, sequence number, and date match the Patient Information Form. The Data manager (or contact person) is notified on any discrepancies to investigate before processing samples.
3. Specimens are logged in our electronic data spreadsheet. All hard copy paperwork is permanently retained on file. The patient's paper file contains the Patient Information Form, a photocopy of the ECOG Material Submission Form specific for the Treatment Protocol and the electronically received notification from ECOG of the assigned protocol sequence number. These are filed according to clinical trial protocol in a limited access area.
4. If applicable, correlative studies are performed before cell material is set aside for banking.
5. Any material available for banking is aliquoted to ensure storage under various conditions.
6. Data are entered on ECOG's myeloma database by the laboratory technologist.

To store cells under various conditions provides flexibility for future research studies. Unless otherwise dictated by a protocol, the following general "sample handling" represents the most common sequence of events that is followed by the MTB.

All samples are handled using **sterile technique**.

Ten milliliters of peripheral blood is drawn in a 10cc red top(clot) tube. The client is asked to spin the specimen down at 2500 rpm for 10 minutes and remove the **serum**. The serum is placed in a plastic screw top vial provided in the kit and shipped to the MTB. Five hundred microliters are removed for a correlative study. The remainder of the serum is banked in 1-ml aliquots using 1-ml Nunc cryotubes. These crytotubes are placed into 2-inch cardboard freezing boxes with grids stored at -70^{o}C. ECOG specimens are stored separately in boxes labeled with "ECOG" and the specified study protocol number. The location is recorded on a freezer map and electronically in the data spreadsheet.

Fourteen milliliters of **peripheral blood** is drawn into two 7cc yellow top (ACD solution B) tubes. Five hundred microliterss are removed for a correlative study. Prior to banking, monocuclear cells (MNC) must be isolated. Isolation of MNC ensures the removal of mature granulocytes, red cells and dead cells yielding lymphocytes, moncytes and plasma cells. MNC are obtained by centrifugation of specimens, diluted with RPMI (cell culture media), through a density gradient (Ficoll-Hypaque) at 400xg for 20 minutes at room temperature. MNC removed from the Ficoll interface are washed in RPMI. and resuspended in S10 (RPMI with 10% fetal calf serum). A cell count is performed. Cells are frozen in sterile freezing media; S20 with 10% dimethylsulfoxide (DMSO) in 1-ml Nunc vials with 5 - 30 x 10^6 cells/vial. If cell count is less than 5 x 10^6 transfer the cells to a 2-ml sarstedt screw top tube. Spin for 5 minutes in the Sorvall Biofuge set at 13. Remove supernatant. Snap freeze remaining pellet in liquid nitrogen. These vials are placed into 2-inch cardboard freezing boxes with grids stored at -70^{o}C for initial freezing. When full, this box is placed in permanent storage of liquid nitrogen. The location is recorded on a freezer log sheet and electronically in the data spreadsheet.

Two to three milliliters of a "redirect" bone marrow are drawn and placed into a Plasma Cell Labeling Index (PCLI) tube. The client is asked to process the sample according to the procedure provided in the kit. The MNC are isolated using the Ficoll-Hypaque procedure (detailed in the previous paragraph). A cell count is performed. Twelve single well cytospin **slides** with approximately 75,000 cells per well are made (using 1 x 10^6 cells). Three of the slides are used for a correlative study. The remaining nine slides are banked for future use. These slides are allowed to air dry and wrapped in aluminum foil. Each packet is labeled and stored at -70^{o}C in a 3-inch box labeled "ECOG" and the specified study protocol number. The location is recorded on the electronic data spreadsheet.

Five to ten milliliters of a "redirect" bone marrow are drawn into either a 7cc yellow top (ACD solution B) or 10cc yellow top (ACD solution A) tube according to protocol submission requests. The tube is spun at 400xg for 10 minutes. The **bone marrow plasma** is removed and aliquoted into 1-ml Nunc cryotubes with a minimum volume of 250 microliters per vial. These crytotubes are placed into 2-inch cardboard freezing boxes with grids stored at -70^{o}C labeled "ECOG" and the specified study protocol number. The location is recorded on a freezer map and electronically in the data spreadsheet. The remaining red cells are lysed using ACK potassium chloride lysis solution, leaving a nucleated cell product. To process add 1-ml of RPMI to the remaining bone marrow sample, after which the entire sample (maximum of 5- ml) is brought up to a total volume of 50-ml with ACK Lyse. After centrifugation at 400xg for 5 minutes, the supernatant is discarded and the cell pellet is resuspended in 50-ml of RPMI. Following centrifugation, cells are resusupended in 1 to 5-ml of bead buffer and cells are counted. Twelve double well cytospin **slides** are made with approximately 75,000 cells per well are made (using 1.875 x 10^6 cells). Slides are banked for future use. These slides are allowed to air dry and wrapped in aluminum foil. Each packet is labeled and stored at -70^{o}C in a 3-inch box labeled "ECOG" and the specified study protocol number. The location is recorded on the electronic data spreadsheet. The isolation of CD138+ cells is performed as follows (using cells post-ACK Lyse as described above). Washed cells (no more than 80 x 10^6 cells per tube) are resuspended in 90 microliters of bead buffer [phosphate buffered saline pH 7.2 (PBS), supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA] per 10 x 10^6 total cells. MACS CD138 MicroBeads are added at a ratio of 1 microliter beads/1 x 10^6 cells) and cells are incubated on ice for 15 minutes. Cells are then washed, resuspended in 0.5-ml of the bead buffer, and separated into CD138+ and CD138- cells on the AutoMACS. To maximize yield, when the sample is almost completely drawn up an additional 0.5-ml bead buffer is added. A cell count is performed on the **CD138+ fraction**. The cells are distrubuted as follows:

0 to 3 x 10^6: Qiagen buffer for future RNA extraction

4 to 6 x 10^6: 2 x 10^6 Trizol for future DNA and Protein extraction

Remaining Qiagen buffer for RNA extraction

7 to 10 x 10^6: 2/3 Qiagen buffer for future RNA extraction

1/3 Trizol for DNA and Protein extraction

>10 x 10^6: 4 x 10^6 Qiagen buffer for RNA extraction

4 x 10^6 Trizol for DNA and Protein extraction

Remaining in sterile freezing media (S20 with 10% dimethylsulfoxide)

These crytotubes are placed into 2-inch cardboard freezing boxes with grids stored at -70^{o}C. ECOG specimens are stored separately in boxes labeled with "ECOG" and the specified study protocol number. The location is recorded on a freezer map and electronically in the data spreadsheet. To ensure the purity of the plasma cells post-purification, a single well cytospin slide made from 5,000 cells is made and sort purity is assessed using fluorescein conjugated anti-kappa and AMCA conjugated anti-lambda. If the plasma cell percentage is above 5% in the initial bone marrow sample, the purity of plasma cells in the sort is greater than 95% and the kappa to lambda ratio is greater than 99:1 or less than 1:99.

The RNA will be used for gene-array expression analyses, Northern and RT-PCR assays and the DNA for sequencing, polymerase chain reaction (PCR), telomere length studies and array-based comparative genomic hybridization (CGH).

All labeling of slides and cryotubes is done with permanent-ink marking pens that are resistant to moisture and ensure that writing remains legible under extreme temperature conditions. Each is labeled with the sequence number, patient initials, ECOG protocol number, date of sample, type of sample (BM, PBL, 138+ BM) and cell count.

All equipment used in the processing and storing of specimens undergo semiannual or annual preventative maintenance. This includes piptes, centrifuges, refrigerators, freezers, sterile hoods, flow cytometer, spectrophotometer, cytospin centrifuge and microscopes.

Freezers that are part of the MTB are located both within the confines of the Hematology Research Laboratory and in a separate freezer room, adjacent to the laboratory space. Temperature and performance of all freezers are monitored by two complimentary alarm systems that provide coverage 24 hours a day, seven days a week: Andover Control System, a computerized system, and a central station monitored system. Both alarm systems are checked during the semiannual preventive maintenance for each freezer. Each freezer has an inherent audible alarm, independent of these two alarm systems. An emergency phone number list is used by both alarm systems and has also been provided to the Mayo Security Department. People on this list are staff members of the ECOG Myeloma Tissue Bank, the laboratory director several members of the Hematology Research Laboratory. In the event that one freezer needs to be emptied, the Mayo Clinic provides a freezer cooled to the desired temperature 24 hours a day, 7 days a week within 1-2 hours. Mayo Clinic also provides preventive maintenance and repairs on all freezers.

The Stabile Building on the downtown Mayo Clinic campus is constructed with "fire controlling" materials. The floor design and building materials are meant to confine a fire up to 2 hours. The Stabile building also has an automated sprinkler system. Notifier is the computerized system used throughout the Mayo Clinic Campus. This system automatically notifies the Mayo Clinic operators and facilities (maintenance and security). This system dispatches fire trucks to the appropriate site until the cause of the alarm has been determined.

The MTB has established the following guidelines for **tissue bank integrity**.

1. All samples (serum, plasma, slides, bone marrow and peripheral blood cells, RNA, DNA and protein) contained in the tissue bank are labeled with sequence number, patient initials, study number, date and type of sample. Serum, plasma and slides are stored at -20^0C or -70^0C. Bone marrow and peripheral blood cells are cryopreserved in DMSO and stored in liquid nitrogen. Prior to extraction cells for RNA are stored in Qiagen buffer and cells for DNA/Protein are stored in Trizol. Their location is recorded on freezer maps and electronic spreadsheets according to the study they are enrolled in.
2. The ECOG Myeloma Laboratory Committee and the ECOG group chair reviews all requests for samples. No samples are released prior to their approval. Samples are sent to the requesting investigator with the following identifiers; sequence number and date sample procured. Once samples are pulled the remaining number of vials/slides is recorded in the appropriate electronic spreadsheet.

These banked samples are available to investigators. Once a request for specimens is received, Kim Henderson the ECOG Myeloma Tumor Biology Technologist checks the repository for availability of the particular sample type to assure feasibility of the study. This begins an active correspondence with the investigator to assure that the specimens available are suitable and sufficiently characterized. If the number and biologic properties of specimens are confirmed in the tissue bank are satisfactory to the investigator and, if necessary, to the statistician involved in the design of the study, the requesting investigator is asked to submit a protocol to the ECOG Laboratory Scientific Advisory Committee. Once approved it is sent to NCI for review or for file, as appropriate. In addition, ECOG's "Laboratory Investigator Agreement" needs to be signed by the investigator prior to initiation of the study. This agreement outlines the responsibilities of the investigator when she/he conducts trials utilizing ECOG resources. The investigator's IRB approval to conduct the proposed research must be submitted to the MTB.

At this point, the samples will be released from the MTB and mailed under appropriate conditions to the investigator. The electronic data base is adjusted showing "when", "what type" and "to whom" the specimens have been released.