A rapid and robust molecular diagnostic approach for multiple myeloma


UKMF Spring Day, 19/03/2014
Structural genetic aberrations shape myeloma biology

- Chromosomal translocations:
  - t(4;14)
  - t(14;16)
  - t(14;20)

- Copy number alterations:
  - gain(1q)
  - del(17p)
  - [del(1p)]

• 2 or more adverse lesions → specific high risk group

• Target group for novel treatment approaches

Boyd et al, Leukemia 2012
Myeloma genetics – clinical impact

Knowledge about genetic lesions in myeloma

• Substantial for innovative clinical trials
• High risk trial
• Impact on clinical management
  • Treatment initiation
  • Intensification
  • Consolidation/maintenance strategies
• Targeted therapies (e.g. MMSET)
Myeloma genetics – clinical impact

Changing requirements regarding testing

- Standardisation/operator independence of assays
- Automation
- DNA/RNA based
  - Enable further analyses from same material
  - e.g. BRAF mutation testing
- Decrease turnover time

Also:
- *Prepare for when the world expert in myeloma cytogenetics retires…*
Novel PCR-based molecular diagnostic tests

BM Aspirate → CD138 selection → DNA RNA

- CNA [MLPA]
- TL assay [Taqman]

100,000 cells minimum

48 hours
Single to 24 samples
PCR based copy number detection

Multiplex ligation-dependent probe amplification (MLPA)

Hybridisation + PCR

Locus specific product length

PCR product separation + quantification (capillary electrophoresis)

Software generated copy number prediction

→ Sample reception to result: 48 hours
MLPA copy number detection – Myeloma Panel

12 regions of interest assessed in 1 reaction mix

FAF1/CDKN2C  
FAM46C

CKS1B

RB1  
DIS3

TRAF3

TP53

HRD

CYLD

WWOX

Prognostically relevant

Deletions

Gains

Reference

Not examined

No examination

12 regions of interest assessed in 1 reaction mix
Comparison MLPA vs. SNP array copy number

Myeloma IX cases (n=86) with matched 500k array, MLPA and iFISH analyses available

<table>
<thead>
<tr>
<th>Locus/Gene</th>
<th>MLPA</th>
<th>iFISH</th>
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<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>1p [CDKN2C] loss</td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td>1q21 [CKS1B] gain</td>
<td>95%</td>
<td>89%</td>
</tr>
<tr>
<td>17p [TP53] loss</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>Hyperdiploidy [chr3,chr9,chr15 ]</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>1p [FAF1] loss</td>
<td>88%</td>
<td>94%</td>
</tr>
<tr>
<td>1p [FAM46C] loss</td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td>16q [CYLD] loss</td>
<td>94%</td>
<td>96%</td>
</tr>
<tr>
<td>16q [WWOX] loss</td>
<td>100%</td>
<td>93%</td>
</tr>
</tbody>
</table>
MLPA to detect 1q amplification

MLPA for CKS1B gains and amplifications

PFS depending on clustering group

p = 0.038

ICR The Institute of Cancer Research
qRT-PCR-based translocation detection

Multiplexed TaqMan qRT-PCR reaction for 10 translocation-specific genes

Hierarchical algorithm to predict translocation based on spiked gene expression (TC classification)

Comparison iFISH MyIX (n=255)
- Sensitivity 95%
- Specificity 92%
- Reproducibility 100%

Kaiser et al., Leukemia 2012
PCR based diagnostic assays – prognostic validation

n=501 trial cases

PFS

OS

del(17p)

Adverse lesions

p<0.001

p<0.001
PCR based diagnostic assays – summary

MLPA and translocation assay

- Fast
- Robust
- Flexible throughput
- Standard equipment
- No bioinformatics required
- Technically and prognostically validated
Thank you!
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Participating Centres

Myeloma CTU Leeds
Making the discoveries that defeat cancer

Unrivalled track record

ICR
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