THE TERRY FOX PAN-CANADIAN MULTIPLE MYELOMA MOLECULAR MONITORING COHORT STUDY (THE M4 STUDY)

PROJECT LEADER: Tony Reiman

PRINCIPAL INVESTIGATORS: Donna Reece
Nizar Bahlis
François Bénard
Suzanne Trudel

CO-INVESTIGATORS: Aldo Del Col
Jonathan Sussman
Trevor Pugh
Chris Venner
Rodger E. Tiedemann
Matthew Cheung

SUPPORTED BY: Terry Fox Research Institute

TABLE OF CONTENTS

1.0 OBJECTIVES

1.1 Primary Objective

To compare PET scans, immunoglobulin gene sequencing (IgS), and multiparameter flow cytometry (MFC) – alone, and in combination – to detect minimal residual disease (MRD) in multiple myeloma patients.

1.2 Secondary Objectives

To explore mechanisms of drug resistance, circulating tumour DNA analyses, and characterization of myeloma progenitors as potential future additions to myeloma characterization and monitoring protocols.

To establish the health care costs and benefits associated with MRD assessment.

To measure quality of life experienced by MM patients undergoing MRD testing, as it relates to treatment effects, their disease, and their individual patient characteristics.

1.3 Study Summary

This prospective cohort study aims to enroll 250 newly diagnosed MM patients across Canada. Blood and bone marrow biospecimens will be collected at multiple time points from each patient for research. PET scans will be performed on a subset of patients at defined time points. Questionnaire data will be collected to inform economic and quality of life analysis of the patient cohort. IgS and MFC MRD assays will be performed on the collected bone marrow specimens and their relative sensitivity and cost will be compared to one another and to PET scans. An algorithm for integrating these different methods of MRD evaluation into clinical practice will be developed that strikes the best balance in terms of diagnostic and therapeutic utility, cost and quality of life in the Canadian context.

Additional research exploring mechanisms of drug resistance, profiling of circulating tumour DNA, and characterizing myeloma progenitor populations that persist despite treatment will be conducted. These promising areas of investigation aim to bring more novel methods of myeloma disease characterization and monitoring closer to clinical application.

This protocol outlines the study procedures for participating clinical sites regarding patient selection, enrollment, informed consent, collection of study data and biological specimens for research. Some information is included throughout this protocol document in brief, regarding the planned laboratory research and analysis plan. The Research and Development (R&D) Plan for this study, which contains much more detailed and complete information about the planned research, is being provided to the clinical sites along with this protocol. The R&D Plan must be submitted along with this protocol to the local Research Ethics Board and any other institutional review committee as required for local study activation. This protocol document and key attachments (myeloma diagnostic criteria, schedule of events, informed consent document, laboratory manual) includes the information that will be needed on a daily basis to enable sites to enroll patients to this study.

It is important to note that clinical data on patients in the M4 study will be collected by also enrolling them in the separate Canadian Multiple Myeloma Database (CMM-DB) study, which has its own protocol and informed consent process. Sites participating in M4 must also participate in the CMM-DB. M4 data will subsequently be linked to CMM-DB patient outcomes data for analysis. No identifying patient information will be collected in the M4 study, and each patient will be assigned an alphanumeric identifier code that can only be linked back to the patient by the study team at the participating clinical site. The M4 patient identifier will be entered into the CMM-DB and used to link the two datasets.

2.0 BACKGROUND INFORMATION AND RATIONALE

2.1 Disease Characterization and Monitoring in Multiple Myeloma

Multiple myeloma (MM) is characterized by the accumulation of malignant, monoclonal plasma cells in the bone marrow (BM) with unique, patient-specific rearrangements of immunoglobulin (Ig) heavy and light chain genes. Most MM patients' serum contains a preponderance of secreted monoclonal immunoglobulin (M protein), including both intact immunoglobulin and free light chains. *[González et al., 2007]* For many years, standard MM disease monitoring involved BM aspirates and biopsies to assess the abundance of plasma cells, and the quantitation of serum M protein using electrophoresis. However, while the measurement of M protein by electrophoresis generally reflects the abundance of monoclonal plasma cells, it is not comparable between patients, and does not always adequately reflect changes in disease burden even within the same patient. The development of a robust serum free light chain (sFLC) assay improves sensitivity but is still limited, as there are some patients in whom little or no myeloma Ig is secreted *[Rajkumar et al., 2011]*; most patients go on to relapse despite negative electrophoresis results.

2.2 The Search for Better Methods to Monitor Minimal Residual Disease

In patients who have a complete response to therapy (as defined by electrophoresis and sFLC assays), more sensitive methods can detect and quantify MRD – and there is now a convergence of highly effective myeloma therapies that produce deep remissions and advances in the technology of MRD monitoring.

2.2.1 Multiparameter Flow Cytometry

For many years, BM flow cytometry has been used to detect and quantify clonal plasma cells, as a means of diagnosing MM and monitoring MRD. Earlier versions of MFC incorporated 2 to 4 colour instruments, allowing the simultaneous detection of a limited number of plasma cell surface proteins using fluorescent antibodies, with sensitivities of ~1:10,000 cells. *[Rajkumar et al., 2011]* Multiparameter, 4-6 colour BM MFC for MRD post-transplant was found to be a powerful prognostic marker in the UK Myeloma IX *[Rawstron et al., 2013; Rawstron et al., 2015]* study and the Spanish Grupo Español de Mieloma (GEM2000/GEM2005) *[Paiva et al., 2008]* studys. Newer MFC techniques using at least 8 colours to identify residual plasma cells have sensitivities comparable to conventional BM IgH polymerase chain reactions (PCR) at 1 in 100,000 cells, with the advantages of being less expensive and applicable to most patients but requiring the analysis of freshly obtained marrow. *[Mailankody et al., 2015]* The most current methods using 10-12 colour MFC ("Next generation flow", "Eurotube") are proposed to have sensitivities of ~1 in 1 million cells, although prospective validation of this level of sensitivity is still needed. Post-transplant MFC can discriminate between patients destined for long-term

survival and those likely to have early relapse, particularly when paired with a thorough baseline assessment of myeloma cell biology.

2.2.2 Immunoglobulin Gene Sequencing

Next-generation IgS is being validated in several patient cohorts and again demonstrates the powerful prognostic significance of obtaining the deepest possible remission. *[Schinke et al., 2015]* Amplification of Ig heavy and light chain variable sequences followed by next-generation sequencing can identify unique sequences that characterize an individual patient myeloma clone in >90% of cases without the need to develop consensus primers, a vast improvement in applicability over older PCR-based methods. *[Martinez-Lopez et al., 2014]* Further, the ability to sequence individual DNA strands allows for quantification of clonal sequences without needing to develop a standard qPCR curve for each patient. The sensitivity of this method for clonal sequence detection is as low as 1 clonal sequence per million cells. Intriguingly, as Ig transcripts are highly abundant in myeloma cells, the sensitivity of RNA-based IgS is probably even greater (though less quantitative) than that for DNA. Blood-based IgS with DNA or RNA is comparably sensitive to 6-colour BM MFC and holds promise as a means to reduce the needed frequency of BM examinations for sensitive disease monitoring. *[Vij et al., 2014]*

Comparing MFC and IgS, MFC is applicable to virtually all patients and is widely available (though many Canadian laboratories would require technology upgrades to implement 10-12 colour MFC). MRD can be assayed by MFC without the need to compare to the diagnostic sample but requires a fresh sample. On the other hand, IgS is currently felt to be more sensitive; in the Intergroupe Francophone Du Myélome/Dana-Farber Cancer Institute (IFM/DFCI) 2009 study, IgS has been found to be more sensitive than 7-colour MFC and to discriminate patients with better or worse prognoses depending on IgS-defined MRD status, even amongst MFC-defined MRD-negative patients. *[Avet-Loiseau et al., 2015]* IgS can also be conducted using frozen material, but is more costly and requires a pre-treatment sample; in 5-10% of patients the clonal sequence cannot be determined for IgS. Nonetheless, while the expense and availability of this technology is currently limiting, it is expected that these limitations will be rapidly overcome as next generation sequencing is increasingly incorporated into clinical laboratories.

2.2.3 Whole Body Imaging of Myeloma to Monitor Disease

Another approach to monitoring MM disease burden is diagnostic imaging, which can detect disease outside of BM and blood, and has the potential to overcome sampling error inherent in BM aspiration. The most promising imaging modality for the detection, staging, prognosis and treatment response assessment of MM is PET, specifically $_{18}$F-FDG PET/CT. $_{18}$F-FDG is taken up by metabolically active myeloma cells, revealing their anatomic distribution particularly when combined with concomitant CT imaging. Clearance of $_{18}$F-FDG-PET-detected myeloma following treatment provides prognostic information that is potentially comparable or complementary to that achieved with blood and BM-based assays. An Italian study of patients undergoing autologous stem cell transplant (ASCT) initially demonstrated the potential power of post-transplant $_{18}$F-FDG-PET, *[Zamagni et al., 2015]* and a recent analysis of a larger French study in collaboration with the Dana-Farber Cancer Institute has shown similar results. *[Moreau et al., 2015]* In contrast, a smaller study incorporating highly active novel agents without transplant at Memorial Sloan-Kettering Cancer Centre suggested that $_{18}$F-FDG-PET did not add to the information gained from molecular MRD monitoring. *[Korde et al., 2015]*

2.2.4 Improving Patient Outcomes through MRD Monitoring

The results of large randomized studies have made it customary for clinicians to offer continuous, indefinite therapy, yet there is research to suggest that this practice may not be optimal for all MM patients. Mayo Clinic data using conventional electrophoresis and immunofixation results to assess remission status post-ASCT, without MFC or IgS, demonstrated high rates of overall survival (OS) even without maintenance therapy for those in a conventionally defined stringent complete response (sCR). *[Kapoor et al., 2013]* The UK and Spanish studies demonstrated that MRD assays can identify the subset of patients in CR who achieve these excellent results, with or without maintenance therapy. In the UK study, PFS appeared superior with the addition of maintenance therapy even in patients who achieved MRD-negative status, but OS remained excellent in MRD-negative patients even without maintenance. A large IFM/DFCI randomized study incorporating MRD testing following discontinuation of maintenance therapy after 12 months recently demonstrated that with modern frontline therapy, MRD-negative status with either $_{18}$F-FDG-PET or IgS predicts high rates of PFS, with MRD-positive patients showing significant relapse rates after stopping maintenance therapy. *[Avet-Loiseau et al., 2015]* Taken together, these results suggest that standard risk, MRD-negative patients might not need indefinite therapy. Conversely, in the UK study high-risk patients who remained MRD-positive had such low PFS rates that this group of patients should potentially be targeted for identification of new treatment strategies. An algorithm indicating when each of these assays should be performed during the clinical course can become the standard for adoption into future clinical studies, and indeed those conducted globally, as well as in clinical practice.

2.3 Developing New Approaches to Myeloma Molecular Characterization and Monitoring

The heterogeneity of the myeloma clone within a given patient is becoming better understood and will clearly have therapeutic implications; it is crucial to understand the characteristics of those MM cells that survive therapy and mediate relapse, and how to best target these cells. The mechanisms of action and resistance to current and future therapies continue to be elucidated and have yet to be incorporated into clinical practice, but will be key to understanding in real time how myeloma cells are escaping therapy, so that clinicians can intervene early to improve patient outcomes. Building upon the MRD monitoring component of the present study, there will also be several novel lines of investigation integrated into the cohort, to identify more sophisticated ways to characterize and monitor MM clones in each patient.

2.3.1 Monitoring Mechanisms of Drug Resistance

While lenalidomide is deemed to be the current "standard" maintenance regimen post-transplant, it remains unclear which patients can benefit from this costly approach. Further, the majority of patients acquire resistance to IMiDs within a couple of years of initiating therapy. *[Benboubker et al., 2014]* Based on previous work, IMiD resistance appears to result from one of three events:

1. Loss or decreased expression of the substrate receptor cereblon (*CRBN)*, the adaptor within the Cul4a cullin-RING finger ubiquitin E3 ligase that is required for IMiD activity (~ 30% of patients);

2. Splicing of exon 10 within the *CRBN* IMiD binding domain (also known as thalidomide binding domain or TBD) (~ 20% patients); and

3. Up-regulation of c-*MYC* expression or a "switch" in the enhancer or superenhancer, converting *MYC* transcription from an *IKZF1*-responsive to an *IKZF1*-non-responsive enhancer. (~50% patients).

The mechanisms that lead to resistance to IMiDs had remained largely elusive; decreased expression of *CRBN* is not the sole mechanism. *[Bolli et al., 2014; Chapman et al., 2011]* It is proposed in this study that it is possible to reliably identify these events and hence accurately predict IMiD resistance through genomic studies. This will then not only allow precise and rational drug selection but will also lead to the discovery of novel means to enhance the activity and/or overcome the resistance to this class of drugs, as well as provide the clinician with a valuable tool to adjust therapy prior to the development of clinical resistance and organ damage.

2.3.2 Circulating Tumour DNA (ctDNA) Sequencing Panel

With the development of sensitive molecular platforms, it is now possible to detect cancer-associated genetic alterations from cell-free DNA (cfDNA) (e.g., point mutations, copy number variations, chromosomal rearrangements), offering the potential for blood-based biomarkers. *[Schwarzenbach et al., 2011]* A number of studies have shown correlations between the presence of tumor-associated genetic alterations in blood and PFS/OS in breast cancer patients. *[Schwarzenbach et al., 2012; Silva et al., 2002]* Similarly, mutations present in plasma of advanced-stage non-small cell lung cancer (NSCLC) patients predicts for poor outcome to first-line chemotherapy. *[Nygaard et al., 2013]*

Further studies in diffuse large B-cell lymphoma, colon and breast cancer have demonstrated the utility of cfDNA for detection of residual disease and as a powerful predictor of recurrence. *[Cristofanilli et al., 2004; Reinert et al., 2015; Roschewski et al., 2015]* Additionally, information acquired from a single biopsy provides a spatially and temporally limited snapshot of a tumor and might fail to reflect its heterogeneity. The requirement of painful BM aspirates for surveillance can result in the refusal of patients to provide sequential BM samples. The use of blood-based biomarkers can avoid the limitations of traditional "single-site" biopsy and allow for non-invasive serial sampling and analysis of the entire tumor genome.

The investigators recently have employed a more flexible, scalable strategy of ultra-deep, full-length gene sequencing of ctDNA from patients with MM, as an alternative to BM aspirates currently used for clinical testing. A clinical-grade ctDNA sequencing panel to query all frequent genomic alterations in MM and thus possibly guide personalized approaches to MM care (with a particular focus on prognostic markers, recurrent actionable somatic mutations, MRD assessment and genomic alterations associated with drug sensitivity) will be applied to patients in the present study.

2.3.3 Multiple Myeloma Progenitors

The absence of a cure in the vast majority of patients, despite advances in therapy and the realization of deep clinical responses, points to the existence of drug-resistant tumor cells with tumor initiating capability, *[Matsui et al., 2008]* though these cells have remained elusive and poorly characterized in MM. In previous work, phenotypic or functionally distinct subpopulations of tumor clone cells ("progenitors") in MM were identified, which recapitulate the physiologic maturation stages between B cells and plasma cells (PCs) and that include Xbp1s- MM cells that appear innately insensitive to PIs. *[Leung-Hagesteijn et al., 2013]* These drug-resistant Xbp1s- MM progenitor subpopulations may be an important root cause of MM relapse and contribute to the current failure to cure MM *[Chng et al., 2014]*; however, comprehensive data is lacking.

Although Xbp1s- MM progenitors appear PI insensitive, it is unknown whether these cells respond to or can survive treatments such as high-dose melphalan and ASCT; furthermore it is unknown whether or not they are fully malignant (genetically identical to the mature PCs that constitute the MM tumor bulk cells) or if they can produce MM relapse and disease in patients. The optimal means to target these cells is unknown, and it is unclear if they will respond to emerging immunotherapeutics (which are likely to become the next standard of care).

To achieve deeper and more durable therapeutic responses for patients, another aim of this study is to leverage the recent identification of MM progenitor subpopulations to help guide the development of better therapeutic strategies that address residual disease in MM. The extent and characteristics of progenitor MRD in MM will be examined to determine which if any tumor subpopulations preferentially survive treatment and that should be better targeted. Treatments will be compared to determine if any have superior activity against specific tumor subpopulations. The mutational landscape of MM plasma cells and MM progenitors will be compared over longitudinally acquired serial samples from patients treated with standard MM therapies to determine if MM progenitor subpopulations possess or lack mutations identified in concurrent MM PC subpopulations, or if MM progenitor subpopulations contribute to later relapsed PC populations. This will help define the extent to which dynamic transitions occur between these tumor subpopulations and whether progenitor subpopulations contribute to evasion of standard therapy. As well, from this characterization of restricted antigen expression on MM progenitors, we aim to provide a basis for broader immunotherapeutic strategies in MM that may provide more durable responses against tumors that demonstrably contain marked intra-clonal functional and genetic diversity. Together, these studies will substantially clarify the roles of MM progenitor subpopulations in therapeutic evasion, relapse and tumor evolution and will provide a framework for targeting of these subpopulations in the clinic.

3.0 STUDY POPULATION

This is a pan-Canadian prospective cohort study run through the University of New Brunswick with financial support from the Terry Fox Research Institute. The aim is to recruit 250 patients over the course of the five years of the study.

3.1 Eligibility Criteria

Questions about eligibility criteria should be addressed prior to registration. For a patient to be eligible for admission to the study:

- Age ≥ 19 years;
- Ability to give informed consent;
- Diagnosed with active multiple myeloma (refer to Appendix I for IMWG definition);
- Also enrolling in the CMM-DB project; and
- Previously untreated and eligible for autologous stem-cell transplantation (ASCT).

Patients who are going to be treated on a clinical trial are also eligible to participate in this study if they meet the other eligibility criteria.

3.2 Informed Consent

Patient consent must be appropriately obtained in accordance with applicable local and regulatory requirements. Each patient must sign a consent form prior to enrollment in the study to document their willingness to participate.

4.0 ENTRY AND REGISTRATION PROCEDURES

All eligible patients enrolled in the study by the participating treatment centre will be assigned a serial number which must be used on all documentation and correspondence with the coordinating centre (University of New Brunswick). The following information will be required:

- Study code (M4 XX.XX)
- Informed consent version date, date signed by patient, name of person conducting consent discussion and date signed

Note: The validity of study results depends on the authenticity of and the follow-up of all patients entered into the study. Under no circumstances, therefore, may an allocated patient's data be withdrawn prior to final analysis, unless the participant withdraws from the study and requests that data collection/submission cease from the point in time of withdrawal.

All eligible patients admitted to the study will be followed by the coordinating centre. It is the responsibility of the physician in charge to satisfy himself or herself that the patient is indeed eligible before requesting registration.

5.0 PLAN FOR CLINICAL EVALUATION AND DATA COLLECTION

M4 study data – i.e. clinical data, blood and bone marrow samples, lab test results, economic data and patient-reported outcomes – will be collected from all patients, and PET scan data will be collected on all patients at baseline and on approximately 80 (those in complete response (CR)) after 12 months of maintenance therapy (more detail is provided in *Table 1*). BM for flow MRD will be collected at three time points only: baseline, 100 days post-ASCT and after 12 months of maintenance therapy. Clinical data will be collected via the CMM-DB Project, under a separate protocol and consent process. M4 data and CMM-DB data will be linked for subsequent analysis, with the remaining data and biospecimens collected in the present M4 study.

Multiple myeloma diagnosed and patient consented

↓

Treatment plan, baseline M4 data collection*

↓

Baseline ^{18}F-FDG-PET scan (all patients)

↓

Initial therapy

↓

M4 data collection

↓

Stem cell mobilization and collection

↓

Autologous stem cell transplantation (ASCT)

↓

M4 data collection 100 days following ASCT*[#]

↓

Maintenance therapy

↓

M4 data collection* and ^{18}F-FDG-PET scan (on 80 patients in CR)
after 12 months of maintenance therapy

↓

M4 data collection every 6 months
until end of study, relapse or death

**BM for flow MRD collected at this time point*
[#] A subset of up to 20 patients will have this assessment at 1-3 days post-transplant instead

Table 1. Schedule of Visits

Research and Treatment Visits	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9+**	**R**
Before treatment begins: Patient and researcher discuss the study and sign informed consent. Patient is screened for inclusion or exclusion in study.	**X**									
Data Collection for Research		Start chemotherapy		Stem cell transplant		Maintenance therapy				
Blood collection~	**X**		**X**		**X**		**X**	**X**	**X**	**X**
Bone marrow collection~	**X**^		**X**		**X**^		**X**^	**X**	**X**	**X**
PET/CT scan*	**X**						**X**			
Self-report quality of life questionnaires	**X**		**X**		**X**		**X**	**X**	**X**	**X**
Occupation Recording	**X**									
Study nurse completes Resource Utilization Form	**X**		**X**		**X**		**X**	**X**	**X**	**X**

~In addition to amounts collected for clinical purposes.

** All patients to receive a PET/CT at baseline; an additional 80 patients (those in CR) will also receive one after 12 months of maintenance therapy.*

^BM for flow MRD collected at this time point.

Year 1 Visits: Prior to treatment (#1); Following chemotherapy (#3); Following stem cell transplant, most participants will undergo assessment at day 100 (±20 days), before starting maintenance therapy; up to 20 patients will be asked to undergo assessment instead at 1-3 days post-transplant (#5). **Year 2 Visits**: After 12 months of maintenance therapy (#7), and 6 months after Visit #7 (#8). **Years 3 and beyond**: Every 6 months (#9+) until the study is completed, myeloma relapses, or death; **R (Relapse):** Additional visit will occur in case of relapse.

6.0 CRITERIA FOR MEASUREMENT OF STUDY ENDPOINTS

To assess the level of response in patients, the following guidelines from the International Myeloma Working Group will be used. It is expected that these tests form part of the standard of care for myeloma patients, but it is recognized that there are variations in local approaches to response assessment. The following table outlines the recommended standard of care tests for response evaluation. It is strongly recommended that this schedule of standard response evaluation be followed as closely as possible. The available data on standard response assessment will be captured on M4 study patients in the CMM-DB protocol in which all M4 patients are required to enroll, and will be used as part of the M4 analysis. However, in the end, the standard of care response evaluation for each patient is left to the discretion of the attending physician.

Table 2. *Required Baseline and Follow-Up Tests for Response Assessment [Kumar et al., 2016]*

	Every response assessment time point	If electrophoresis shows no measurable protein	At suspected CR	At suspected progression (clinical or biochemical)
SPEP	X	◦◦	X	X
Serum immunofixation	◦◦	X	X	X
UPEP	X	◦◦	X	X
Urine immunofixation	◦◦	X	X	◦◦
Serum FLC	X	◦◦	X	X
Bone marrow aspirate	X	◦◦	X	X
Plasmacytoma imaging				
Serum M-spike, urine M-spike, involved Ig FLC or bone marrow not meeting above criteria, but at least one lesion that has a single diameter of ≥2 cm	X	◦◦	X	◦◦
Haemoglobin, serum calcium, creatinine	X	◦◦	◦◦	X

CR = complete response. SPEP = serum protein electrophoresis. UPEP = urine protein electrophoresis. FLC = free light chain. IMWG = International Myeloma Working Group. X = test performed. ◦◦ = test not performed.

7.0 STATISTICAL CONSIDERATIONS

7.1 Objectives and Design

This is an investigator-initiated multicentre study assessing three techniques for the detection of multiple myeloma MRD.

7.2 Primary Endpoints and Analyses

Our primary comparison is the sensitivity of MFC versus IgS in patients who meet the conventional definition of complete remission post-treatment. We expect ~50% of patients, or 125 patients, to be eligible for this comparison.

7.3 Sample Size and Power

Based on prior studies we expect that approximately 60% (75 of 125) of patients will show evidence of MRD by MFC. We will then have 80% power with a 1-sided alpha of 0.05 to detect an increase in sensitivity of 11% for IgS compared to MFC.

7.4 Secondary Endpoints and Analyses

- Comparisons of the sensitivity of MFC and IgS with PET scans to detect MRD
- The prognostic significance of MRD assessment on PFS and OS
- The economic impact of MRD testing
- QOL in patients undergoing MRD testing
- Exploration of leading edge methods of myeloma characterization and monitoring
    - Prognostic significance of ctDNA profiles
    - Sensitivity and specificity of bioassays of drug resistance
    - Characterization of myeloma progenitor populations that persist in the MRD state

For further details about the planned laboratory, clinical, economic, quality of life and statistical analyses, please refer to the R&D Plan for this study that was approved by the Terry Fox Research Institute Executive and an International Review Committee. The R&D Plan is to be submitted with this protocol to participating sites for the information of those conducting local institutional review, including the Research Ethics Board.

8.0 ETHICAL, REGULATORY AND ADMINISTRATIVE ISSUES

8.1 Inclusivity in Research

This study does not exclude individuals from participation on the basis of attributes such as culture, religion, race, national or ethnic origin, colour, mental or physical disability (except incapacity), sexual orientation, sex/gender, occupation, ethnicity, income, or criminal record, unless there is a valid reason (i.e. safety) for the exclusion.

In accordance with the Declaration of Helsinki and the Tri-Council Policy Statement (TCPS), vulnerable persons or groups will not be automatically excluded from a clinical study (except for incompetent persons) if participation in the study may benefit the patient or a group to which the person belongs. However, extra protections may be necessary for vulnerable persons or groups. It is the responsibility of the local investigator and research ethics board (REB) to ensure that appropriate mechanisms are in place to protect vulnerable persons/groups. In accordance with TCPS, researchers and REBs should provide special protections for those who are vulnerable to abuse, exploitation or discrimination. As vulnerable populations may be susceptible to coercion or undue influence, it is especially important that informed consent be obtained appropriately.

Centres are expected to ensure compliance with local REB or institutional policy regarding participation of vulnerable persons/groups. It is the centre's responsibility to ensure compliance with all local SOPs.

Persons who cannot give informed consent (i.e., mentally incompetent persons, or those physically incapacitated, such as comatose persons) are not to be recruited into this study. It is the responsibility of the local investigator to determine the subject's competency, in accordance with applicable local policies and in conjunction with the local REB (if applicable).

8.2 Obtaining Informed Consent

It is expected that consent will be appropriately obtained for each participant/potential participant in this study, in accordance with ICH-GCP section 4.8. The centre is responsible for ensuring that all local policies are followed.

Additionally, in accordance with GCP 4.8.2, this study may require that participants/potential participants be informed of any new information may impact a participant's/potential participant's willingness to participate in the study.

Based upon applicable guidelines and regulations (Declaration of Helsinki, ICH-GCP), a participating investigator (as defined on the participants list) is ultimately responsible, in terms of liability and compliance, for ensuring informed consent has been appropriately obtained. It is recognized that in many centres other personnel (as designated on the participants list) also play an important role in this process. In accordance with GCP 4.8.5, it is acceptable for the Qualified Investigator to delegate the responsibility for conducting the consent discussion.

It is required that each participant sign a consent form prior to their enrollment in the study to document his/her willingness to take part. Written informed consent will be obtained for all study procedures, the planned analyses of the biospecimens and data, linkage to clinical outcomes data

in the CMM-DB project, and long-term banking of biospecimens and data for future research. Ongoing REB approval will be maintained for curating the M4 biobank and associated data, and future REB approval will be required for any new research to be conducted using the M4 biobank and data. It may also be required, as indicated above, that participants/potential participants be informed of new information if it becomes available during the course of the study. In conjunction with GCP 4.8.2, the communication of this information should be documented.

The use of translators is allowed in obtaining informed consent. Provision of translators is the responsibility of the local centre. Centres should follow applicable local policies when procuring or using a translator for the purpose of obtaining informed consent to participate in a clinical study.

In accordance with ICH-GCP 4.8.9, if a subject is unable to read then informed consent may be obtained by having the consent form read and explained to the subject.

8.3 Discontinuation of the Study

If this study is discontinued for any reason all centres will be notified in writing of the discontinuance and the reason(s) why. If the reason(s) for discontinuance involve any potential risks to the health of patients participating on the study or other persons, this information will be provided to centres as well.

If this study is discontinued at any time by the centre (prior to closure of the study by the coordinating centre), it is the responsibility of the qualified investigator to notify the coordinating centre of the discontinuation and the reason(s) why.

Whether the study is discontinued by the coordinating centre or locally by the centre, it is the responsibility of the qualified investigator to notify the local Research Ethics Board and all clinical study subjects of the discontinuance and any potential risks to the subjects or other persons.

8.4 Retention of Patient Records and Study Files

All essential documents must be maintained in accordance with ICH-GCP.

9.0 REFERENCES

Avet-Loiseau H, Corre J, Lauwers-Cances V, Chretien M, Robillard N, Leleu X, et al. 191 Evaluation of Minimal Residual Disease (MRD) By Next Generation Sequencing (NGS) Is Highly Predictive of Progression Free Survival in the IFM/DFCI 2009 Study. 2015.

Benboubker L, Dimopoulos MA, Dispenzieri A, Catalano J, Belch AR, Cavo M, et al. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. N Engl J Med 2014 09/04;371(10):906- 917.

Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I, et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun 2014;5:2997-2997.

Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011 03/24;471(7339):467-472.

Chng WJ, Dispenzieri A, Chim C, Fonseca R, Goldschmidt H, Lentzsch S, et al. IMWG consensus on risk stratification in multiple myeloma. Leukemia 2014 02;28(2):269-277.

Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004 08/19;351(8):781-791.

González D, van dB, García-Sanz R, Fenton JA, Langerak AW, González M, et al. Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood 2007 11/01;110(9):3112-3121.

Kapoor P, Kumar SK, Dispenzieri A, Lacy MQ, Buadi F, Dingli D, et al. Importance of achieving stringent complete response after autologous stem-cell transplantation in multiple myeloma. J Clin Oncol 2013 12/20;31(36):4529-4535.

Korde N, Roschewski M, Zingone A, Kwok M, Manasanch EE, Bhutani M, et al. Treatment With Carfilzomib-Lenalidomide-Dexamethasone With Lenalidomide Extension in Patients With Smoldering or Newly Diagnosed Multiple Myeloma. JAMA Oncol 2015 09;1(6):746-754.

Kumar S, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lanc Onc 2016; 17(8): e328-e346.

Leung-Hagesteijn C, Erdmann N, Cheung G, Keats JJ, Stewart AK, Reece DE, et al. Xbp1s-Negative Tumor B Cells and Pre-Plasmablasts Mediate Therapeutic Proteasome Inhibitor Resistance in Multiple Myeloma. Cancer Cell (Science Direct) 2013 09/09;24(3):289-304.

Mailankody S, Korde N, Lesokhin AM, Lendvai N, Hassoun H, Stetler-Stevenson M, et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. Nat Rev Clin Oncol 2015 05;12(5):286-295.

Martinez-Lopez J, Lahuerta JJ, Pepin F, González M, Barrio S, Ayala R, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood 2014 5/15;123(20):3073-3079

Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Res 2008 01/01;68(1):190-197.

Moreau P, Attal M, Karlin L, Garderet L, Facon T, Macro M, et al. 395 Prospective Evaluation of MRI and PET-CT at Diagnosis and before Maintenance Therapy in Symptomatic Patients with Multiple Myeloma Included in the IFM/DFCI 2009 Study. 2015.

Nygaard AD, Garm Spindler K, Pallisgaard N, Andersen RF, Jakobsen A. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. Lung Cancer 2013 03;79(3):312-317.

Paiva B, Vidriales M, Cerveró J, Mateo G, Pérez J,J., Montalbán M,A., et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. Blood 2008 11/15;112(10):4017-4023.

Rajkumar SV, Harousseau J, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. The Lancet Oncology 2014; 15(12): e538-48.

Rawstron AC, Child JA, de Tute R,M., Davies FE, Gregory WM, Bell SE, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. J Clin Oncol 2013 07/10;31(20):2540-2547.

Rawstron AC, Gregory WM, de Tute R,M., Davies FE, Bell SE, Drayson MT, et al. Minimal residual disease in myeloma by flow cytometry: independent prediction of survival benefit per log reduction. Blood 2015 03/19;125(12):1932-1935.

Reinert T, Schøler L,V., Thomsen R, Tobiasen H, Vang S, Nordentoft I, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. Gut 2015 02/04.

Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol 2015 05;16(5):541-549.

Schinke C, Deshpande S, Mitchell A, Faham M, Patel P, Thanendrarajan S, et al. 2979 Impact of Minimal Residual Disease in High and Standard Risk Multiple Myeloma. 2015.

Schwarzenbach H, Eichelser C, Kropidlowski J, Janni W, Rack B, Pantel K. Loss of heterozygosity at tumor suppressor genes detectable on fractionated circulating cell-free tumor DNA as indicator of breast cancer progression. Clin Cancer Res 2012 10/15;18(20):5719-5730.

Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011 06;11(6):426-437.

Silva JM, Silva J, Sanchez A, Garcia JM, Dominguez G, Provencio M, et al. Tumor DNA in plasma at diagnosis of breast cancer patients is a valuable predictor of disease-free survival. Clin Cancer Res 2002 12;8(12):3761-3766.

Vij R, Mazumder A, Klinger M, O'Dea D, Paasch J, Martin T, et al. Deep sequencing reveals myeloma cells in peripheral blood in majority of multiple myeloma patients. Clin Lymphoma Myeloma Leuk 2014 04;14(2):131-139.e1.

Zamagni E, Nanni C, Mancuso K, Tacchetti P, Pezzi A, Pantani L, et al. PET/CT Improves the Definition of Complete Response and Allows to Detect Otherwise Unidentifiable Skeletal Progression in Multiple Myeloma. Clin Cancer Res 2015 10/01;21(19):4384-4390.

APPENDIX I – DEFINITION OF MULTIPLE MYELOMA

Clonal bone marrow plasma cells ≥10% OR biopsy-proven bony or extramedullary plasmacytoma AND any one or more of the following CRAB features and myeloma-defining events (MDEs):

1. Evidence of end organ damage attributable to the underlying plasma cell proliferative disorder, specifically:

    a. *Hyper**c**alcemia*
    Serum calcium >0.25 mmol/L (>1mg/dL) higher than upper limit of normal or >2.75 mmol/L (>11mg/dL)

    b. ***R**enal Insufficiency*
    Creatinine clearance <40 mL/min or serum creatinine >177µmol/L (>2mg/dL)

    c. ***A**nemia*
    Hemoglobin >20g/L below lowest limit of normal, or <100g/L

    d. ***B**one lesions*
    One or more osteolytic lesion on skeletal radiography, CT, or PET/CT. If bone marrow has <10% clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement

    e. *Any one or more of the following biomarkers of malignancy (MDEs):*
    - 60% or greater clonal plasma cells on bone marrow examination
    - Serum involved / uninvolved free light chain ratio of 100 or greater, provided the absolute level of the involved light chain is at least 100mg/L
    - More than one focal lesion on MRI that is at least 5mm or greater in size.

APPENDIX II: LAY SUMMARY

Multiple myeloma (MM) is a deadly cancer of the bone marrow that is challenging to manage and treat: the drugs that are currently available attack the cancer in the same way for everyone, but each patient has different types of MM cancer cells and different family traits that predict better or worse outcomes. As well, the ways in which we test to see if the cancer is in remission are not very good at detecting small numbers of cancer cells still in the bone marrow after treatment – and which will, sooner or later cause the patient to get sick again. So, the goal of our research is to improve MM patients' survival and quality of life over time, by finding better ways of a) characterizing each patient's experience with the disease, and b) identifying and tracking the small numbers of cells that remain after treatment.

Our plan is to track 250 patients across Canada over time, who are getting treatment for multiple myeloma. While they are getting treatment, our research will evaluate samples of their blood and bone marrow with newer, more precise laboratory tests. We will also ask patients to take part in two scans of their bodies during their treatment. We think that these tests can help doctors make better treatment recommendations to patients.

We will look at whether one test is better than another, or if we need to use a combination of these tests to have the best information possible to make treatment decisions. At the same time, we will also be looking into how and why some patients' cancer becomes resistant to the treatments over time, and how myeloma cells are able to start growing again after treatment. Our team will also collect information on how each patient's health and quality of life changes during and after treatment, and what are the associated costs with these new approaches – to both the healthcare system overall, and to patients.

Once we have completed our five-year research program, we hope to have created a proven and affordable process of combining these new laboratory tests with our current clinical approach, to create new options to evaluate and treat multiple myeloma.

It is our goal that this research will make a difference, right away and across the world, in how doctors treat multiple myeloma, in how it is studied by scientists, and in how patients advocate for their own healthcare.

APPENDIX III – CONTACT INFORMATION FOR COORDINATING CENTRE AND SITES

COORDINATING CENTRE

University of New Brunswick (Saint John)
Dr. Tony Reiman (anthony.reiman@horizonnb.ca; 506-648-6885)
Dr. Alli Murugesan (Alli.Murugesan@unb.ca)

PARTICIPATING SITES

Saint John Regional Hospital
Dr. Tony Reiman (anthony.reiman@horizonnb.ca; 506-648-6885)

QEII Health Sciences Centre, VG Site
Dr. Darrell White (darrell.white@nshealth.ca; 902-473-4642)

Hôpital Maisonneuve-Rosemont
Dr. Jean Roy (jroy.hmr@ssss.gouv.qc.ca; 514-252-3400 x3404)

McGill University Health Centre
Dr. Michael Sebag (michael.sebag@mcgill.ca; 514-843-1558)

The Ottawa Hospital
Dr. Arleigh McCurdy (amccurdy@toh.ca; 613 737 8899 x71281)

Sunnybrook Odette Cancer Centre
Dr. Matthew Cheung (matthew.cheung@sunnybrook.ca; 416-480-6100 x7672)

UHN Princess Margaret Cancer Centre
Dr. Donna Reece (donna.reece@uhn.ca; 416-946-2824)

CancerCareManitoba
Dr. Rami Kotb (rkotb@cancercare.mb.ca)

Cross Cancer Institute
Dr. Christopher Venner (christopherPaul.venner@albertahealthservices.ca)

University of Calgary
Dr. Nizar Bahlis (nbahlis@ucalgary.ca; 403-944-1880)

Leukemia/Bone Marrow Transplant Program of British Columbia
Dr. Kevin W. Song (ksong@bccancer.bc.ca)
Dr. Heather Sutherland (hsutherl@bccancer.bc.ca)