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10TH ARIZONA MYELOMA NETWORK

GENETICS AND MYELOMA

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Progression of myeloma

Bone marrow stromal cell dependence

IL-6 dependence

Angiogenesis

Bone destruction

Increased DNA-labeling index

Normal long-lived plasma cell

Germinal-center B cell

MGUS

Smoldering myeloma

Intramedullary myeloma

Extramedullary myeloma

Myeloma cell line

Germinal-center B cell
Prognostication

ISS Staging

Gene Expression Profiling

FISH (m-SMART)

Metaphase Cytogenetics

Imaging

- Measures metabolic activity
- Identifies macrofocal disease
- Detects extramedullary disease
- CT-portion very sensitive in detecting osteolytic lesions
- CT-PET has prognostic implications
- Medicare approved

CT-PET scanning in Multiple Myeloma

A: MRI-FL normal
B: MRI-FL between 1-7
C: MRI-FL >7

Deaths / N
45 / 191 62 / 218 85 / 202

5-Year Estimate
73% (65,80) 67% (59,74) 54% (46,62)

P-val: Overall<0.001, A vs. B=0.42, A vs. C<0.001, B vs. C=0.001
GENETIC STUDIES IN MYELOMA

• Metaphase karyotyping
  – Usually normal diploid in MGUS, <10% abnormal in SMM, 33% abnormal in untreated MM, >60% in end-stage RRMM

• Inter-phase FISH (i-FISH)
  – >90% abnormal
  – Best done with light-chain counterstaining

• Gene expression profiling (GEP) of CD138-purified plasma cells
  – Identifies 7 molecular subgroups
  – 70-gene model highly prognostic regardless of therapy applied, also in relapse; changes with progression

• Mutational analysis
  – Identifies drug-able targets
GENETIC STUDIES IN MYELOMA

Sky Karyotype

Gene Array: Normal vs MM

CA13 – Tri-FISH

Gene Expression

High
Low

120 genes

MM +/- Bone Disease

No Lytic Lesions > 3 Lytic Lesions

DKK1, FRZB, CENPA, TYMS, TTK, MAD2L1, PCNA, PRKDC, CCNF, AML1B
WNT10B, ARHE, LNHR, SLAM, TACI, PIM1, IL6R, BIK, BAX, CFLAR
Myeloma is not one disease: 7 distinct molecular subgroups characterized by unique gene expression patterns and clinical features

70-Gene Risk Score Identifies High Risk Myeloma

70 GENES IDENTIFIED & VALIDATED

TT2 (n =351)

13% IDENTIFIED TO HAVE HIGH-RISK MM

70-gene model outperforms B2M, albumin, LDH, free-lites, metaphase cytogenetics in terms of predicting outcome
Hyper-octaploidy

SKY KARYOTYPE

Identifies with different fluorochromes the individual chromosomes and permits analysis of breakpoints, translocations, deletions, and amplifications.
Role of chromosome 1q in creating genetic havoc

A 1q21 × 2, 1p12 × 3

B 1q21 × 2, 1p13 × 3

C 1q21 × 2, 1p32 × 2

D MYCN × 3

E MYCN × 3

F 1q21 × 3

G 1q21 × 3

H 1q21 × 4

I 1q21 × 3

Sawyer et al Blood 2015
Figure 2. Partial karyotypes from controls 2 and 3 demonstrating the origin of JTIq12 and a model for the origin of 1q12 aberrations. (A) Partial karyotypes of control #2 demonstrating CN gains of 1q21 and CN losses in RCs. Normal chromosomes 1 (left bracket), RC9(q11) by both SKY (chromosome 1 yellow, chromosome 9 white) and FISH (9q11) (aqua) (middle bracket), and progression of CN aberrations resulting from an additional jump of JTIq12 to the RC12(q11) (SKY, chromosome 12 pink) and FISH (right bracket). CN of 1q21 is 4 with the loss of both 9q and 12q in the RCs. (B) Control 3 shows a chromosome 1 by both G-banding and FISH (left bracket) with a 1q21 CN of 2 resulting from a JTIq12 to the telomere of 1p and an isochromosome 1q (right bracket). (C) Model for the origin of CN gains of 1q21. Characterization of aberrations is identified following hypomethylation of 1q12 pericentromeric heterochromatin. Normal chromosome 1 (A) is depicted with a centromere (black) and FISH probes 1q12 (red) and 1q21 (green) (far left). Transient aberrations include cells with decondensation of the 1q12 region (B), triradial of 1q12 resulting in a CN gain of 1q21 (C), and cells with breakage in the 1q12 (red) pericentromeric heterochromatin (D). Extra copies of the JTIq12 originating from a triradial usually either translocate to the telomeric region of an RC (E) or alternatively to the pericentromeric region of an RC (F). Copies of JTIq12s that do not successfully translocate to an RC (G) result in acentric copies of chromosome 1q21 and are subsequently encapsulated into micronuclei and lost from the cell. FISH hybridizations to metaphase chromosomes are shown in inverse 4,6-diamidino-2-phenindole to delineate G-banding patterns.
HYPER-HAPLOID MYELOMA

- Hyperhaploid clones (24-34 chromosomes) were identified in 33 patients with multiple myeloma (MM) identifying a novel numerical cytogenetic subgroup. Strikingly, all hyperhaploid karyotypes were found to harbor monosomy 17p, the single most important risk stratification lesion in MM. A catastrophic loss of nearly a haploid set of chromosomes results in disomies of chromosomes 3, 5, 7, 9, 11, 15, 18, 19, and 21, the same basic set of odd-numbered chromosomes found in trisomy in hyperdiploid myeloma. **All other autosomes are found in monosomy, resulting in additional clinically relevant monosomies of 1p, 6q, 13q, and 16q.**

- Hypotriploid subclones (58-68 chromosomes) were also identified in 11 of the 33 patients and represent a duplication of the hyperhaploid clone. Analysis of clones utilizing interphase fluorescence in-situ hybridization (iFISH), metaphase FISH, and spectral karyotyping identified either monosomy 17 or del17p in all patients. Amplification of 1q21 was identified in eight patients, demonstrating an additional high-risk marker. Importantly, our findings indicate that current iFISH strategies may be uninformative or ambiguous in the detection of these clones, suggesting this patient subgroup maybe under-reported.

- **Overall survival for patients with hyperhaploid clones was poor, with a five-year survival rate of 22.9%. These findings identify a distinct numerical subgroup with cytogenetically defined high-risk disease.**

Rare, 33 cases of >1000, Male predominance, 72%, Younger age, median – 50yr
Figure 1
Figure 2
Figure 3
Figure 4
MRI & FDG-PET IN MM

Sagittal STIR MRI

Sagittal FDG PET

Ant MIP FDG PET
INTRA-CLONAL HETEROGENEITY IN MYELOMA

• Gleaned from
  – Non-uniform response in individual patients
    • MRI-defined focal lesions resolve more slowly and can persist in CR while still harboring MM cells
  – Differences in genetics when examined in different sites in same patient
    • Especially when comparing random iliac crest marrow and CT-guided FNA from focal lesions
      – Differences involving MM-cell GEP risk, although subtypes usually identical
      – Differences in bone marrow micro-environment
Discrepancies in Cytogenetic Abnormalities and GEP Parameters Reveal Intra-Patient Heterogeneity

**Discrepancies in Presence of CA and GEP70 Risk-Scores**

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<th>FL GEP70 Risk</th>
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<td>Total</td>
<td>61</td>
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**Higher Cases PR (Proliferation) Subgroup in FL**

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<th>LB</th>
<th>MF</th>
<th>MS</th>
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Clinical Significance of Focal Lesion Sampling

Paired Samples: Superior outcomes linked to absence of high-risk GEP70 score in both RBM and FL sites.
GEP DIFFERENCES IN DIFFUSELY INFILTRATIVE (DI) VERSUS FOCAL NODAL (FN) GROWTH IN MM:
TOWARD DEVELOPING FOCAL LESION-DIRECTED THERAPIES

MM-GEPC: DKK1 IN FN

ME-GEPC: 206 GENES DIFFER IN FN v DI
Focal Lesion MM Cell and Micro-Environmental Signatures are Biologically Linked

**FL ME:**
- **Hematopoiesis**
  - ITGA2B/CD41B
  - RHCE
  - MKI67
- **Bone**
  - BGLAP
- **Anti-inflammatory**
  - Adiponectin

**FL PC:**
- **Ig Genes**
  - CD27
  - CXCR4
- **AP-1 genes**
  - RHOB
  - NR4A1
- **KLF6**
  - TXNIP

**Focal Lesion**
- **Reactive Stroma**
  - Collagen genes
  - Fibronectin
  - Periostin
  - MMP11
  - FAP
  - VEC/Angiogenesis
  - EDNRA
  - FLT1
  - Th17/Tregs
  - IL17A
  - FOXP3

**Adhesion**
- CAM-DR

**Angiogenesis**
- Immune suppression

**Drug Resistance**
- Immature phenotype
- Dormancy/Stemness
Genetic heterogeneity in Myeloma
Recurrent mutations in MAP kinase, P53 and NFkB pathways

Lohr et al, Cancer Cell 2014
GEP and mutations

Prominence of PR and MF subgroups among mutations
GEP and mutations

F1 alterations: all treated samples, n=182

Mutation Count Per Ptn across GEP70

Mutation Count Per Ptn across molecular subtypes

CD-1  CD-2  HY  LB  MF  MS  PR
FoundationOne Stats at MIRT

- 191 unique patients reported
- 121 with actionable mutations with therapy suggestions
- 70 no therapies
- 12 unique patient had no reportable mutations
- Majority of mutations are KRAS, TP53, NRAS, BRAF, RB1
FoundationOne Stats at MIRT

- 57 unique patients with KRAS mutations
- 35 unique patients with NRAS mutations
- 12 unique patients with BRAF mutations

Patient treated based on F1 recommendations
- 33 patients have received Trametinib
- 8 patients have received Vermurafenib or Dabrafenib
MIRT Case #1

- 55 yo M
- non-secretory MM dx in 2007, low risk by GEP70
- Treated on TT3A
- Relapse #1 in 03/2012 w/ GEP70 high risk; now secretory disease
- Salvage therapy with: Pomalidomide, Carfilzomib, metronomic therapy x2, VDT-PACE + HSC boost
- Last treatment resulted in extended hospital admission for 3 months d/t side effects.
- FMI testing revealed KRAS G12D mutation
- Patient initiated therapy with trametinib 2mg po QD on 8/17/13 (79 days after last prior therapy)
MIRT Case #1

- Side effects: Rash and transient LFT elevation neither requiring dose reduction
- Quick response in KFLC levels
MIRT Case #2

- 52 yo male
- IgG kappa MM diagnosed in 12/31/03
- Initially treated with DCEP by local oncologist
- Recurrence with extramedullary disease in the liver in 2007
- Salvage therapies: VRD, TACE, PAC-MED + HSC boost
- FMI on 8/13/13 revealed a KRAS Q61H mutation
- Started trametinib 2mg daily po on 9/18/13 (28 after completing last therapy)
MIRT Case #2

- Side effect: Grade 1 rash
- Complete resolution of liver lesion (by PET)

8/31/13

9/21/13

11/20/13

Patient continues on single agent trametinib
MIRT Case #3

- 67 yo M
- IgG kappa MM, low risk by GEP70 and GEP80
- Treated on TT3 in 2003
- Relapsed in 2009 >> Carfilzomib
- 2012 extramedullary disease
- Salvage with VTD-PACE and PAC-MED
- 9/2013 extraorbital mass noted >> metronomic therapy
- 9/2013 F1 shows BRAF V600E mutation
MIRT Case #3

• Started therapy with single agent Dabrafenib in 10/2013
• Patient in CR as of Jan 2014
THANKS

• Patients and their families for their trust and endurance
• Referring doctors
• Colleagues and nursing staff

• My mentor, Dr. Emil J Freireich

• My family
  – Wife Kathy & Children: Britta, Eva, Bart