Multiple myelomas

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Outline

1. Background
2. Risk stratification schemes
3. Mutational landscape
4. Implications for treatment
5. Goals and challenges
Pathobiology: Cost of immunity

- PCs begin as activated B cells that clonally expand in the GC of the LN and there undergo affinity maturation via somatic hyper-mutation at the *IGH* locus
- Antibodies are refined via class switch recombination
- Both processes require DNA double strand breaks, resulting in aberrant chromosomal translocations in up to 1% of events: ~1,000 cells per day thus generate an abnormal translocation
- A fraction of these can result in oncogene deregulation
Conventional Prognostic Factors

Host

- Age, comorbidities, frailty
- Low albumin

Disease

- Adverse cytogenetics
- High LDH
- **High B2M**
- Circulating PCs
- Extra-medullary
- Tumor resistance

**Ultra-high-risk: mOS < 2yrs**

1. Co-occurrence of multiple adverse cytogenetics
2. Co-occurrence of (adverse cytogenetics) + (ISS III) + either (high LDH or no CR)

Griepp et al., *JCO*. 2005

Boyd et al., *Leukemia*. 2012
Cytogenetics

- Conventional karyotyping challenging due to inherently low proliferation of plasma cells
- FISH challenging due to variable degree of myeloma cells, and advanced with plasma cell fractionation

<table>
<thead>
<tr>
<th>~60% = Hyperdiploid (#47 – 74 chromosomes)</th>
<th>~40% = Nonhyperdiploid</th>
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<tbody>
<tr>
<td>Recurrent trisomies of odd chromosomes (3, 5, 7, 9, 11, 15, 19)</td>
<td><em>IGH (14q32)</em> translocations</td>
</tr>
<tr>
<td>del 13&lt;sup&gt;1&lt;/sup&gt;; gains 1q; del 1p; del17p</td>
<td></td>
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\[1\] Constitutes high risk only when present on karyotype; if FISH only, is considered standard risk

<table>
<thead>
<tr>
<th>Risk category</th>
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<tr>
<td>Standard risk</td>
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<tr>
<td>High risk</td>
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<table>
<thead>
<tr>
<th>Translocations</th>
<th>Frequency</th>
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<tr>
<td>11;14 (<em>cyclinD1</em>)</td>
<td>19%</td>
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<tr>
<td>6;14 (<em>CCD3</em>)</td>
<td>1%</td>
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<tr>
<td>4;14 (<em>FGFR3</em>)</td>
<td>13%</td>
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<tr>
<td>14;16 (<em>MAF</em>)</td>
<td>4%</td>
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<tr>
<td>14;20 (<em>MAFB</em>)</td>
<td>1%</td>
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Benefit from PI

- High risk
Gene Expression Microarray

- Hypothesis: to develop a prognostically relevant molecular classification scheme based on the MM transcriptome
- Methods:
  1. Newly diagnosed patients treated with ASCT (n = 351 in the training set and n = 214 in the test set)
  2. Samples enriched for PCs
  3. GEP with Affymetrix Microarray
  4. Unique genes exhibiting high variability of expression across the training set were retained (n = 1559)
  5. Unsupervised hierarchic clustering of average linkage used to identify disease subgroups
7 Genetic signatures

2 High risk groups: 1 reveals overexpression of proliferation genes and 1 defined by the t(4;14) translocation (→ **MMSET/FGFR3** expression)
Early disease-related death correlated with gene expression extremes

Identified a 70-gene set with a differential expression pattern in 13% of patients

Gene signature score retained significance after multivariable adjustments for clinical and genetic data, including ISS stage

GEM

- Translated the 70-gene result into a commercial test: MyPRS (Signal Genetics, NY)
GEM

- Notably, the 70-gene risk score outputs markedly increased “high-risk” scores (76% from 13%) at relapse, suggesting “evolution”
- Score also predicts survival post-relapse

GEM: Implementation

1. **2015 NCCN** Unanimously agreed “although GEP is not currently *routinely* used in clinical practice [for MM] during diagnostic workup, GEP is a useful tool and may be helpful in selected patients to estimate the aggressiveness of disease”

2. **2014 IMWG** Consensus statement “no evidence so far” to suggest altering treatment based on risk groups
Beyond GEM: NG Sequencing

Genome Sequencing

Hypotheses

1. Integrating point mutation and CNV analyses will identify ‘significant’ mutations

2. Mutation profile and clonal compositions differ between (non)hyper-diploid and (un)treated cohorts

3. Contribution of subclones can be inferred from a single biopsy
Lohr et al. Methods

- 203 tumor-normal pairs subjected to massively parallel sequencing
  - 177 whole exome sequencing: Illumina HiSeq: 89x depth average coverage
  - 26 whole genome sequencing: Illumina HiSeq: 30x depth average coverage
  - 153 subjected to CNV analysis (Affymetrix SNP array)

- Mutation validation performed on 140 mutations, with a success rate of 90.4%
Eleven mutated genes met statistical significance: 65% of patients

An additional 6 “gene sets” of mutations met statistical significance (primarily members of cell-cycle pathways e.g. CDKN1B, CCND1)
  - Borderline significance alone, but reach significance as a “pathway”: 51% of patients

Leaves 18% of patients without obviously functionally important mutations – suspect rare events or focal CNAs or translocations
Lohr et al. Results: Associations

No strong statistically significant association between particular mutations and clinical features, tumor ploidy, or history of prior treatment.
Lohr et al. Results: Clonality

- Calculated the CCF (cancer cell fraction) for each “significant” mutation using allelic fraction, copy number at that locus, and tumor purity
- Clonal heterogeneity evident in all patients: 3-7 clones most common
- No purely clonal mutations (i.e. putative “drivers”)
- Occasionally multiple “significant” mutations found within 1 patient, even within 1 pathway (e.g. NRAS + BRAF or KRAS)
  - But, not in the same cell of a patient (i.e. they represented subclones when together)
“Significant” mutations more often clonal in previously-treated compared to untreated patients, suggesting treatment may accelerate the fixation of certain subclones by eliminating less fit clones (p = 0.007 by Wilcoxon rank-sum test)
BRAF inhibitors cause MAP kinase pathway up-regulation and growth stimulation in *KRAS* and *NRAS* mutated cells

Genome Sequencing, cont.

Hypotheses

• The subclonal architecture of MM evolves with time and treatment: NGS provides sufficient resolution to describe this

• Identification of "driver" mutations or mutation "signatures" can inform disease pathogenesis and prognosis
Bolli et al., Hypothesis

Janiszewska M, Polyak K. *Cell Stem Cell* 2015
Whole exome sequencing in 84 samples from 67 patients

DNA isolated from CD138+ BM cells and compared with “control” PBMC DNA

Some patients sampled at relapse (n = 15); interval of 299 days (77-618)

Affymetrix Genome-Wide Human SNP Array performed in 9/84 samples

Sequencing performed on Illumina HiSeq2000 platform; minimum depth of 30x, average depth of 236x

Various bio-informatics techniques to identify point mutations, followed by validation
Bolli et al. Results

- Median of 52 mutations (range 21 – 488) found per patient
- Concurrent *BRAF* and *NRAS* or *KRAS* mutations at diagnosis in the same patient: generally in subclonal fractions
Bolli et al. Results

- With serial samples (n = 15), the fraction of tumor cells harboring each variant was examined
- Four patterns of evolution deduced

- a) No change
- b) Differential clonal response
- c) Linear
- d) Branching evolution
Genetics over time

- MM is well designed to study clonal dynamics since samples are obtainable and the disease has a relatively prolonged course
- 1 patient with several serial (paired) samples from diagnosis to terminal PCL

Keats et al., Blood 2012

**Clonal competition with alternating dominance in multiple myeloma**

Jonathan J. Keats, Marta Chesi, Jan B. Egan, Victoria M. Garbitt, Stephen E. Palmer, Esteban Braggio, Scott Van Wier, Patrick R. Blackburn, Angela S. Baker, Angela Dispenzieri, Shaji Kumar, S. Vincent Rajkumar, John D. Carpten, Michael Barrett, Rafael Fonseca, A. Keith Stewart, and P. Leif Bergsagel

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Clonal evolution

- WGS identifies waxing/waning of SNV events and describes a “shifting dominance”
- Gives new meaning to “PR”

Keats et al., *Blood* 2012
Subclonal diversity

- Melchor et al., ASH 2014: assessed subclonality levels (437 patients)
- Median # subclones = 5
- No clear association with subclonal # and outcome
- But, subclonal diversity was associated:
  - High diversity index: PFS = 13 mo vs 27 mo
  - No dominant subclone (> 25%) PFS = 22 vs 28 mo
- Increased subclonal diversity → worse outcome
Genomics: Functional Relevance

- **“Common” mutation**
  Coded genome, epigenome, noncoding mutations, CNVs, expression levels
  \[\text{Computational analysis}\]

- **Probable alteration in protein function**
  Toxicities and complexities of therapy combinations
  Driver-mutational heterogeneity (across patients)
  Clonal heterogeneity, adaptability, and evolution (within patient)
  \[\text{Not easy}\]

- **Clinically useful?**
Genomics: Functional Relevance

- RNA-seq + WES on 14 samples
- Majority (64%) of mutations are not expressed – likely not functionally relevant
- Nonsense-mediated RNA decay is not thought to explain the majority of these findings
- Limited study: small n; no data on introns; etc.

Rashid N et al. *Blood* 2014
Genomics: implications

- Minor subclones can be clinically relevant
- Repeat “biopsy” will be increasingly valuable in targeting therapy
- Current cell-line models are too homogeneous for optimal drug development
- Concept of multidrug combinations are supported, ala HIV medicine

TT3a

- **Induction:** VTD-PACE x 2 cycles
  - (Bortezomib/Thalidomide/Dexamethasone/Cisplatin/Doxorubicin/Cyclophosphamide/Etoposide)
- **Transplant:** Mel 200 x2 cycles
- **Consolidation:** VTD-PACE x2
- **Maintenance:** VTD x1yr, TD x2yr
TT3a

- 5 yr PFS = 65%; 5 yr OS = 74%
- PFS-based cure-fraction estimate = 33% (or 70% if 5 yr landmark applied)

Barlogie et al., ASH 2014
TT4, TT5

- Began in 2008
- Stratifies to low/high risk disease according to gene expression signature
- TT4 continues TT3 for low-risk (~85%) disease
- TT5 augments therapy to dose-dense for the ~15% of high-risk patients
BRAF inhibition

- Two case reports of vemurafenib for R/R MM
- BRAF mutations may have increased prevalence in EM MM
  - 7/379 (de novo) →
  - 4/7 (EM disease)
- 54 yo MD, s/p RVD, ASCT, m-L, PD, CD, Cyt
- Ongoing response at 4 mo

Sharman JP et al., Clin Lymph Myel Leuk 2014
MEK inhibition

- Genomic profiling (405 cancer-related, 24 frequently rearranged genes) on 351 patients that had progressive disease (median prior regimens = 5, range 1-20)
- Patients with KRAS, NRAS, or BRAF activation (n = 60) considered for therapy with trametinib
- SD = 30
- PR = 8
- VGPR = 2
- CR = 3

Heuck et al., ASH 2014
Summary

- Growing body of genomics data in myeloma
- Thus far appears to significantly enhance prognostic classification
- Await robust predictive capacity, including the identification of actionable lesions
- Clonal heterogeneity defines myeloma and likely impacts treatment response
- Data beyond DNA level will be required and may explain why therapies targeted at mutant oncogenes may be ineffective or result in paradoxical effects
Thanks
Pam Becker
David Coffey
Mike Schmitt
Jesse Salk

Questions?