CCUS and Clonal Monocytosis of Clinical Significance

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DISCLOSURE

• I have the following financial relationships:
  Contracted Research: Celgene
Question

- In patients with cytopenia or monocytosis, who should undergo targeted DNA sequencing as part of their investigations?
  - All patients
  - All those referred to a haematologist
  - All those undergoing a bone marrow for investigation
  - Only those with borderline bone marrow features
  - None
% patients with mutations

CMML

>90% of cases

AML

MDS

90% of cases

Adapted from Itzykson et al, 2013

Ley et al, 2013

Papaemmanuil et al, 2013
Myeloid Malignancy

DNA Methylation
- TET2
- DNMT3A
- IDH1/2

Chromatin Modification
- ASXL1
- EZH2

RNA Splicing
- SF3B1
- SRSF2
- U2AF1
- ZRSR2

Signalling
- KRAS
- NRAS
- FLT3
- KIT
- CBL

Cohesin
- STAG2

Transcription
- RUNX1
- BCOR

Tumour Suppressors
- TP53
- WT1

Adapted from Cazzola et al, 2013
Potential application in diagnosis of chronic myeloid malignancies.....

- Remains heavily reliant on morphology
- Challenging in those with <5% blasts
- Cytogenetic abnormality detected in only 30-50% of cases

- Idiopathic cytopenia with undetermined significance (ICUS)
  - Proportion progress to MDS or AML
Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression

Catherine A. Cargo,1 Nicola Rowbotham,1 Paul A. Evans,1 Sharon L. Barrans,1 David T. Bowen,2 Simon Crouch,3 and Andrew S. Jack1

- Aimed to molecularly characterise those cytopenic patients with the most clinically significant disease
- fail to meet current diagnostic criteria

Investigation of Cytopenia

Bone Marrow Biopsy
Non-Diagnostic

Time = \( n \) months

Bone Marrow Biopsy
AML / MDS

Pre-clinical MDS

Cargo et al, Blood.2015; 126(21):2362-5
## Targeted Sequencing Panel

<table>
<thead>
<tr>
<th>Functional Pathway</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Methylation</td>
<td>TET2, DNMT3A, IDH1, IDH2</td>
</tr>
<tr>
<td>Chromatin Modification</td>
<td>ASXL1, EZH2</td>
</tr>
<tr>
<td>Splicing</td>
<td>SF3B1, SRSF2, U2AF1, ZRSR2</td>
</tr>
<tr>
<td>Transcription Factors</td>
<td>NPM1, RUNX1, BCOR, WTI, TP53</td>
</tr>
<tr>
<td>Signalling</td>
<td>FLT3, NRAS, KRAS, CBL, cKIT, JAK2, MPL, CSF3R</td>
</tr>
<tr>
<td>Cohesin complex</td>
<td>STAG2</td>
</tr>
<tr>
<td>Other</td>
<td>SETBP1, CALR</td>
</tr>
</tbody>
</table>
Results – pre-diagnostic sample

- Driver mutation and/or structural variant identified in 91% of non-diagnostic samples (63/69)

Cargo et al, Blood.2015; 126(21):2362-5
Is there a clinical benefit to detecting mutations early?

Cargo et al, Blood. 2015; 126(21):2362-5
Mutation Patterns in PB can predict a myeloid malignancy in BM

<table>
<thead>
<tr>
<th>Gene</th>
<th>N. of Mutated Patients</th>
<th>Positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Number</td>
<td>Isolated N (%)</td>
</tr>
<tr>
<td>TET2</td>
<td>128</td>
<td>25 (19.5)</td>
</tr>
<tr>
<td>ASXL1</td>
<td>96</td>
<td>14 (14.6)</td>
</tr>
<tr>
<td>SRSF2</td>
<td>94</td>
<td>6 (6.4)</td>
</tr>
<tr>
<td>SF3B1</td>
<td>77</td>
<td>31 (40.3)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>59</td>
<td>17 (28.8)</td>
</tr>
<tr>
<td>RUNX1</td>
<td>50</td>
<td>2 (4)</td>
</tr>
<tr>
<td>JAK2</td>
<td>35</td>
<td>9 (25.7)</td>
</tr>
<tr>
<td>TP53</td>
<td>33</td>
<td>14 (42.4)</td>
</tr>
<tr>
<td>U2AF1</td>
<td>32</td>
<td>8 (25)</td>
</tr>
<tr>
<td>IDH2</td>
<td>25</td>
<td>1 (4)</td>
</tr>
<tr>
<td>EZH2</td>
<td>25</td>
<td>3 (12)</td>
</tr>
<tr>
<td>ZRSR2</td>
<td>22</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>NRAS</td>
<td>21</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>IDH1</td>
<td>20</td>
<td>4 (20)</td>
</tr>
<tr>
<td>STAG2</td>
<td>20</td>
<td>4 (20)</td>
</tr>
</tbody>
</table>

RUNX1, EZH2, CBL, TP53, NRAS, CUX1 or IDH2 – PPV 0.86-1.0

Malcovati et al, Blood. 2017; 129(25): 3371-8
Is the presence of a mutation diagnostic of MDS?

is not yet fully understood; thus, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia, where these mutations may be commonly found. Further study is required to determine the optimal management and monitoring of such patients and
Somatic mutations in healthy individuals

- Somatic mutations identified in small proportion of healthy individuals
- Increase in frequency with age

Somatic mutations in healthy individuals

Risk of progression?

- Presence of a mutation
  - Haematological malignancy more common by factor of 11
  - VAF >10% - increased by factor of 50

Overall risk 0.5% per year
1% in those with VAF>10%

Jaiswal et al, NEJM, 2014
“CHIP”
(Clonal Haematopoiesis of Indeterminate Potential)

- Absence of definitive morphological evidence of a haematological neoplasm
- Does not meet diagnostic criteria for PNH, MGUS or MBL
- Presence of a somatic mutation associated with haematological neoplasia at a variant allele fraction of at least 2%
Does the genomic profile differ between healthy individuals and suspected MDS?

- **Allele fraction**
  - Median AF=39.9% (vs 9-10%\(^1\))
  - Only 1 patient harboured an isolated mutation <20%
  - Healthy individuals who subsequently developed a haematological malignancy – mean VAF 25.2%\(^1\)

- **No. of mutations per patient**
  - Majority of patients had ≥2 mutations
  - Only 8% of those with age related clonal haematopoiesis had ≥2 mutations\(^1\)

\(^1\)Jaiswal et al, NEJM, 2014; DOI: 10.1056/NEJMoa1408617
How frequent are somatic mutations in cytopenic patients?

- 35-38% of ICUS patients have ≥1 mutation
- Cohort size 144-249 patients

Kwok et al, Blood. 2015
Malcovati et al, Blood. 2017
## Clonal Cytopenia of Undetermined Significance

<table>
<thead>
<tr>
<th></th>
<th>CHIP</th>
<th>ICUS</th>
<th>CCUS</th>
<th>Lower risk MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytopenia</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Clonality</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>% Blasts</td>
<td>&lt;5%</td>
<td>&lt;5%</td>
<td>&lt;5%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Overall risk</td>
<td>Very low</td>
<td>Very low</td>
<td>???</td>
<td>Low</td>
</tr>
</tbody>
</table>

Adapted from - Steensma et al, Blood. 2015; 126(1): 9-16
Are mutations clinically relevant in cytopenic patients?

- 56/154 (36%) had ≥1 mutation
  - Mutated vs Unmutated - HR 13.93 (P<0.001)
  - 10yr probability of progression 96% vs 15% (p<0.001)

- Highly specific mutation pattern
  - Spliceosome mutations
  - Co-mutation patterns involving TET2, ASXL1 or DNMT3A
    - RUNX1, EZH2, CBL, BCOR, CUX1, TP53 or IDH1/IDH2

Malcovati et al, Blood. 2017; 129(25): 3377-8
Impact on survival

Highly specific mutation pattern
ICUS vs MDS

Malcovati et al, Blood. 2017; 129(25): 3371-8
### Diagnostic criteria for CMML

**Table 11. Diagnostic criteria for CMML**

<table>
<thead>
<tr>
<th>CMML diagnostic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent PB monocytosis $\geq 1 \times 10^9/L$, with monocytes accounting for $\geq 10%$ of the WBC count</td>
</tr>
<tr>
<td>Not meeting WHO criteria for <em>BCR-ABL1</em>+ CML, PMF, PV, or ET*</td>
</tr>
<tr>
<td>‡The presence of mutations in genes often associated with CMML (eg, <em>TET2</em>, <em>SRSF2</em>, <em>ASXL1</em>, <em>SETBP1</em>) in the proper clinical contest can be used to support a diagnosis. It should be noted however, that many of these mutations can be age-related or be present in subclones. Therefore, caution would have to be used in the interpretation of these genetic results.</td>
</tr>
<tr>
<td>‡An acquired clonal cytogenetic or molecular genetic abnormality is present in hemopoietic cells‡</td>
</tr>
<tr>
<td>or</td>
</tr>
<tr>
<td>‡The monocytosis (as previously defined) has persisted for at least 3 mo and</td>
</tr>
<tr>
<td>‡All other causes of monocytosis have been excluded</td>
</tr>
</tbody>
</table>
Molecular profile in bone marrow samples

- A total of 207 patients had a BM performed
- Of those with a diagnosis
  - Mutation detected in 99%
- Of those without a diagnosis
  - Mutation detected in 57%
- In those with a mutation
  - No difference in no. of mutations or VAF between diagnostic and non-diagnostic groups

Cargo et al, Blood, 2019; 133(12):1325-1334
OS correlated strongly with no. of mutations

$p < 0.0001$

$p = 0.002$

Cargo et al, Blood, 2019; 133(12):1325-1334
<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>OR, 95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>p&lt;0.0001</td>
<td>1.04(1.02-1.06)</td>
</tr>
<tr>
<td>ASXL1</td>
<td>p&lt;0.0001</td>
<td>2.10(1.45-3.05)</td>
</tr>
<tr>
<td>CBL</td>
<td>p=0.003</td>
<td>2.12(1.30-3.47)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>p&lt;0.0001</td>
<td>2.87(1.73-4.76)</td>
</tr>
<tr>
<td>KRAS</td>
<td>p=0.13; NS†</td>
<td>1.69(0.85-3.33)</td>
</tr>
<tr>
<td>NRAS</td>
<td>p=0.002</td>
<td>2.11(1.32-3.36)</td>
</tr>
<tr>
<td>RUNX1</td>
<td>p&lt;0.0001</td>
<td>2.75(1.74-4.37)</td>
</tr>
<tr>
<td>SRSF2</td>
<td>p=0.41; NS†</td>
<td>1.18(0.80-1.72)</td>
</tr>
<tr>
<td>TET2</td>
<td>p=0.30; NS†</td>
<td>0.82(0.57-1.19)</td>
</tr>
<tr>
<td>EZH2</td>
<td>p&lt;0.0001</td>
<td>4.88(2.52-9.44)</td>
</tr>
<tr>
<td>STAG2</td>
<td>p&lt;0.0001</td>
<td>22.39(7.48-67.01)</td>
</tr>
</tbody>
</table>

Cargo et al, Blood, 2019; 133(12):1325-1334
What is the clinical phenotype of non-diagnostic\textsuperscript{mut} patients?

Cargo et al, Blood, 2019; 133(12):1325-1334
Non-diagnostic\textsuperscript{mut} patients are indistinguishable from CMML

<table>
<thead>
<tr>
<th>% of monocytes expressing CD56</th>
<th>% CD64\textsuperscript{+} monocytes of leucocytes</th>
<th>% of monocytes expressing CD14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMML vs NDM</td>
<td>p=0.45; NS</td>
<td>CMML vs NDM</td>
</tr>
<tr>
<td>CMML vs NDU</td>
<td>p&lt;0.0001</td>
<td>CMML vs NDU</td>
</tr>
<tr>
<td>NDM vs NDU</td>
<td>p&lt;0.0001</td>
<td>NDM vs NDU</td>
</tr>
</tbody>
</table>

 Cargo et al, Blood, 2019; 133(12):1325-1334
Peripheral Blood is predictive of a bone marrow diagnosis

- Peripheral blood mutation analysis highly predictive of diagnosing a myeloid malignancy in bone marrow
  - PPV 0.97, NPV 1.0

- High concordance between mutations detected in PB and BM (96%)

<table>
<thead>
<tr>
<th>BM</th>
<th>PB</th>
<th>Mutation detected in PB?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Cargo et al, Blood, 2019; 133(12):1325-1334
Clonal Monocytosis of Clinical Significance

- Patients with a persistent monocytosis failing to meet morphological criteria of CMML (or other myeloid malignancy)
  - Presence of a somatic mutation associated with CMML (most commonly TET2, SRSF2, ASXL1)

- Mutation spectrum, immunophenotypic features and overall survival indistinguishable from CMML
  - Presumptive evidence of disease
Who should have sequencing performed?

- Caution is required when introducing new test.
Diagnostic algorithm

Bone marrow for investigation of cytopenia/monocytosis

Morphological assessment

Exclude other malignancy

Flow Cytometry/Triaging

Dysplasia

Mutation screening (copy number)

Mutation detected

≥1 mutation

? > 10-20% VAF

MDS/CMML

ICUS

No abnormality

Consider alternative causes

?PB Screening

CCUS/CMCS
Impact on laboratory and clinical practice

- Would require a standardised approach
  - Panel design and minimum genes to sequence
  - Analysis and interpretation
    - grading of mutations
- Appropriate infrastructure within laboratories
  - Data processing and storage
  - Bioinformatic support
- Clinical impact
  - High frequency of mutations would significantly increase the number of patients attending haematology clinic
Summary

- *Mutation analysis provides key information even in those failing to meet current diagnostic criteria*

- **CCUS**
  - Patients have worse overall survival and progressive cytopenias even in the absence of morphological disease
  - Mutations can predict the development of overt disease
  - Absence of a mutation has a high negative predictive value
  - Provides potential window of opportunity for intervention
    - Therapeutic intervention

- **CMCS**
  - Mutation profile, immunophenotypic features and outcome indistinguishable from CMML
  - Peripheral blood is clinically informative
Acknowledgements

- **HMDS**
  - Paul Evans
  - Sharon Barrans
  - Jan Taylor
  - Paul Glover
  - Matthew Cullen
  - Molecular department

- **ECSG**
  - Simon Crouch
  - Alex Smith

- **MSKCC**
  - Elli Papaemmanuil
  - Kelly Bolton
Abstract #1712

- Targeted sequencing predicts the development of myeloid malignancies and clinical outcome in patients with unexplained cytopenia

- Saturday, December 7

- 5.30-7.30pm
Question

- In patients with cytopenia or monocytosis, who should undergo targeted DNA sequencing as part of their investigations?
  - All patients
  - All those referred to a haematologist
  - All those undergoing a bone marrow for investigation
  - Only those with borderline bone marrow features
  - None