Title: New Approaches to Molecular Imaging of Multiple Myeloma

Ravi Vij¹, Kathryn J. Fowler² and Monica Shokeen²*

¹Division of Hematology and Oncology, Washington University School of Medicine, Saint Louis, Missouri; ²Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri

*Corresponding author: mshokeen@wustl.edu

Corresponding Author: Monica Shokeen (Mallinckrodt Institute of Radiology, Department of Radiology, 4525 Scott Avenue, St. Louis, MO 63110, Phone: 314-362-8979, Fax: 314-747-5190, Email: mshokeen@wustl.edu)

First Author: Ravi Vij (Division of Hematology and Oncology, Washington University Medical School, St.Louis, MO 63110, Phone: 314-454-8204, Email: rvij@dom.wustl.edu)

Running Title: Molecular Imaging of Multiple Myeloma
Abstract: Molecular imaging plays important role in detection and staging of hematological malignancies. Multiple myeloma (MM) is an age-related hematological malignancy of clonal bone marrow plasma cells characterized by destructive bone lesions and is fatal in the vast majority of patients. Traditional skeletal survey and bone scans have sensitivity limitations for osteolytic lesions manifested in MM. Progressive biomedical imaging technologies such as low dose CT, molecularly targeted PET, MRI, and the functional-anatomical hybrid versions (PET-CT, PET-MRI) provide incremental advancements in imaging MM. Imaging with PET and MRI using molecularly targeted probes are promising precision medicine platforms that might successfully address the clinical ambiguities of myeloma spectrum diseases. The intent of this focus article is to provide a concise review of the present status and promising developments on horizon such as the new molecular imaging biomarkers under investigation that can either complement or potentially supersede existing standards.

Key words: Multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS), PET, MRI, PET/CT, PET/MRI, receptor very late antigen-4 (VLA-4), metabolic imaging
MULTIPLE MYELOMA (MM)

MM is the second most common age-related hematologic malignancy in the United States and is incurable in the vast majority of patients. MM is a malignancy of clonal bone marrow plasma cells whose DNA has undergone characteristic class switch recombination and somatic hypermutation(1). In addition to hallmark genetic mutations, the bone micro-environmental elements play a critical role in the MM pathogenesis(2). MM is preceded by a premalignant stage called monoclonal gammopathy of undetermined significance (MGUS) with a 0.5-1% incidence to MM(3). Smoldering multiple myeloma (sMM) is an intermediate clinical stage in which the risk of incidence to MM is 10% per year(3). The International Myeloma Working Group (IMWG) diagnostic criteria for premalignant and malignant MM has been elegantly summarized by Rajkumar et. al.(3). Due to the aging population, the incidence of MM is expected to increase along with the associated costs. Total healthcare costs in the first year after diagnosis of MM is $118,353(4). Advancements in targeted therapy as well as the success of stem cell transplantation has contributed to improvements in the 5-year survival rate in MM (26.3% in 1975 versus 46.6% in 2011) (5). Promising new agents are currently under development for relapsed and refractory MM (6). The treatment regimen for MM is dictated by patient eligibility for autologous or allogeneic stem cell transplantation. About 80% of MM patients treated at our institution are transplant eligible. Majority of these patients receive combination therapy of Bortezomib (proteasome inhibitor), Revlimid (an immunomodulatory drug) and Dexamethasone (corticosteroid); although treatments are tailored around patient age and comorbidities. Despite the success in 5-year survival, challenges of relapse and acquired drug resistance remain in MM. The remissions are transient and vast majority of patients
eventually relapse and die from progressive disease. The mechanisms by which premalignant myeloma (MGUS and sMM) progresses to MM are complex and not known. Malignant myeloma plasma cells accumulate in the bone marrow and cause disruption of the bone homeostasis leading to bone destruction and marrow failure (Figure 1). Consequently, the risk of skeletal related events, such as fractures, is very-high in MM patients and continues to rise even with treatment(7). Malignant plasma cells are generally avid secretors of immunoglobulins; therefore, MM and its obligate precursor state, MGUS are readily detected using serum and/or urine markers (either intact immunoglobulin or free light chains) in majority of cases. However, serum markers are insufficient to distinguish premalignant MGUS and sMM from fully transformed MM. The diagnosis of MM requires very high monoclonal tumor burden and/or end organ damage such as lytic bone lesions. Evaluation of progression and treatment response is also confounded in 10% of MM patients who display an oligo-secretory phenotype (defined as serum M-protein < 1g/dL and urine M-protein < 200 mg/24 h) (8). The timely and accurate diagnosis of MM is important because a delay in the diagnosis of MM can be detrimental to the patient’s outcome. Imaging might provide critical information such as predicting high-risk fracture sites, visualization of non- and oligo-secretory MM tumors and assessing treatment response at various stages of disease (9).

The current clinical imaging practice for MM includes an initial diagnostic full skeleton radiographic survey for evaluation of lytic bone lesions (recommended by International Staging System)(10). Skeletal survey involves acquiring a series of radiographs (plain 2D films) to cover the entire skeleton or common anatomic regions appropriate for the clinical indications of the whole spine. Despite the advantage of this fast, relatively low cost
imaging option, a key limitation of radiographic skeletal survey is its low sensitivity to
detect early osteolytic lesions as typically lesions can only be detected after 30-50% of
mineralized bone destruction has occurred(11). Low dose whole-body (WB) computed
tomography (CT) is now frequently used in MM and has higher sensitivity to radiographs
for superimposed skeletal regions such as scapulae, ribs and sternum(12). Additionally,
CT is better for detecting extra-osseous lesions and for radiotherapy planning as
compared to conventional radiography (13). PET and MRI have high sensitivity and
specificity for providing molecular, functional and metabolic information in MM patients.
Recent advances in functional PET and MR imaging for MM are discussed below.

PET IMAGING IN MM

Functional PET imaging is widely used to assess medullary and extra-medullary disease
(EMD), providing diagnostic factors such as maximum standardized uptake values
(SUVmax), quantifying number of focal lesions, and identifying diffuse bone marrow
infiltration. Metabolism in cancer cells is altered as compared to normal cells. PET
imaging of tumor-metabolism using 18F-fluorodeoxyglucose (18F-FDG) has been widely
used in the clinic for staging, treatment planning and monitoring of response(14). Several
reviews on tumor cell metabolism imaging are available including a comprehensive article
by Plathow et. al.(15). Although, the majority of clinical metabolic PET imaging in MM is
performed with 18FDG, 18FDG has significant limitations for MM. MM cells are
hypoproliferative, do not consistently overexpress GLUT-1 transporter, and distinguishing
a benign lesion from a low-metabolic MM lesion can be difficult to achieve. Over a third
of intramedullary myeloma lesions can go undetected by 18FDG-PET(16). There is an
unmet need for myeloma-specific diagnostic imaging agents. New tracers targeting
different molecular signatures, and therefore biological properties of myeloma, will enhance knowledge of disease progression and lead to personalized patient management.

**LIPID METABOLISM**

**11C-ACETATE/PET IN MM.**

A variety of cancer cells including myeloma cells can metabolize exogenous acetate for de-novo membrane biosynthesis through fatty acid synthase (FAS) and enter the tricarboxylic acid cycle (TCA cycle)(17). FAS is overexpressed in MM cells and has been shown to sustain the biogenesis of lipids from extracellular acetate(18). Okawa et. al. have shown the expression of FAS in primary myeloma cells as well as in cell lines, and demonstrated apoptosis upon pharmacological inhibition of FAS *in vitro*(19). 11C-Acetate is a promising clinical PET tracer that has been shown to be sensitive in bone metastases, primarily prostate cancer and is being evaluated for cancers that have limited avidity for 18FDG(20). Clinically, in a small prospective study Lin et. al. showed significant correlation between systemic tumor burden as measured by percentage of bone marrow plasma cell infiltrates and 11C-acetate marrow uptake \((r=+0.63, p=0.01)\), and a higher number of focal lesions were detected using 11C-acetate compared to 18FDG (13 versus 10)(21). Ho et al. demonstrated that 11C-acetate had enhanced sensitivity over 18FDG (84.6% versus 57.7%) in detecting diffuse infiltration and focal lesions in MM patients. They also demonstrated a correlation of 11C-acetate marrow uptake with the clinical serum \(\beta\)-2-microglobulin levels, and a reduction in 11C-acetate uptake after treatment which was associated with systemic measures of response(22). These data support additional 11C-
acetate-PET and $^{18}$FDG-PET comparison studies in newly diagnosed and refractory patients.

$^{11}$C/$^{18}$F-CHOLINE/PET IN MM.

Radiolabeled choline ($^{11}$C or $^{18}$F) and its analogues are precursors for biosynthesis of cellular membrane phospholipids, and are used as metabolic PET markers of membrane metabolism and turnover. In a small study with 10 patients Nanni et. al. reported $^{11}$C-choline to be better at identifying myeloma lesions in the bone as compared to $^{18}$FDG (37 versus 22)\(^{(23)}\). There have been reports of incidental findings of MM or solitary plasmacytoma by choline-PET\(^{(24)}\). Additional pre-clinical and clinical evaluations will help correlate myeloma hallmarks with choline metabolism and uptake mechanisms.

AMINO ACID-PET IMAGING IN MM.

Amino acid transporter targeted probes represent a promising class of imaging agents that target the increased rates of amino acid transport by cancer cells\(^{(25)}\). Tumor uptake of amino acid tracers primarily reflect the rate and mechanism of transport rather than other metabolic fates such as protein synthesis. $^{11}$C-methionine ($^{11}$C-MET) is a potential amino acid PET tracer for MM\(^{(26)}\). Luckerath et. al. demonstrated significantly high uptake of radiolabeled MET in myeloma cells as compared to FDG, and there was differential MET uptake in myeloma cell lines (with high uptake in cell lines of worse prognosis)\(^{(27)}\). L-type amino-acid transporter 1 (LAT1) mediates sodium-independent cellular transport of amino acids for protein synthesis and other metabolic pathways, and high levels of LAT-1 correlate with proliferating cancers. Isoda et. al. have demonstrated the expression of LAT1 by immunohistochemistry in 100 MM patients and found LAT1 in 56% of patients. $^{18}$F-labeled amino acid, 3,4-dihydroxy-6-$^{18}$F-fluoro-L-phenylalanine ($^{18}$F-
DOPA), is a tracer for imaging LAT-1, and warrants evaluation as a PET marker of prognosis and therapeutic planning and response in MM.

**RECEPTOR TARGETED PET IMAGING IN MM**

MM re-sculpts the bone microenvironment by facilitating neo-angiogenesis, recruitment of tumor associated macrophages and activating osteoclasts while inhibiting osteoblasts, thereby causing a vicious cycle of tumor growth and bone destruction. A grim result of this interplay is that majority of MM patients are diagnosed only after pathologic bone fracture. Integrins are glycoprotein cell receptors that transmit signals bi-directionally across the plasma membrane by undergoing conformational changes in response to stimuli from the intra-cellular products and extra-cellular components(28). Interactions of the tumor cell surface integrins with the stromal environment play a defining role in the pathogenesis of MM. Activated form of the receptor very late antigen-4 (VLA-4; also known as integrin $\alpha_4\beta_1$) is present in high-levels on MM cells. VLA-4 is a critical mediator of myeloma cell adhesion to the bone marrow stroma, and promotes MM cell trafficking, proliferation and drug resistance. We have previously demonstrated the sensitive and specific molecular imaging of activated VLA-4 in MM tumors using the PET radiopharmaceutical, $^{64}$Cu-CB-TE1A1P-LLP2A(29). We are currently developing VLA-4 targeted radiopharmaceuticals for translation into humans to image myeloma spectrum diseases and compare with $^{18}$FDG-PET. The chemokine receptor-4 (CXCR4) is another key receptor that plays an important role in MM pathogenesis. Philipp-Abbrederis et. al. recently demonstrated imaging of advanced MM in humans using the CXCR4 targeted PET probe, $[^{68}\text{Ga}]$Pentixafor(30).

**MRI IN MM**
The role of MRI in imaging MM relies on two primary functions: improved sensitivity for detecting pathologic lesions, and the potential for predictive and prognostic imaging biomarkers. In regards to sensitivity of disease detection, WB MRI offers high soft tissue contrast resolution which in turn yields superior sensitivity compared to conventional radiography for visualization of focal and diffuse tumor infiltration of the bone marrow in untreated patients\(^{31}\). The updated criteria for diagnosis of MM by IMWG recommends MRI as part of initial assessment\(^{3}\), and MRI is also considered particularly beneficial in patients with smoldering MM\(^{32}\). Hillengass and coworkers in a study of 149 patients with asymptomatic MM demonstrated that patients with greater than one focal lesion had significantly shorter progression-free survival than those without or with only one focal lesion \((P<0.001)^{33}\). Beyond sensitivity, there has been much interest in developing prognostic and predictive imaging biomarkers using the functional capabilities of MRI. One such example is dynamic contrast-enhanced MRI (DCE-MRI) using gadolinium-based contrast agents. Increased angiogenesis of the bone marrow is associated with the transition from premalignant states to MM. In a prospective clinical trial, 30 patients were evaluated for the levels of angiogenesis from MGUS to frank malignancy\(^{34}\). The kinetic parameters, \(K^{\text{trans}}\) (transendothelial transport of gadolinium from vascular compartment to the tumor interstitium (wash in)) and \(K^{\text{ep}}\) (reverse transport of gadolinium back into the vascular space (wash out)) derived from DCE-MRI of lumbar vertebrae were compared with bone marrow microvessel density (MVD) and serum panel of 17 angiogenic markers. The study found moderate-strong correlation between MVD and \(K^{\text{ep}}\) in all patients \((r=0.59; P=0.001)\), and weak-moderate correlation between MVD and \(K^{\text{trans}}\) in all patients \((r=0.43; P=0.03)\). It should be noted that DCE is not a WB application and
is done on a limited region such as to evaluate a plasmacytoma or a specific anatomic region. For evaluation of cellularity of the lesion or quantification of the distribution of plasma cells in bone marrow, apparent diffusion coefficient (ADC) values derived from diffusion weighted imaging (DWI) sequences are used. DWI can noninvasively quantify altered diffusion, volume, and flow permeability of new vessels. The relationship between tumor and background marrow ADC values is complex and depends on the degree of marrow activation and the status of the tumor. In a pilot study of 11 patients with metastatic osseous lesions, median global ADC values acquired by semi-automated segmentation of the DWI data allowed for differentiation of responders from non-responders(35). In a prospective trial of 26 patients with MM and baseline/follow-up WB-DWI imaging, there was a significant change in ADC values following therapy which was reproducible between multiple readers(36). Additional small number of studies have shown similar results suggesting that DWI/ADC imaging is a potential response biomarker platform(37-39). While DWI/ADC data provide insight into tumor cellularity and disease activity, the interpretation of these images can be complicated by physiological factors such as age and bone marrow activation due to physical activity and infection.

PET/MRI
In recent years simultaneous PET/MRI platforms have become available for clinical use. These hybrid systems can combine the molecular data of PET with the anatomic and functional data of MRI. The benefits of simultaneous acquisition are that two previously separate exams can now be performed in a single imaging session, there is improved registration between modalities, and dynamic PET and DCE could be done simultaneously. Drawbacks of hybridizing PET with MRI rest mainly on issues related to
attenuation correction of the PET data. MR based attenuation correction does not take into account cortical bone; however, vendors and researchers are actively investigating the potential impact of this on quantitative evaluation of osseous lesions while working toward improved technology. In regards to workflow challenges, it is essential to focus on patient tolerance and comfort when designing whole body PET/MRI protocols \((40)\). Minimization of MRI sequences to what is essential to answer the clinical or research question is advised. In the absence of WB PET/MRI, WB-PET imaging and MRI of the spine and pelvis is recommended. Additionally, any known or concerning areas of disease involvement may be targeted for imaging. PET/MRI protocols that are being optimized at our institution for prospective MRI of MGUS, sMM and MM patients are summarized in Table 1. Figure 2 demonstrates an example of a fused MR-PET image showing an active site of MM involvement in a lumbar vertebral body. Studies aimed at evaluating PET/MRI as a diagnostic tool for MM will provide more insights into the benefits of this promising imaging platform.

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Figure 1. Simplified overview of molecular markers targeted by PET and MRI. Multiple myeloma (MM) cells and the microenvironment possess anatomical and functional biomarkers for imaging. Myeloma cells primarily reside in the bone marrow compartment disrupting the bone microenvironment and also altered metabolism.
Table 1. MRI sequences to include in WB-PET/MRI examinations for evaluation of marrow lesions

<table>
<thead>
<tr>
<th>Recommended MR Sequence</th>
<th>Recommended Area of Evaluation</th>
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<tr>
<td>T1-weighted turbo-spin echo (TSE) sequence</td>
<td>Evaluate cortex (normally dark) and marrow infiltration (marrow darker than normal).</td>
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<tr>
<td>T2-weighted fat suppression post-contrast images</td>
<td>T2 weighted fat suppressed images may show areas of marrow edema and replacement which are often brighter than background fat containing marrow.</td>
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<tr>
<td>T2 HASTE (ssfse)</td>
<td>Fast acquisition T2 weighted sequence that allows for full body coverage and anatomic detail of organs and soft tissues.</td>
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<tr>
<td>Diffusion weighted imaging (DWI)/apparent diffusion coefficient (ADC)*</td>
<td>Bone marrow, cellularity of lesion. Possible biomarker of treatment response.</td>
</tr>
<tr>
<td>Dynamic contrast enhancement MRI (DCE-MRI)*</td>
<td>Surrogate for perfusion and permeability. Performed on a limited region such as a plasmacytoma.</td>
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<tr>
<td>T1 DIXON AC</td>
<td>Dual echo GRE sequence that is acquired at in-phase and opposed phase echo times with generation of fat only and water only images through DIXON method. These images are utilized to create the mumap for attenuation correction of the PET data.</td>
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* DCE-MRI and DWI sequences may be applied in a more focused way to characterize specific sites of disease and potentially add value in assessing tumor response. DCE is not a whole body method.
Figure 2. Axial MR and fused MR-PET images show an active site of MM involvement in a lumbar vertebral body (arrows). **A**-fused single shot turbo spin echo T2, **B**-Fused DWI-PET, **C**-Fused ADC map-PET, **D**-Fat suppressed turbo spin echo T2, **E**-DWI, **F**-ADC. Note the bright signal intensity on the T2 weighted (D) and diffusion images (E) with corresponding dark signal intensity on the ADC (F) denoting restriction in diffusion, a correlate for increased cellular density.

*Image collected at Washington University Clinical PET/MR Scanner*