Risk Classification in Myeloma: FISH Testing and Beyond

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Disclosures

• I have no conflicts of interest to disclose
Outline

• What do we mean by “risk” in multiple myeloma?
  • High risk versus Standard risk
  • What are the systems that we use to stratify risk?

• What are chromosomes, genes, and mutations?
  • Review DNA structure and what all the letters and numbers mean
  • What are some common chromosomal abnormalities in multiple myeloma and why do they happen?

• What is the significance of these chromosome abnormalities and do they impact treatment decisions?
Perhaps a better name for this cancer should be…

The multiple myelomas — current concepts in cytogenetic classification and therapy

Shaji K. Kumar* and S. Vincent Rajkumar
What do we mean by “risk”? 

• High risk = more likely to be difficult to control  
• Standard risk = more likely to be easier to control  

• How do we make this assessment? 

• In myeloma, we often use the word “Stage” to refer to risk
International Staging System (ISS)

• Lab tests:
  • Albumin
  • Beta-2 microglobulin

(Greipp, San Miguel et al. 2005)
Revised International Staging System (R-ISS)

- Lab tests
  - Albumin
  - Beta-2 microglobulin

- Chromosome abnormalities

(Palumbo, Avet-Loiseau et al. 2015)
What are Genes and Chromosomes?
What are Genes and Chromosomes?

- DNA is the molecule carrying the instruction manual for how cells do their jobs.
- DNA has individual sections of instructions called “genes”, which each encode a specific set of instructions to make RNA and then proteins.
- Genes are all located at specific locations, and long segments of DNA with many genes are organized into “chromosomes”
- There are 23 unique chromosomes in the human genome, and every cell should have 2 copies of each (1 from each parent)
What do Chromosomes have to do with Myeloma?

• When DNA is functioning normally, cells do the job they are supposed to do.
• One important factor that results in cells becoming cancer is damage to DNA.
• Each type of cancer has patterns of DNA damage that lead to development of that cancer.
Perspective

- Multiple myeloma is a cancer of plasma cells, a part of the immune system.
- Plasma cells are fully mature, differentiated white blood cells.
- Specific mutations that cause myeloma are believed to only affect the plasma cells, not the other cells in the body.

https://en.wikipedia.org/wiki/Haematopoiesis
Plasma cell development

## Two main categories of chromosomal abnormalities

<table>
<thead>
<tr>
<th>IgH Translocations</th>
<th>Hyperdiploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 40% of cases</td>
<td>• 50-60% of cases</td>
</tr>
<tr>
<td>• Gene responsible for making the Immunoglobulin Heavy Chain (IgH) on chromosome 14 gets combined abnormally with another gene that tells cells to grow</td>
<td>• Extra copies of chromosomes, usually odd numbered ones (3, 5, 7, 9, 11, 15, 19)</td>
</tr>
<tr>
<td>• Most common partners are</td>
<td></td>
</tr>
<tr>
<td>• 15-20%: Cyclin D1: t(11;14)</td>
<td></td>
</tr>
<tr>
<td>• 10-15%: MMSET: t(4;14)</td>
<td></td>
</tr>
<tr>
<td>• 5%: MAF: t(14;16)</td>
<td></td>
</tr>
<tr>
<td>• 5%: others</td>
<td></td>
</tr>
</tbody>
</table>
Two main categories of chromosomal abnormalities

**IgH Translocations**

- IgM
- Light chain: Kappa, lambda
- Heavy chain: IgA, IgG, IgM

**Hyperdiploidy**

- IgA
- or
- IgG
Other common changes

• +1q (gain or amplification)
• del(13q)
• del(17p)
• del(1p)
• del(14q)
• del(16q)

• Smaller mutations at the gene level
  • KRAS/NRAS
  • BRAF

  • These aren’t be picked up by conventional testing
How do we test for these changes?

• Bone marrow biopsy
• Separate out the plasma cells from other healthy cells
• 2 tests:
  • Karyotype/cytogenetics
  • FISH (fluorescent in situ hybridization)
Karyotype (Cytogenetics)


https://www.wikiwand.com/en/Virtual_karyotype
Example of abnormal karyotype

• Downsides:

• Time intensive
• Only able to detect big changes
• Cells need to be actively dividing, so this often appears normal in MM

https://www.researchgate.net/figure/Hyperdiploid-karyotype-GTG-banded-with-numerical-and-structural-changes_fig1_43531882
FISH

• Specific probes used to detect regions of interest

• Pros:
  • We already know what to look for, so we can ask to look for just the specific changes we want to know
  • Doesn’t require a dividing cell

• Cons:
  • This will only detect the abnormality that you are “fishing” for
  • Can’t detect small mutations, just whether the areas are present or combined with the other probe

FISH

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Example of a cytogenetics report

B. FISH analysis: CD138+ cells

<table>
<thead>
<tr>
<th>Probe</th>
<th># nuclei examined</th>
<th>% positive for abnormality</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1/IGH</td>
<td>200</td>
<td>23.0% fusion</td>
<td>0-3%</td>
</tr>
<tr>
<td>FGFR3/IGH</td>
<td>200</td>
<td>16.5% split IGH</td>
<td>0-6%</td>
</tr>
<tr>
<td>IGH/MAF</td>
<td>200</td>
<td>17.5% split IGH</td>
<td>0-5%</td>
</tr>
<tr>
<td>IGH/MAFB</td>
<td>200</td>
<td>20.5% split IGH</td>
<td>0-6%</td>
</tr>
<tr>
<td>IGH break apart</td>
<td>200</td>
<td>16.0% IGH rearranged</td>
<td>0-6%</td>
</tr>
<tr>
<td>D13S319</td>
<td>200</td>
<td>22.0% monosomy 13</td>
<td>0-4%</td>
</tr>
<tr>
<td>ATM</td>
<td>200</td>
<td>2.0%</td>
<td>0-6%</td>
</tr>
<tr>
<td>TP53</td>
<td>200</td>
<td>4.0%</td>
<td>0-8%</td>
</tr>
<tr>
<td>CKS1B,CDKN2C</td>
<td>200</td>
<td>12.0% gain 1q</td>
<td>0-6%</td>
</tr>
</tbody>
</table>

C. FISH analysis: CD138+ cells

<table>
<thead>
<tr>
<th>Probe</th>
<th># nuclei examined</th>
<th>% positive for abnormality</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 5</td>
<td>200</td>
<td>1.0%</td>
<td>0-4%</td>
</tr>
<tr>
<td>Chromosome 7</td>
<td>200</td>
<td>0.5%</td>
<td>0-3%</td>
</tr>
</tbody>
</table>

RESULT:
A. 43,X,-X,der(7)t(1;7)(q21;p15),-10,t(11;14)(q13;q32),-13,-13,?add(16)(p13.3)[1]/46,XX[19]

• This patient has t(11;14), -13, and gain(1q)
Which cytogenetics are “high risk”

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**Table 1. Standard Risk Factors for MM and the R-ISS**

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Serum β₂-microglobulin &lt; 3.5 mg/L, serum albumin ≥ 3.5 g/dL</td>
</tr>
<tr>
<td>II</td>
<td>Not ISS stage I or III</td>
</tr>
<tr>
<td>III</td>
<td>Serum β₂-microglobulin ≥ 5.5 mg/L</td>
</tr>
<tr>
<td>CA by iFISH</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)</td>
</tr>
<tr>
<td>Standard risk</td>
<td>No high-risk CA</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Serum LDH &lt; the upper limit of normal</td>
</tr>
<tr>
<td>High</td>
<td>Serum LDH &gt; the upper limit of normal</td>
</tr>
</tbody>
</table>

**A new model for risk stratification for MM**

- **R-ISS stage**
  - I: ISS stage I and standard-risk CA by iFISH and normal LDH
  - II: Not R-ISS stage I or III
  - III: ISS stage III and either high-risk CA by iFISH or high LDH

Abbreviations: CA, chromosomal abnormalities; iFISH, interphase fluorescent in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; R-ISS, revised International Staging System.

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**Table 1. Molecular cytogenetic classification and risk stratification of multiple myeloma (MM).**

<table>
<thead>
<tr>
<th>Cytogenetic abnormality</th>
<th>Gene/chromosome (s) affected</th>
<th>Risk stratification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary cytogenetic abnormality</td>
<td>Trisomies of one or more odd-numbered chromosomes</td>
<td>Standard risk</td>
</tr>
<tr>
<td>Trisomic MM</td>
<td>CCND1</td>
<td>Standard risk</td>
</tr>
<tr>
<td>t (11;14) MM</td>
<td>FGFR3 and MMSET</td>
<td>High risk</td>
</tr>
<tr>
<td>MAF MM</td>
<td>C-MAF</td>
<td>High risk</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>MAF-B</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>Standard risk</td>
</tr>
</tbody>
</table>

**Secondary cytogenetic abnormality**

- Gain (1q) 1q
- Del (17p) p53
- p53 mutation p53

**Other** Variable

*Presence of any two high-risk cytogenetic abnormalities is considered double-hit MM. Presence of any three or more high-risk cytogenetic abnormalities is considered triple-hit MM.

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Palumbo, et al, JCO 2015

What do I consider to be “high risk”? 

- t(4;14) 
- t(14;16) 
- t(14;20) 
- del(17p) 
- +1q 
- del(1p) 

- Combinations of multiple “high risk” cytogenetic abnormalities = “ultra high risk”? 

- Others: 
  - Plasma cell leukemia 
  - Extramedullary disease
Summary of cytogenetic risk

• What does it mean to have “high risk” or “standard risk” disease?

• High risk = more likely to be difficult to control
• Standard risk = more likely to be easier to control

• Expectations are important. They help to frame decision making
  • Can change risk:benefit ratio about side effects versus effectiveness of treatment
  • When to relax and when to be more aggressive
  • For some patients, this can impact treatment decisions

• But cytogenetics aren’t the only factor.
  • Many patients with high risk disease do well for a long time.
  • Some patients with “standard risk” have early progression and myeloma is more difficult to control
What will we use in the future?

- Gene expression profiling

(Shaughnessy Jr, Zhan et al. 2005)
What might we use in the future?

- Gene expression profiling
- Next generation sequencing

(Walker, Mavrommatis et al. 2018)
What might we use in the future?

- Gene expression profiling
- Next generation sequencing
- Whole genome sequencing
What might we use in the future?

• Gene expression profiling
• Next generation sequencing
• Whole genome sequencing
• Single cell sequencing
What might we use in the future?

• Gene expression profiling
• Next generation sequencing
• Whole genome sequencing
• Single cell sequencing
• All of the above?
What might we use in the future?

- Gene expression profiling
- Next generation sequencing
- Whole genome sequencing
- Single cell sequencing
- All of the above?

- None of the above?

(Costa, Chhabra et al. 2019)
What impact do cytogenetics have on treatment today?

• As of right now, nothing definitive, but we’re moving in that direction
Proposed Management Schemes by Risk

Mayo

a

Newly Diagnosed Myeloma: Transplant Eligible

- **Standard Risk**
  - VRd x 3-4 cycles

- **High Risk**
  - VRd or Dara-VRd* x 3-4 cycles

- **Early ASCT**
- **Lenalidomide maintenance**
- **Lenalidomide maintenance**
- **Delayed ASCT at relapse**
- **Bortezomib-based maintenance**

Emory

b

Newly Diagnosed Myeloma: Transplant Ineligible

- **Standard Risk**
  - VRd x 8-12 cycles

- **High Risk**
  - VRd x 8-12 cycles

- **Lenalidomide maintenance**
- **Bortezomib-based maintenance**

(Rajkumar and Kumar 2020)
Early hints of targeted therapy?

- Venetoclax in t(11;14)

Kumar, et al, Blood 2017
Kumar, et al, Lancet Oncol 2020
What impact do cytogenetics have on treatment today?

• As of right now, nothing clear cut, but we’re getting there

• No studies have clearly shown that we should be treating patients differently based on their cytogenetics

• While cytogenetics have helped us to identify trends about prognosis among all patients with myeloma, for individual patients the most important thing is:

  How things are going for you or your loved one!

• Myeloma is like a person – the first impression can shape your opinion of it, but it takes a lifetime to really get to know it.
Thank you!

Questions?

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