Minimal residual disease in smoldering myeloma

Malin Hultcrantz, MD, PhD
Research fellow
Myeloma Service, Memorial Sloan-Kettering Cancer Center
• MRD by flow cytometry and NGS
• Genetic landscape
• Future directions
Korde et al, JAMA Oncology, 2015

17 patients with smoldering myeloma

8 cycles of CRd combination therapy

- Carfilzomib 20/36 mg/m²
  - day 1, 2, 8, 9, 15, 16
- Lenalidomide 25 mg/day
  - day 1–21
- Dexamethasone 20/10 mg
  - day 1, 2, 8, 9, 15, 16, 22, 23

Stable disease or better?

24 cycles of extended dosing

- Lenalidomide 10 mg/day
  - day 1–21
Patients and treatment response

<table>
<thead>
<tr>
<th>BEST TREATMENT RESPONSE AFTER COMBINATION THERAPY</th>
<th>SMOLDERING MYELOMA (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>16 (94%)</td>
</tr>
<tr>
<td>non-CR</td>
<td>1 (6%)</td>
</tr>
</tbody>
</table>

CR = complete response

Korde et al, *JAMA Oncology*, 2015
MRD assessment

- MRD assessment at CR
- Multiparametric flow cytometry
  - Two tube eight color flow cytometry, sensitivity $10^{-5}$
- Next generation sequencing for VDJ rearrangement using LymphoSIGHT
  - Sensitivity $10^{-6}$

Korde et al, *JAMA Oncology*, 2015
## MRD assessment in SMM

<table>
<thead>
<tr>
<th>Method</th>
<th>Status</th>
<th>SMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRD by two-tube 8 color flow cytometry</td>
<td>Negative</td>
<td>16/17 (94%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1/17 (6%)</td>
</tr>
<tr>
<td>MRD by VDJ next generation sequencing</td>
<td>Negative</td>
<td>9/12 (75%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>3/12 (25%)</td>
</tr>
</tbody>
</table>

Korde et al, JAMA Oncology, 2015
• MRD by flow cytometry and NGS

• Genetic landscape

• Future directions
Mutational landscape

SMM patients n=17, NDMM patients=39

Tumor only DNA from CD₁₃₈+ plasma cells from baseline

Whole exome sequencing, median coverage of 125x (range 105-185x)

Mailankody et al, ASCO, 2016
Median number of mutations per patient

Non-synonymous mutations

Median number = 53

Newly diagnosed multiple myeloma
N=39

Median number = 52

Smoldering myeloma
N=17

Mailankody et al, ASCO, 2016
Significantly recurrent mutations of individual genes in multiple myeloma

• KRAS
• NRAS
• BRAF
• CYLD
• FAM46C
• TRAF3
• DIS3
• IRF4
• HIST1H1E
• ACTG1
• TP53
• LTB
• PRDM1
• RB1
• MAX

Patients with mutations in significantly recurrent multiple myeloma genes

New diagnosed multiple myeloma: n/N(%)=17/39 (44%)

Smoldering myeloma: n/N (%)=1/17 (6%)

NDMM versus SMM, Fisher’s exact test: P=0.005
• MRD by flow cytometry and NGS

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Future directions

• Relationship between molecular MRD positivity and immunophenotype?

• Relationship between mutations at MRD and clinical phenotype?

• Prognostic impact of the above?
Lessons learned from AML
Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia

Jeffery M. Klco, MD, PhD; Christopher A. Miller, PhD; Malachi Griffith, PhD; Allegra Petti, PhD; David H. Spencer, MD, PhD; Shamika Ketkar-Kulkarni, MS; Lukas D. Wartman, MD; Matthew Christopher, MD, PhD; Tamara L. Lamprecht, BS; Nicole M. Helton, BS; Eric J. Duncavage, MD; Jacqueline E. Payton, MD, PhD; Jack Baty, BA; Sharon E. Heath; Obi L. Griffith, PhD; Dong Shen, PhD; Jasreet Hundal, MS; Gue Su Chang, PhD; Robert Fulton, MS; Michelle O’Laughlin, BS; Catriona Fronick, BS; Vincent Magrini, PhD; Ryan T. Demeter, BE; David E. Larson, PhD; Shashikant Kulkarni, MS, PhD; Bradley A. Ozenberger, PhD; John S. Welch, MD, PhD; Matthew J. Walter, MD; Timothy A. Graubert, MD; Peter Westervelt, MD, PhD; Jerald P. Radich, MD; Daniel C. Link, MD; Elaine R. Mardis, PhD; John F. DiPersio, MD, PhD; Richard K. Wilson, PhD; Timothy J. Ley, MD

Figure 4. Day 30 Mutation Clearance by Gene for 50 Acute Myeloid Leukemia Cases

- **DNMT3A (13 cases)**
  - 3/15 variants with day 30 VAF < 2.5%

- **TET2 (7 cases)**
  - 5/10 variants with day 30 VAF < 2.5%

- **IDH1 and/or IDH2 (12 cases)**
  - 8/12 variants with day 30 VAF < 2.5%

- **NPM1 (18 cases)**
  - 18/18 variants with day 30 VAF < 2.5%

- **FLT3 (17 cases)**
  - 22/22 variants with day 30 VAF < 2.5%

- **KRAS and/or NRAS (14 cases)**
  - 14/16 variants with day 30 VAF < 2.5%

- **Spliceosome (6 cases)**
  - 4/6 variants with day 30 VAF < 2.5%

- **Cohesin (5 cases)**
  - 5/5 variants with day 30 VAF < 2.5%

- **TP53 (3 cases)**
  - 3/3 variants with day 30 VAF < 2.5%

VAF indicates variant allele frequency. Serial VAF measurements demonstrating the clearance patterns of several recurrently mutated acute myeloid leukemia genes in the set of 50 cases. Orange lines indicate a day 30 VAF of 2.5% or more; blue lines indicate a day 30 VAF of less than 2.5%.
Mutation clearance and outcomes

Figure 5. Association Between Mutation Clearance and Outcomes

A. Event-free survival for all patients

- Log-rank P < 0.001
- Day 30 VAF < 2.5%
- Day 30 VAF ≥ 2.5%

B. Overall survival for all patients

- Log-rank P = 0.003
- Day 30 VAF < 2.5%
- Day 30 VAF ≥ 2.5%

Klco et al, JAMA 2015
Profiling at diagnosis and MRD

Diagnosis

Treatment

MRD

M+ / M-

F+ M+

F+ M-

F- M+

F- M-

M=mutations by NGS, F=flow cytometry
Flow sort and sequencing of MRD

F+ M+
F+ M-
F- M+
F- M-

Sort

Deep seq
DNA barcoding

Custom panel seq

Schmitt et al, PNAS, 2012
Future studies at MSKCC

• Profiling of MRD in smoldering and newly diagnosed myeloma

• Profiling of precursor disease
  – Case control study
  – iStopMM
Collaborators

Memorial Sloan-Kettering Cancer Center
Ola Landgren
Elli Papaemmanuil
Sham Mailankody
Neha Korde
Ahmet Dogan
Myeloma Service

Karolinska Institute
Leonie Saft

University of Iceland
Sigurdur Y Kristinsson
Sigrún H Lund

University of Milan
Niccolò Bolli

TGen
Jonathan Keats
Austin Christofferson
Thank you!

Malin Hultcrantz, MD PhD
Research Fellow, Myeloma Service
Memorial Sloan-Kettering Cancer Center
Email: hultcram@mskcc.org
Schmitt et al, PNAS, 2012
Duplex sequencing

A mutation occurs on both strands → Add Adaptors → Ligate and PCR → Sequence

Random error → True variant → Consensus

Create single strand consensus sequence from every unique molecular tag → Create duplex sequences based on molecular tags and sequencing primers → Rare variant
UMI deep sequencing

UMI=unique molecular identifiers