MOLECULAR DIAGNOSTICS OF MULTIPLE MYELOMA

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Disclosures
Objectives

- Define MGUS (monoclonal gammopathy of undetermined significance) and multiple myeloma
- Discuss the molecular pathogenesis of the initiation and progression of MGUS and multiple myeloma
- Discover diagnostic cytogenetics and molecular techniques for myeloma characterization and risk stratification
- Learn diagnostic limitations in plasma cell neoplasms
  - Intratumoral heterogeneity
  - Isolation of plasma cells
- Preview novel myeloma diagnostics
  - E.g. Next generation sequencing (NGS), gene expression profiling (GEP)
Multiple Myeloma 101

- Malignant neoplasm of **Plasma cells**
  - Terminally differentiated non-dividing cell of B-lymphoid lineage
  - Synthesize immunoglobulin (antibodies) that are antigen specific
  - Clonal plasma cells typically secrete one antibody (heavy chain + light chain → monoclonal protein)

- **Incredibly heterogeneous**; multiple disease types (low risk to high risk)

- **Genome instability**
  - *Almost all patients with multiple myeloma are cytogenetically abnormal*

- All cases thought to progress through **precursor disease (MGUS)**
Disease Definitions

- **MGUS** – annual risk of progression 1%
  - M-protein < 3 g/dL
  - Clonal plasma cells in BM <10%
  - Absence of end-organ damage (CRAB criteria)

- **Smoldering myeloma (SMM)** – annual risk of progression 10%(5yrs) 3%(5yrs) 1%(10yrs)
  - M-protein ≥ 3 g/dL
  - Clonal plasma cells in BM ≥ 10%
  - Lack of CRAB end-organ damage

- **Clinical myeloma**
  - M-protein, clonal plasma cells, AND CRAB end-organ damage
  - (New) BM plasmacytosis ≥ 60%; (new) abnormal sFLC \( \kappa/\lambda \) ratio ≥ 100 (kappa) or ≤ 0.01 (lambda); (new) focal BM lesions detected by PET-CT and/or MRI in asymptomatic individuals
**There are two MGUS types**

- **Lymphoid/lymphoplasmacytic MGUS** = IgM MGUS
- **Plasma cell MGUS** = Non-IgM MGUS (IgG > IgA > Ig light chain > IgD > IgE)
  - Lifelong follow-up recommended given **1%/year risk of malignant progression**
  - Risk factors for progression: non-IgG M-protein; M-protein > 1.5 g/dL; **abnormal FLC ratio**, hypogammaglobulinemia
    - Bone marrow, bone survey indicated
- **Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial**
  - Prediagnostic serum samples consistently demonstrated MGUS in the years before the malignant diagnosis in 71 patients who developed myeloma in 10 yr f/u

Blood 2014, 123(3) 305-307

M-protein–related diseases

- AL amyloidosis
- MGUS (MIDD, ITG, FS, etc)
- Other M-protein–related diseases

Survival

Irreversible end-organ damage

Tumor progression

- MGUS
- Intermediate- and high-risk MGUS
- Low-risk SMM
- High-risk SMM
- Active MM
- End-organ damage (CRAB)
- EMM, PCL

Accumulation of genetic, epigenetic, and microenvironmental abnormalities
Myeloma Pathogenesis
Myeloma Pathogenesis #1

- Inciting event and cytogenetic abnormalities ➔ MGUS
  - Thought to be product of abnormal plasma cell response to antigenic stimulation
  - Monoclonal immunoglobulin production
Myeloma Pathogenesis
Translocations

- Oncogene (CCND1, MYC, etc.) juxtaposed to IgH enhancer, resulting in aberrant overexpression
- Growth and replication via novel:
  - Transcription factors
  - Growth factor receptors
  - Cell cycle mediators
Translocations – common involved oncogenes

- **t(11;14) 11q13 – cyclin D1**
- **t(12;14) 12p13 – cyclin D2**
- **t(14;16) 16q23 – c-maf**
- **t(6;14) 6p21 – cyclin D3**
- **t(8;14) 8q24 – c-myc (usually secondary) or mafA**
- **t(4;14) 4p16.3 – multiple myeloma set domain & fibroblast growth receptor 3**
- **t(14;20) 20q11 – mafB**
Multiple myeloma is *odd*

- Almost all MM tumors are aneuploid (numerical abnormalities of chromosomes)
- Reflects genetic instability
- **Hyperdiploid** group:
  - 48-75 chromosomes ➔ usually 49-56
  - Generally affects *odd-numbered chromosomes* (except 1, 13, 17)
  - Low incidence of structural chromosome abnormalities
- Overexpression of genes on affected chromosomes
Cytogenetic Abnormalities

MM abnormality

High risk
- $\text{IGH-MMSET}(\text{FGFR3}); t(4;14)$
- $\text{IGH-MAF}; t(14;16)$
- $\text{IGH-MAFB}; t(14;20)^*$
- $\text{IGH-MAFA}; t(8;14)^*$

Favorable/Standard Risk
- $\text{IGH-CCND1}; t(11;14)$
- $\text{IGH-CCND3}; t(6;14)^*$
- $\text{IGH-CCND2}; t(12;14)^*$

Hyperdiploidy

*Rare (together < 3% of MM)
Universal cyclin dysregulation is initiating step

- Deregulation of the G1/S cell cycle transition point
  - via the overexpression of cyclin D genes, an event shown to be a key early molecular abnormality in myeloma
Myeloma Pathogenesis #2

- Progression from MGUS to MM
  - Additional genetic insults
  - Changes in the bone marrow microenvironment
Progression of MGUS/myeloma

Secondary abnormalities – cell cycle dysregulation

- Aberrant expression of cyclin proteins
- Disruption of the retinoblastoma pathway (checkpoint in cell cycle)
  - translocations, mutations, loss of, hypermethylation of:
    - cyclin D kinase inhibitors (CdkI)
    - CDKN2A (p16), CDKN2C
    - Tumor suppressors

TP53 (p53) deletion or mutation ➔ p21 ➔ G1/S dysregulation

RB inactivation (chromosome 13 deletion)
Secondary abnormalities – signaling pathways

Cytokines, growth, and survival factors (e.g., TNF-α, IL-6, and IGF-1)

Uncommon in MGUS
Myeloma Pathogenesis #3

- Extramedullary progression
  - Peripheral
  - Solid tissues / body cavities
multiple myeloma DIAGNOSTICS
Bone marrow evaluation
Bone Marrow Aspirate
Conventional Cytogenetics

- Proliferating cells only, low overall sensitivity
- Fresh tissue
- 20 metaphases analyzed
- Labor intensive, expensive
Fluorescence in situ hybridization (FISH)

- Hundreds of cells, in interphase (not dividing)

- Plasma cell selection:
  - Staining with cytoplasmic immunoglobulin
  - Cell sorting and plasma cell labeling (purification) with anti-CD138-coated magnetic beads

- Pros: sensitive, specific

- Cons: Labor intensive, very targeted probes
Table 5  New proposed International Myeloma Working Group molecular cytogenetic classification

<table>
<thead>
<tr>
<th>Percentage of patients</th>
<th>Clinical and laboratory features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyperdiploid</strong></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>More favorable, IgG-κ, older patients.</td>
</tr>
<tr>
<td><strong>Non-hyperdiploid</strong></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Aggressive, IgA-λ, younger individuals</td>
</tr>
<tr>
<td><strong>Cyclin D translocation</strong></td>
<td>18</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Upregulation of CCND1; favorable prognosis; bone lesions. Two subtypes by GEP</td>
</tr>
<tr>
<td>t(6;14q)(p21;32)</td>
<td>2</td>
</tr>
<tr>
<td>t(12;14)(p13;q32)</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>MMSET translocation</strong></td>
<td>15</td>
</tr>
<tr>
<td>t(4;14)(p16;q32)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Upregulation of MMSET; upregulation of FGFR3 in 75% unfavorable prognosis with conventional therapy; bone lesions less frequent</td>
</tr>
<tr>
<td><strong>MAF translocation</strong></td>
<td>8</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Confirmed as aggressive by at least two series</td>
</tr>
<tr>
<td>t(14;20)(q32;q11)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>One series shows more aggressive disease.</td>
</tr>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unknown effect on outcome but presumed aggressive.</td>
</tr>
<tr>
<td><strong>Unclassified (other)</strong></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Various subtypes and some with overlap</td>
</tr>
</tbody>
</table>
Role of Cytogenetics and FISH

- Conventional karyotyping
  - No abnormality better than any
    - The detection of a clone reflects dividing cells
  - High-risk
    - del(13)
    - Hypodiploidy
  - Favorable
    - Hyperdiploidy
    - t(11;14)

- FISH
  - High-risk
    - del(17p)
    - t(4;14)
    - t(14;16)
    - 1p/q abnormalities
  - Favorable
    - Hyperdiploidy
    - t(11;14)
Poor prognosis is multifactorial

- Newly diagnosed myeloma pts < 66 yo (IFM – French study)
- No proteasome (bortezomib) inhibition
- Overall survival according to number of poor prognostic factors
  - Age > 55
  - β2-microglobulin > 5.5 mg/L (albumin)
  - t(4;14) (11%) 
  - Del(17p) (5.4%)
  - 1q gains (33%)

J Clin Oncol June 1, 2012 vol. 30 no. 16 1949-1952
Other Studies

- Plasma cell DNA content and proliferation
  - Now **flow cytometry-based** at Mayo Clinic
    - % of cells in S-phase, DNA index (0.95-1.05), ploidy, % polytypic plasma cells
  - Supplants *plasma cell labeling index* test, now somewhat outdated
    - Slide-based immunofluorescence staining proliferating cells
    - Measured the % of plasma cells in **S-phase** of cell cycle

- Immunohistochemistry
When will my MGUS patient develop myeloma?

No unequivocal genetic or phenotypic markers to differentiate
Gene Expression Profiling
## Genetic Classification Systems

<table>
<thead>
<tr>
<th>Group</th>
<th>TC</th>
<th>Gene</th>
<th>%</th>
<th>UAMS</th>
<th>HOVON-GMMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D translocation</td>
<td>11q13</td>
<td>CCND1</td>
<td>15%</td>
<td>CD-1 CD-2</td>
<td>CD-1 CD-2</td>
</tr>
<tr>
<td></td>
<td>6p21</td>
<td>CCND3</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12p13</td>
<td>CCND2</td>
<td>&lt;1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSET translocation</td>
<td>4p16</td>
<td>MMSET</td>
<td>15%</td>
<td>MS</td>
<td>MS</td>
</tr>
<tr>
<td>MAF translocation</td>
<td>16q23</td>
<td>c-maf</td>
<td>5%</td>
<td>MF</td>
<td>MF</td>
</tr>
<tr>
<td></td>
<td>20q12</td>
<td>mafB</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8q24</td>
<td>mafA</td>
<td>&lt;1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>D1</td>
<td>CCND1</td>
<td>33%</td>
<td>HY</td>
<td>HY CD-1 NFkB CTA PRL3</td>
</tr>
<tr>
<td></td>
<td>D1+D2</td>
<td>CCND1 + CCND2</td>
<td>7%</td>
<td>PR</td>
<td>PR CTA</td>
</tr>
<tr>
<td>Other</td>
<td>None</td>
<td>No CCND</td>
<td>2%</td>
<td>PR</td>
<td>PR CTA PRL3</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>CCND2</td>
<td>18%</td>
<td>LB</td>
<td>LB CTA PRL3</td>
</tr>
</tbody>
</table>
Next Generation Sequencing

The Future of Molecular Diagnostics

Table 2. MM molecular subgroups potentially suitable for future targeted trials

<table>
<thead>
<tr>
<th>Molecular feature</th>
<th>Current detection method</th>
<th>Future detection method</th>
<th>Potential targeted treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)</td>
<td>FISH</td>
<td>Targeted NGS, RO-PCR, GEP</td>
<td>Proteasome inhibitors (?), MMSET inhibitors, FGFR3 inhibitors, MEK inhibitors</td>
</tr>
<tr>
<td>t(14;16), t(14;20) Overexpression of MAF or MAFB</td>
<td>FISH</td>
<td>Targeted NGS, RO-PCR, GEP</td>
<td>MEK inhibitors</td>
</tr>
<tr>
<td>ISS/FISH high risk Combination of t(4;14) or t(14;16)/t(14;20), del(17p) and/or gain(1q)</td>
<td>FISH, SSCP</td>
<td>Targeted NGS, RO-PCR + FISH/SSCP, GEP + FISH/SSCP</td>
<td>Treatment intensification, novel drugs</td>
</tr>
<tr>
<td>BRAF V600E mutation</td>
<td>Various (SSCP, Sanger sequencing)</td>
<td>Targeted NGS</td>
<td>BRAF inhibitors</td>
</tr>
<tr>
<td>Unfavorable GEP</td>
<td>GEP</td>
<td>Validated GEP signature, GEP-derived RO-PCR (?)</td>
<td>Novel inhibitors targeting overexpressed genes, eg, AURKA inhibitors</td>
</tr>
<tr>
<td>Absence of unfavorable features</td>
<td>FISH</td>
<td>Targeted NGS, RO-PCR + FISH, GEP + FISH</td>
<td>Combinations of established agents, innovative maintenance strategies</td>
</tr>
</tbody>
</table>

RQ-PCR indicates real-time quantitative PCR.

Clonal heterogeneity (genomic chaos)

- Multiple subclones at time of diagnosis
- Certain clones dominate the malignancy at any one point in disease course
  - therapy imparts selection pressures ➔ subclones emerge
- Hazard to targeted therapy
  - Mutations often present in subclones (e.g. BRAF)
- Model for treatment resistance; lack of curability

Clinical Examples
Mayo FISH panel

-13/13q-, *RB1/LAMP1*
- t(11;14), *CCND1/IGH*
- t(14;var), *IGH*
- 17p-, *TP53/Cen17*
- +3/+7, Cen3/Cen7
- +9/+15, Cen9/Cen15
- 1q gain, *TP73/CKS1B*
- t(8;var), *MYC*

Reflex if IGH rearrangement not with *CCND1*:
- t(4;14)(p16.3;q32) *FGFR3/IGH*
- t(14;16)(q32;q23) *IGH/MAF*
- t(14;20)(q32;q12) *IGH/MAFB*
- t(6;14)(p21;q32) *CCND3/IGH*
mSMART

Mayo Stratification for Myeloma And Risk-adapted Therapy

Newly Diagnosed Myeloma

High-Risk
- FISH<br>  - Del 17p<br>  - t(14;16)<br>  - t(14;20)<br>- GEP<br>  - High risk signature

Intermediate-Risk<sup>a</sup>
- FISH<br>  - t(4;14)<sup>d</sup><br>  - 1q gain<br>  - Complex karyotype<br>  - Metaphase Deletion 13 or hypodiploidy<br>  - High PC S-phase<sup>f</sup>

Standard-Risk<sup>a,b</sup>
- All others including:<br>  - Trisomies<br>  - t(11;14)<sup>e</sup><br>  - t(6;14)
Future Direction

- Role of molecular/cytogenetic testing after treatment
- Role of minimal residual disease in optimizing management and prognosis
- Effect of proteasome inhibitors, IMiDs, and total therapy (TT) on prognosis – will conventional prognostic indicators still stand?
- Comprehensive genomic tools for risk stratification and targeted therapy
- Intratumoral heterogeneity
  - Technologies generally reflect the predominant clonal population
Take-away

- Myeloma is extremely heterogeneous but always progresses through a benign precursor stage (MGUS, smoldering disease)
- MGUS is of two types: IgM and non-IgM
- MGUS progresses at an annual rate of 1% to myeloma requiring treatment
- Genomic instability is present from the earliest stages
- FISH is the mainstay of current molecular diagnostics
- Next generation sequencing is likely to play a very significant role in the near-term future
- Risk stratification is multifactorial; clinical, laboratory, genetic data
- Clonal heterogeneity is a major limitation to diagnosis and effective therapy