Molecular Pathogenesis of Multiple Myeloma:

Ig translocations
hyperdiploid vs non-hyperdiploid
CYCLIN D dysregulation
other oncogenic events

Michael Kuehl
MM: post-germinal center tumor of long-lived BM PC

Germinal Center
- Hypermutation
- Antigen selection
- IgM switching

Lymph Node
- Immature B cell
- Mature B cell
- Lymphoblast
- Short-lived PC (3 days)
- IgM
- G, A, E, D

Bone Marrow
- Plasmablast > Long-lived PC (30 days)
- VDJ recombination
- Immature B cell
Germinal Center B cell

Normal Long-lived Plasma cell

Stages: pre-malignant MGUS > MM > PCL > HMCL

- bone marrow stromal cell dependence
- IL-6 dependence
- increased DNA labelling index
- angiogenesis
- bone destruction

MGUS

Smoldering MM

Intra-medullary MM

Extra-medullary MM (PCL)

Myeloma Cell Line (HMCL)
B-cell-specific DNA modifications: 
Translocation & mutations in tumors

Early B-cell development: 
VDJ recombination

Germinal center: 
IgH switch 
recombination 
Somatic hypermutation
14q32 translocation breakpoints: JH vs SWITCH

14q32

\[ \text{VH} \quad \text{VDJ} \quad \text{E} \quad \text{E} \]

\[
\begin{array}{c}
\text{der 14} \\
\text{JH} \\
\text{der 11} \\
\text{Switch} \\
\text{der 4}
\end{array}
\]

\[
\begin{array}{c}
\text{CYCLIN D1} \\
\text{FGFR3} \\
\text{MMSET}
\end{array}
\]

telomeric

centromeric
Diversity of IgH translocations in MM

6 recurrent partners 40%

11q13 (CYCLIN D1) 15
6p21 (CYCLIN D3) 3
16q23 (c-MAF > CYCLIN D2) 4
20q11 (MAF B > CYCLIN D2) 2
8q24 (MAF A > CYCLIN D2) 1
4p16 (MMSET&FGFR3) 15

8q24 (c-MYC) 4%

Other (rare) partners 16%

NO IgH translocation 40%
### MYC translocations: a late event in MM

<table>
<thead>
<tr>
<th></th>
<th>Burkitt’s</th>
<th>Myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
<td>Early (initiation)</td>
<td>Late (progression)</td>
</tr>
<tr>
<td><strong>Prevalence</strong></td>
<td>Always</td>
<td>MGUS: absent or rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All MM: 15% (IgH, 4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Advanced MM: 44%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMCL: 91%</td>
</tr>
<tr>
<td><strong>Type of translocation</strong></td>
<td>Simple</td>
<td>Mostly complex</td>
</tr>
<tr>
<td><strong>Ig locus associated</strong></td>
<td>Always</td>
<td>~60% (IgH ~ Ig(\lambda) &gt;&gt; Ig(\kappa))</td>
</tr>
<tr>
<td><strong>B-cell mechanism</strong></td>
<td>Yes</td>
<td>No (off in PC &amp; tumors)</td>
</tr>
<tr>
<td><strong>Heterogeneity</strong></td>
<td>No</td>
<td>Sometimes</td>
</tr>
</tbody>
</table>

* c-MYC >> N-MYC > L-MYC
IgH location of 42 cloned MM breakpoints

- 4p16
- 11q13, 6p21
- 16q23, 20q11
- other

5’ breakpoints

3’ breakpoints
Structure of IgH and Igλ Translocations
metaphase chromosomes: 48 tumors & 33 HMCL

<table>
<thead>
<tr>
<th>IgH partners</th>
<th>#</th>
<th>simple TLC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Recurrent</td>
<td>42</td>
<td>83%</td>
</tr>
<tr>
<td>MYC</td>
<td>21</td>
<td>24%</td>
</tr>
<tr>
<td>Other</td>
<td>26</td>
<td>19%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Igλ partners</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Recurrent</td>
<td>3</td>
<td>33%</td>
</tr>
<tr>
<td>MYC</td>
<td>10</td>
<td>20%</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>33%</td>
</tr>
</tbody>
</table>

* Simple reciprocal translocations
Two kinds of chromosome content in MM

Hyperdiploid (HRD) & non-hyperdiploid (NHRD)
- each ~50% tumors
- NHD poorer prognosis than HRD

HRD (48-75 chromosomes)
- multiple trisomies involving 8 chromosomes
  3, 5, 7, 9, 11, 15, 19, 21

NHRD (<48 and/or >75 chromosomes)
- increased prevalence of IgH translocations
Translocations: HRD vs NHRD MM

metaphase FISH: 48 MM tumors & 33 HMCL

<table>
<thead>
<tr>
<th></th>
<th>HRD</th>
<th>NHRD</th>
<th>HMCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgH TLC</strong></td>
<td>31%</td>
<td>81%</td>
<td>88%</td>
</tr>
<tr>
<td>6 recurrent partners</td>
<td>12</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td>other IgH partners</td>
<td>19</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ig[] TLC</strong></td>
<td>15</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td><strong>MYC TLC</strong></td>
<td>45</td>
<td>46</td>
<td>88</td>
</tr>
</tbody>
</table>
> 2 Ig translocations in an MM tumor cell
(64% of HMCL, 29% of advanced MM, ~5%MGUS)

Rarely involves 2 of the 6 major recurrent partners, but this includes all functionally distinct combinations:

- CYCLIN D1 or CYCLIN D3 + c-MAF or MAF B
- CYCLIN D1 or CYCLIN D3 + FGFR3/MMSET
- FGFR3/MMSET + c-MAF or MAF B

More often a recurrent partner and a rare partner, or two different rare partners

Most often c-MYC and a recurrent or a rare partner
Different roles for translocations in MM

Hyperdiploid pathway
(12% recurrent/ 1° IgH TLC)

Germinal center B cell

Non-hyperdiploid pathway
(67% recurrent/ 1° IgH TLC)

MGUS

Intra-medullary MM

Extra-medullary MM (PCL)

Myeloma Cell Line (HMCL)

2° Ig TLC (IgH ~ Igκ >> Igλ)

MYC TLC
Expression of CCND in PC, MM and other hematopoietic tumors

A. AML ALL NHL

B. CLL

C. BMPC

D. MM

Relative CCND expression vs. Proliferation Index (PI)
### Cyclin D1, D2, D3 Relative RNA Expression in PB, PC, 261 MM, HMCL

#### 8 MM tumor groups

|       | PB  | PC  | 4p  | 16% | MAF | 6p  | 3%  | 11q | 16% | D1  | 31% | D1+D2 | 7% | D2  | 17% | O   | 2% | HMCL |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|

- **CCND1**
- **CCND2**
- **CCND3**
8 TC (Translocation/Cyclin D) groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>% MM</th>
<th>% HRD</th>
<th>% MGUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p</td>
<td>16</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>MAF</td>
<td>7</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>6p</td>
<td>3</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>11q</td>
<td>16</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>D1</td>
<td>31</td>
<td>93</td>
<td>24</td>
</tr>
<tr>
<td>D1+D2</td>
<td>7</td>
<td>95</td>
<td>9</td>
</tr>
<tr>
<td>D2</td>
<td>17</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>none</td>
<td>2</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>261</td>
<td>50%</td>
<td>21</td>
</tr>
</tbody>
</table>

Limited # of samples
Specific gene-expression patterns in 5 largest MM groups:
(maf > 4 > D1 > 11 > D2)

Supervised cluster using 1002 genes that best distinguish the 5 largest MM groups
## Prevalence of K- and N-RAS mutations in 8 MM Groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th># MM</th>
<th>% K-RAS</th>
<th>P value</th>
<th>% N-RAS</th>
<th>P value</th>
<th>% K- or N-RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p</td>
<td>57</td>
<td>14</td>
<td>0.55</td>
<td>3</td>
<td>&lt;.01</td>
<td>17</td>
</tr>
<tr>
<td>MAF</td>
<td>10</td>
<td>20</td>
<td>0.69</td>
<td>10</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>6p</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>11q</td>
<td>58</td>
<td>17</td>
<td>1</td>
<td>24</td>
<td>&lt;.02</td>
<td>41</td>
</tr>
<tr>
<td>D1</td>
<td>73</td>
<td>16</td>
<td>0.71</td>
<td>21</td>
<td>0.04</td>
<td>37</td>
</tr>
<tr>
<td>D1+D2</td>
<td>10</td>
<td>40</td>
<td>0.07</td>
<td>0</td>
<td>0.36</td>
<td>40</td>
</tr>
<tr>
<td>D2</td>
<td>35</td>
<td>20</td>
<td>0.59</td>
<td>3</td>
<td>0.06</td>
<td>23</td>
</tr>
<tr>
<td>none</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>0.36</td>
<td>25</td>
</tr>
<tr>
<td>TOTAL</td>
<td>248</td>
<td>17</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>31</td>
</tr>
</tbody>
</table>

(low number of tumor samples in these groups)
TC groups: differences in lytic bone disease

<table>
<thead>
<tr>
<th>GROUP</th>
<th># MM</th>
<th>%MRI+</th>
</tr>
</thead>
<tbody>
<tr>
<td>4p</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>MAF</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>6p</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>11q</td>
<td>27</td>
<td>94</td>
</tr>
<tr>
<td>D1</td>
<td>57</td>
<td>86</td>
</tr>
<tr>
<td>D1+D2</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>D2</td>
<td>27</td>
<td>67</td>
</tr>
<tr>
<td>none</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>all</td>
<td>172</td>
<td>79</td>
</tr>
</tbody>
</table>

Limited # of samples
Survival of different tumor groups after high-dose chemo + stem cells

Avet-Loiseau, Blood 2002
Selective progression of MM to HMCL

<table>
<thead>
<tr>
<th></th>
<th>TUMOR</th>
<th>HMCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 recurrent IgH translocations</td>
<td>40%</td>
<td>76%</td>
</tr>
<tr>
<td>D1 group</td>
<td>35%</td>
<td>NONE</td>
</tr>
<tr>
<td>D1 + D2 group</td>
<td>8%</td>
<td>3%</td>
</tr>
</tbody>
</table>
A Translocation/CYCLIN D (TC) classification of MM
(based on 6 recurrent Translocations & Cyclin D expression)

Analogous to classification of ALL based on translocations and ploidy

Based on apparent unifying and early dysregulation of a CYCLIN D gene in MGUS and MM

Gene-expression patterns associated with groups

Biological and clinical features associated with groups

Prognosis and response to different treatments associated with groups

Tumor phenotype appears to be determined by early pathogenic events, consistent with hypothesis that MM represents several different diseases
# p18 RNA and proliferation index

16 normal PC; 33 HMCL; 261 MM tumors

<table>
<thead>
<tr>
<th>PI</th>
<th>#</th>
<th>&lt;0.55</th>
<th>0.55-2</th>
<th>&gt;2</th>
<th>p18&gt;2, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL PC</td>
<td>&lt;1</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>HMCL</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>32</td>
<td>10</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>MM</td>
<td>&lt;1</td>
<td>151</td>
<td>19</td>
<td>127</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>60</td>
<td>3</td>
<td>49</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>50</td>
<td>6</td>
<td>14</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>normalized p18INK4c RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>p18&gt;2, %</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>60</td>
</tr>
</tbody>
</table>
Fig. 6 Effect of transfected p18 on growth of HMCL

A. Growth of GFP Infected Cells

- L363 mock
- L363 p18
- OPM2 p18
- JIM 3 p18
- KMS12pe p18

B. OPM2 in S-Phase
6 Days Post Infection

<table>
<thead>
<tr>
<th>Percent of Cells in S-Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
</tr>
<tr>
<td>p18</td>
</tr>
</tbody>
</table>

C. Western blot

- OPM2 WT 100 ug
- OPM2 p18 15% inf, 100 ug
- L363 WT 12.5 ug
- L363 WT 25 ug
- L363 p18 100% inf, 12.5 ug
- Jim3 100 ug
The RB pathway can be altered by sequential events in an MM tumor.
activating mutations: N, K- RAS, FGFR3

increased DNA labeling index

bone destruction

angiogenesis

Germinal Center B cell

MGUS

Smoldering Myeloma

Intramedula Myeloma

Extra-medula Myeloma

Myeloma Cell Line

Karyotypic & epigenetic abnormalities

Secondary (Ig) TLC

activating mutations: N, K- RAS, FGFR3

p18, RB inactivation

MYC dysregulation

p53 mutation
Most MGUS/MM cells are not cycling

Is there a stem cell in MGUS/MM? If so, what is the phenotype of the stem cell?
A few thoughts about treatment of MM

Classification schemes (TC, PI, and others):

different tumor phenotype => different responses to different therapies

Targeted therapy, e.g., small-molecule inhibitors of FGFR3 in 15% of tumors with t(4;14)

Tumor/bone marrow stromal interaction as a therapeutic target
Should MGUS be treated?

- Diagnosed by simple blood tests
- Same age dependence as MM
- Prevalence about one million (~ 50,000 new cases per year)
- Disseminated in bone marrow (cf normal BM plasma cells)
- Overall survival marginally less than age-matched controls
  UNLESS there is primary amyloidosis (AL) with pathological Ig protein deposits in critical tissues
- Non-IgM MGUS > MM at rate of 0.5-3% per year depending on level of monoclonal Ig (i.e., tumor mass)
- Unable to predict if and when progression to MM will occur
- Tumors with t(4;14) and t(14;16) are rare: ?rapid progression
- Tumors with t(11;14) somewhat enriched; especially in AL
COLLABORATORS

Kuehl lab
Current
Leslie Brents
Conny Cultraro
Ana Gabrea
Ben Ristau
Adriana Zingone

Alumni
Amel Dib
Tim Peterson
Marina Martelli
Ying Qi
Yaping Shou
Laura Raducha-Grace

Mayo Clinic (Arizona)
Leif Bergsagel
Marta Chesi
Rafael Fonseca

U. Arkansas
John Shaughnessy
Jeff Sawyer
Bart Barlogie

NCI
Lou Staudt
Lanny Kirsch
Thomas Reid