Myeloma Biomarkers
February 19, 2024

Tech Support
1-719-234-7952
Resources

- Resource tab includes
  - Speaker bios
  - Copy of the slide presentation
  - Exhibit Hall

Submit your questions throughout the program!
MMRF Research Initiatives

1. MMRF Myeloma Accelerator Challenge (MAC) Grants
   - Broad, multi-institutional research grants designed to advance clinical trial concepts in the areas of
     • High-risk newly diagnosed multiple myeloma (NDMM)
     • High-risk smoldering myeloma (SMM)
   - Each research network will be funded up to $10M over 3 years

2. MMRF Horizon Adaptive Platform Trials
   - Paired with MAC grants
   - Done in collaboration with 13 MMRC sites
   - Trials in relapsed/refractory myeloma, high-risk NDMM, high-risk SMM

For more information, visit themmrf.org

2023 Myeloma Accelerator Challenge Program Grant Recipients

Transforming Treatment of High-Risk Myeloma
Network includes Tisch Cancer Center at Mt Sinai, Albert Einstein Medical College, Hackensack University Medical Center, Stanford University Medical Center, UCSF, Washington University of Saint Louis

A Systems Biology Approach to High-Risk Myeloma
Network includes Erasmus Medical Center, Rotterdam; Amsterdam University Medical Centers; Julius Maximilian University of Wurzburg; University of Turin; University of Salamanca

Clinical and Multi-Omics Platforms to Define High-Risk Smoldering Myeloma
Network includes Emory University, Atrium Health Levine Cancer Institute, Icahn School of Medicine at Mt. Sinai, Mass General Hospital, Mayo Clinic, MSKC Institute, Dana-Farber Cancer Institute

Each network will receive $7M over 3 years for a total $21M investment by the MMRF, meant to foster collaboration and advance compelling hypotheses that are ready for rapid testing in clinical trials.
Speakers

**Benjamin T. Diamond, MD**  
Sylvester Comprehensive Cancer Center  
University of Miami, Miller School of Medicine  
Miami, Florida

**Francesco Maura, MD**  
Sylvester Comprehensive Cancer Center  
University of Miami, Miller School of Medicine  
Miami, Florida

Available Biomarkers for Multiple Myeloma: A “How-To” and Perspective on Common Results

**Benjamin T. Diamond, MD**  
Sylvester Comprehensive Cancer Center  
University of Miami, Miller School of Medicine  
Miami, Florida
What qualifies as a biomarker?

- A characteristic that can be objectively measured and evaluated as an indicator of a biologic process
  - Diagnostic: M protein
  - Prognostic: minimal residual disease (MRD)
  - Predictive: t(11;14)
**Common Biomarkers in Your Blood Work**

- B2M: beta-2 microglobulin
- LDH: lactate dehydrogenase
- Albumin
- Light chains and ratio
- SPEP: serum protein electrophoresis
- SIFE: serum immunofixation electrophoresis

R-ISS: Revised International Staging System (with FISH)

Paraprotein: surrogate for disease burden

---

**Monoclonal Protein Is a Product of Monoclonal Plasma Cells**

Tracking and Diagnosing With Free Light Chains Can Be Tricky

Light chain ratio caveats
Sensitive to the denominator

<table>
<thead>
<tr>
<th>Component</th>
<th>Ref range &amp; units</th>
<th>2 mo ago</th>
<th>5 mo ago</th>
<th>7 mo ago</th>
<th>8 mo ago</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free kappa</td>
<td>0.33–1.94 mg/dL</td>
<td>31.87</td>
<td>27.18</td>
<td>37.43</td>
<td>34.21</td>
</tr>
<tr>
<td>Free lambda</td>
<td>0.57–2.63 mg/dL</td>
<td>0.29</td>
<td>0.28</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>Free kappa/lambda ratio</td>
<td>0.26–1.65</td>
<td>109.90</td>
<td>97.07</td>
<td>93.58</td>
<td>117.97</td>
</tr>
</tbody>
</table>

Sensitive to kidney function


Paraprotein for Diagnosis, Risk Stratification, and Monitoring

**Diagnostics**

<table>
<thead>
<tr>
<th></th>
<th>MGUS</th>
<th>SMM</th>
<th>MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal bone marrow plasma cells</td>
<td>&lt;10%</td>
<td>10% to 59% and/or either serum ≥30 g/L or Urine ≥500 mg/24 hours</td>
<td>≥10% or biopsy proven plasmacytoma</td>
</tr>
<tr>
<td>M protein</td>
<td>Serum &lt;30 g/L or Urine ≤500 mg/24 hours</td>
<td>Any level</td>
<td></td>
</tr>
<tr>
<td>Myeloma-defining events (CRAB SLiM criteria)*</td>
<td>None</td>
<td>None</td>
<td>Present</td>
</tr>
</tbody>
</table>

**Risk stratification**

<table>
<thead>
<tr>
<th></th>
<th>High risk</th>
<th>Biochemical diagnosis (SLiM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M protein ≥1.5 g/dL</td>
<td>≥60% BM plasma cell</td>
<td></td>
</tr>
<tr>
<td>Non-IgG MGUS ≥20% BM plasma cell</td>
<td>FLC ratio ≥100</td>
<td></td>
</tr>
<tr>
<td>Abnormal FLC ratio ≥20</td>
<td>&gt;1 focal lesion on MRI</td>
<td></td>
</tr>
</tbody>
</table>

*R: renal insufficiency (creatinine clearance <40 mL/min or serum creatinine >2 mg/dL); A: anemia (Hb <10 g/dL or 2 g/dL < normal); B: bone disease (≥1 lytic lesions on skeletal radiography, CT, or PET-CT)...

### Biomarkers in Response Criteria: Room for Improvement

<table>
<thead>
<tr>
<th>Response</th>
<th>IMWG criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCR</td>
<td>CR as defined below plus normal FLC ratio and absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence</td>
</tr>
<tr>
<td>CR</td>
<td>Negative immunofixation on the serum and urine, disappearance of any soft tissue plasmacytomas, and &lt;5% plasma cells in bone marrow</td>
</tr>
<tr>
<td>VGPR</td>
<td>Serum and urine M protein detectable by immunofixation but not on electrophoresis or &gt;90% reduction in serum M protein plus urine M protein level &lt;100 mg/24 h</td>
</tr>
<tr>
<td>PR</td>
<td>&gt;50% reduction of serum M protein and reduction in 24-hour urinary M protein by &gt;90% or to &lt;200 mg/24 h</td>
</tr>
<tr>
<td></td>
<td>If the serum and urine M protein are unmeasurable, a &gt;50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M protein criteria</td>
</tr>
<tr>
<td></td>
<td>If serum and urine M protein are not measurable and serum free light assay is also not measurable, &gt;50% reduction in plasma cells is required in place of M protein, provided baseline bone marrow plasma cell percentage was &gt;30%</td>
</tr>
<tr>
<td></td>
<td>In addition to the above listed criteria, if present at baseline, a &gt;50% reduction in the size of soft tissue plasmacytomas is also required</td>
</tr>
</tbody>
</table>

sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response

Durie BG et al. Leukemia. 2006;20:1467.

---

### Measuring MRD via Next-Generation Sequencing

Next-generation sequencing approach to MRD measurement

1 myeloma cell in 100,000–1,000,000!

How do we interpret a NGS MRD test?

No residual sequences detected
Estimated MRD value:
0 residual clonal cells (range: 0–2)§
Total nucleated cells evaluated from this sample: 881,232

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen type</th>
<th>Estimated sequence abundance (residual clonal cells per million nucleated cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-yr</td>
<td>Fresh bone marrow</td>
<td>Not detected</td>
</tr>
<tr>
<td>1-yr</td>
<td>Fresh bone marrow</td>
<td>&lt;1††</td>
</tr>
<tr>
<td>Pre-ASCT</td>
<td>Fresh bone marrow</td>
<td>27</td>
</tr>
<tr>
<td>Dx</td>
<td>FFPE slides</td>
<td>40,367</td>
</tr>
</tbody>
</table>

Sample clonality\(^1\) | Total nucleated cells\(^2\) | LOCI | Total sequences\(^3\) | Total unique sequences\(^4\) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>881,232</td>
<td>IGH</td>
<td>149,159</td>
<td>139,144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGK</td>
<td>189,950</td>
<td>109,267</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGL</td>
<td>54,811</td>
<td>40,589</td>
</tr>
</tbody>
</table>

Supplemental sequence information

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Limit of detection (per million cells)(^5)</th>
<th>Limit of quantitation (per million cells)(^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGH – Sequence A</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>IGH – Sequence B</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

How do we interpret a flow cytometry MRD test?

Multiparametric flow cytometry approach to MRD measurement

**Single tube**
- CD117 PC5.5
- CD19 PC7
- CD138 APC
- CD56 APC-R700
- CD45 APC-H7
- CD81 Pacific Blue
- CD38 BV510
- CD38 BV510
- CD27 BV605
- k FITC
- λ PE

Interpretation:
G. Bone marrow, flow cytometry analysis:
NEGATIVE FOR ABNORMAL PLASMA CELL POPULATION

Comment: Bone marrow elements are present in this specimen. An abnormal plasma cell population is not detected. The detection limit of this assay is 0.001% of leukocytes.

Technical data:
- Total analyzed leukocytes: 2,444,000
- Limit of detection: 0.001%
- Total plasma cells: 1687
- Mast cell population: 0.06%
- Immature B-cell population: 3.1%

Flow cytometry analysis has been performed using the following CD and non-CD antibodies:
- Plasma cell myeloma MRD panel:
  - CD45, CD138, CD229, CD319, CD38, CD117, CD56, CD27, CD81, CD19, cyKappa, cyLambda

Why do we care? MRD has more resolution than standard response criteria.

Validation of the International Myeloma Working Group standard response criteria


Why do we care? MRD is prognostic for outcome.

MRD positivity (compared to MRD negativity)\(^1\)

a

<table>
<thead>
<tr>
<th>Study</th>
<th>Hazard ratio</th>
<th>HR</th>
<th>95% CI</th>
<th>\textit{p}-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koren 2015</td>
<td>0.10</td>
<td>0.2</td>
<td>(0.03; 1.2)</td>
<td>0.22</td>
</tr>
<tr>
<td>Moreau 2014</td>
<td>0.40</td>
<td>2.9</td>
<td>(1.6; 4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Palou 2008</td>
<td>0.35</td>
<td>0.3</td>
<td>(0.2; 0.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Shennoh 2013</td>
<td>0.28</td>
<td>1.6</td>
<td>(0.9; 2.8)</td>
<td>0.11</td>
</tr>
<tr>
<td>Random effects model</td>
<td>0.35</td>
<td>0.7</td>
<td>(0.2; 2.4)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

b

<table>
<thead>
<tr>
<th>Study</th>
<th>Hazard ratio</th>
<th>HR</th>
<th>95% CI</th>
<th>\textit{p}-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moreau 2014</td>
<td>0.48</td>
<td>3.9</td>
<td>(1.3; 11.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>Palou 2008</td>
<td>0.48</td>
<td>1.5</td>
<td>(0.7; 3.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Random effects model</td>
<td>0.48</td>
<td>1.0</td>
<td>(0.3; 3.0)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Association of MRD negativity with outcomes\(^2\)

Longitudinal MRD Tracking Provides a Window Into Disease Dynamics

For 108 patients on continuous lenalidomide maintenance:
- Measure MRD status every year
- Patients who sustained MRD negativity for 2 years had no recorded progression at median 19.8 months past the 2-year maintenance landmark


MRD: Final Notes and Future Directions

Consider:
1) Prognostic at the patient level, but as a regulatory end point?
2) Heterogeneity across institutions

MRD end points in trials

MRD-adapted therapy
- Intensification/de-escalation
- Maintenance combination/duration
- De-escalation/cessation

MRD and disease biology

myeloma biomarkers webinar
February 19, 2024

upcoming: genomic biomarkers in smoldering myeloma

Lenalidomide (R)/
dex vs observation

- Mayo 2008
- PETHEMA
- RLM
- Mayo 2018 (20/2/20)
- Pangea

intervention for high-risk SMM (HR-SMM) has produced favorable results.
- Less aggressive, more susceptible?
- Patient fitness?
- Heterogeneous inclusion criteria and biology?


Genomic contextualization of treated HR-SMM

Carfilzomib, Lenalidomide, and Dexamethasone Followed by Lenalidomide Maintenance for Prevention of Symptomatic Multiple Myeloma in Patients With High-risk Smoldering Myeloma

A Phase 2 Nonrandomized Controlled Trial

54 patients with HR-SMM
KRd × 8 cycles ➔ R × 2 yrs
MRD-negative rate: 70%
92.7% PFS rate (MM) at 5 years
- E Hill et al. Abstract 337

- 51 patients with HR-SMM
- Elotuzumab-R+/d × 24 cycles

Structural variant (SV) hot spots
Copy number variants (CNVs) + Single nucleotide variants (SNV) + chromothripsis

Clinical Risk Scores Are Inconsistent in Smoldering Myeloma

- Outcomes
  - Progression (clinical or biochemical)
  - Sustained MRD negativity × 1-year

Genomic Complexity: A Potential Biomarker Associated With Worse Outcome

- Outcomes
  - Progression (clinical or biochemical)
  - Sustained MRD negativity × 1-year
Genomics to Predict Clinical Outcomes

**Francesco Maura, MD**
Sylvester Comprehensive Cancer Center
University of Miami, Miller School of Medicine
Miami, Florida

The advent of novel drugs has resulted in improved overall survival in patients with multiple myeloma. For example, in patients treated with the combination of bortezomib, thalidomide, and dexamethasone (VTD), overall survival has significantly increased compared to historical controls. However, a subset of patients with multiple myeloma has not benefited from newer therapies, reflected in persisting poor clinical outcomes. Conversely, another subset of patients with multiple myeloma has excellent outcomes despite limited therapy. For instance, a combination of thalidomide and dexamethasone (TD) has shown promising results.

Clinical Outcomes in Multiple Myeloma

Prognostication and Risk in Multiple Myeloma

<table>
<thead>
<tr>
<th>ISS</th>
<th>R-ISS*</th>
<th>R2-ISS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>• B2M</td>
<td>• B2M</td>
<td>• B2M</td>
</tr>
<tr>
<td>• Albumin</td>
<td>• Albumin</td>
<td>• Albumin</td>
</tr>
<tr>
<td></td>
<td>• LDH</td>
<td>• LDH</td>
</tr>
<tr>
<td></td>
<td>• t(4:14)</td>
<td>• t(4:14)</td>
</tr>
<tr>
<td></td>
<td>• del17p</td>
<td>• del17p</td>
</tr>
</tbody>
</table>

RELATIVE RISK

- No genomic information included
- Not useful for developing patient-specific tailored therapeutic decisions

Figure generated using BioRender.


Outline

1. Advantage of using genomics as biomarkers in multiple myeloma
2. WGS to maximize the efficacy of our immunotherapy-based strategies in multiple myeloma
3. Genomics to develop individualized risk and strategies for newly diagnosed multiple myeloma
Cancer genomics and clinical outcomes

Cancer genomics, pathogenesis, and heterogeneity
Aims of translational and genomic research

APOBEC

APOBEC is associated with:
- Immunosuppressed immune microenvironment
- Aggressive disease
- Loss of HLA
- Metastasis

APOBEC (SBS2/SBS13)
256 WGS from newly diagnosed MM enrolled in the GMMG-HD6 clinical trial

~90% of patients with MM have evidence of APOBEC mutagenesis

MAF/MAFB:IGH translocations

APOBEC and Clinical Outcomes in Newly Diagnosed Multiple Myeloma

Chromothripsis in Multiple Myeloma

Chromothripsis is when one or more chromosomes are shattered and reassembled in the wrong way.

Chromothripsis can involve key driver genes with impact on our treatment efficacy.

MMRF CoMMpass (752 low coverage long insert WGS from MMRF): 24% of patients have chromothripsis.
### Outline

1. Advantage of WGS: impact of mutational signatures and structural variants in multiple myeloma

2. **WGS to maximize the efficacy of our immunotherapy-based strategies in multiple myeloma**

3. Genomics to develop individualized risk and strategies for newly diagnosed multiple myeloma
What affects the response to immunotherapy?

To be effective, immunotherapy needs a functional immune system.
Resistance to Anti-GPRC5D CAR T/TCE

Antigen loss is the main mechanism of resistance to anti-GPRC5D CART/TCE

Resistance to Anti-BCMA CAR T/TCE

Biallelic deletion of BCMA (<5%)
In 6/12 (50%) patients with WGS at relapse, the progression after anti-BCMA CART/TCE was driven by antigen escape mechanisms.


Outline

1. Advantage of WGS: impact of mutational signatures and structural variants in multiple myeloma

2. WGS to maximize the efficacy of our immunotherapy-based strategies in multiple myeloma

3. Genomics to develop individualized risk and strategies for newly diagnosed multiple myeloma
Individualized Risk Model for Multiple Myeloma (IRMMa)

- MMRF, N=1062
- Moffitt, N=177
- MPG, N=492
- MSKCC, N=109
- UAMS, N=93

Model is driven by deep neural networks

- Clinical
- Demographic
- Ethnic
- Treatment
- Genomics (WES/WGS)

IRMMa: Multistate Model

Phase 1: Induction
- Newly diagnosed multiple myeloma, N=1933
- Progressed, N=285 (14%)

Phase 2: Post-induction
- Alive in remission, N=658 (34%)
- Alive after PD, N=444 (23%)
- Response in induction (P2), N=1511 (78%)
- Progressed, N=756 (39%)
- Death in remission, N=37 (5%)
- Death after PD, N=312 (16%)

Primary refractory patients represent the “real” high-risk multiple myeloma

Individualized Risk Model for Multiple Myeloma (IRMMa)

Validation set GMMG-HD6*
256 NDMM treated with VRD + SCT

Contribution of Genomics in IRMMa Accuracy

https://github.com/UM-Myeloma-Genomics/GCP-MM: copy number signatures (CNV.Sig) to predict from whole-genome and exome sequencing data
https://github.com/UM-Myeloma-Genomics/mmsig: SBS signatures fitting tools to detect APOBEC from whole-genome and exome sequencing data

Heat map showing the predicted treatment variance across 1,933 patients in case of treatment with VRd +/- HDM-ASCT +/- continuous treatment (CT)

PFS, progression-free survival (probability to be alive and in remission at 5 years); VRd, bortezomib, lenalidomide, dexamethasone; HDM-ASCT, high-dose melphalan and autologous stem cell transplantation

IRMMa has been designed for research purpose only. Interpretation of the output should rely on the guide/tutorial
Future Directions...

Summary

- Genomics can provide useful information to predict clinical outcomes and individualized treatment strategies.
- Models like IRMMa (artificial intelligence) can predict individualized risk for each patient, opening a new era for precision medicine.
- Genomics is emerging as the most important force in promoting resistance to novel immunotherapies (CART and bispecific T-cell engagers).
Questions & Answers

Check out our High-Impact Topic VIDEOS

For more information, visit themmrf.org/educational-resources/
MMRF Patient Resources

Myeloma Mentors® allows patients and caregivers the opportunity to connect with trained mentors. This is a phone-based program offering an opportunity for a patient and/or caregiver to connect one-on-one with a trained patient and/or caregiver mentor to share his or her patient journeys and experiences.

No matter what your disease state—smoldering, newly diagnosed, or relapsed/refractory—our mentors have insights and information that can be beneficial to both patients and their caregivers.

Contact the Patient Navigation Center at 888-841-6673 to be connected to a Myeloma Mentor or to learn more.
Join the MMRF Community!

**National Walk/Run Program**
- Atlanta | 10.26.24
- Boston | 10.12.24
- Chicago | 9.8.24
- Dallas | 11.16.24
- Houston | 11.23.24
- Los Angeles | 8.17.24
- National Virtual | 12.14.24
- New York City | 10.5.24
- Philadelphia | 10.19.24
- San Francisco | 8.24.24
- Scottsdale | 12.7.24
- Southeast Michigan | TBD
- Tampa | TBD
- Washington D.C. | 9.28.24

**Other MMRF Event Programs**
- Moving Mountains for Multiple Myeloma
- Half and Full Marathons
- Bike/Road to Victories
- Create Your Own Fundraiser

Upcoming Patient Education Events

**Save the Date**

<table>
<thead>
<tr>
<th>Program</th>
<th>Date and Time</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bispecific Antibodies</td>
<td>Monday, February 26, 2024 11:00 AM – 12:00 PM (ET) 8:00 AM – 9:00 AM (PT)</td>
<td>Jesus Berdeja, MD  Melissa Alsina, MD</td>
</tr>
<tr>
<td><em>Livestream</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Tuesday, March 5, 2024 1:00 PM – 2:00 PM (ET) 10:00 AM – 11:00 AM (PT)</td>
<td>Joshua Richter, MD  Alexander Lesokhin, MD</td>
</tr>
<tr>
<td><em>Livestream</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For more information or to register, visit themmrf.org/educational-resources
Resources

• Resource tab includes
  – Exhibit Hall
  – Speaker bios
  – Copy of the slide presentation
Need help with travel to a clinical study?

- The MMRF has partnered with the Lazarex Cancer Foundation to help provide more equitable access to clinical studies for multiple myeloma patients.
- This partnership is one facet of the MMRF’s commitment to improve diversity and representation in myeloma clinical studies.
- MMRF has provided $100,000 over 2 years to Lazarex to fund travel, lodging, and food for patients (and a travel companion) so that they can participate in clinical studies that are appropriate for them.
- Patients are funded according to income guidelines and will be reimbursed for allowed expenses.
- For more information on this program and to be connected with Lazarex, call our Patient Navigation Center at 1-888-841-6673.

Thank you!