Hematology Case Studies: MPAL & JMML

Nicholas Brehl, M.Ed., MLS (ASCP)CM
Case 1

• 36 year old female

• Symptoms: non-productive cough, fatigue, fever, chills, night sweats

• Physical Exam: Painful cervical lymphadenopathy and lower extremity swelling
## Case 1

### Selected Laboratory Results At Admission

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>$323.4 \times 10^9/L$</td>
<td>$3.6-10.6 \times 10^9/L$</td>
</tr>
<tr>
<td>Blast</td>
<td>96%</td>
<td>0%</td>
</tr>
<tr>
<td>Platelet</td>
<td>$16 \times 10^9/L$</td>
<td>$150-450 \times 10^9/L$</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>13.5 mg/dl</td>
<td>2.7-7.4 mg/dl</td>
</tr>
<tr>
<td>Potassium</td>
<td>8.6 mmol/L</td>
<td>3.5-5.5 mmol/L</td>
</tr>
</tbody>
</table>
Case 1

Bone Marrow Exam

• 100% Cellularity

• Sheets of blasts
  – Slight MPO positivity with negative butyrate esterase
Case 1

Cytogenetics
• 46, XX, t(9;22)(q34;q11.2.) [20]
• BCR-ABL1 positive

Flow
• TdT, MPO (partial), CD34 (partial), HLADR, CD38, CD19, CD10, CD13 (dim), CD15 (partial)
Mixed-Phenotype Acute Leukemia (MPAL)

- 2-5% of acute leukemias
- Representative of 2 or 3 cell lineages
- Biphenotypic versus bilineal
Typical Presentation

• Median WBC $5.4 \times 10^9$/L (range 0.8-278.7 $\times 10^9$/L )

• Bone Marrow Failure
Incidence

• Median age 35 yrs (range 14-81)
• Slight predominance in males
## Historic: EGIL & WHO 2001

<table>
<thead>
<tr>
<th>Points</th>
<th>B Lineage</th>
<th>T Lineage</th>
<th>Myeloid Lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CD79a, cyt IgM, cyt CD22</td>
<td>CD3 (cyt/m), anti-TCR α/β, anti-TCR γ/δ</td>
<td>Anti-MPO Anti-lysozyme</td>
</tr>
<tr>
<td>1</td>
<td>CD19, CD10, CD20</td>
<td>CD2, CD5, CD8, CD10</td>
<td>CD13, CD33, CDw65, CD117</td>
</tr>
<tr>
<td>0.5</td>
<td>TdT, CD24</td>
<td>TdT, CD7, CD1a</td>
<td>CD14, CD15, CD64</td>
</tr>
</tbody>
</table>

## WHO 2008 Diagnostic Criteria

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Markers</th>
</tr>
</thead>
</table>
| Myeloid   | Myeloperoxidase  
OR  
Monocytic differentiation evidenced by at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme |
| T lineage | Cytoplasmic OR surface CD3                                                                                                               |
| B Lineage | **Strong CD19** AND strong expression in at least one of the following: CD79a, cytoplasmic CD22, or CD10  
OR  
**Weak CD19** AND strong expression in at least two of the following: CD79a, cytoplasmic CD22, or CD10 |

WHO: Mixed-Phenotype Acute Leukemia Categories

MPAL with t(9;22)(q34;q11.2) BCR-ABL1
MPAL with t(v;11q23) MLL
MPAL B/Myeloid NOS
MPAL T/Myeloid NOS
MPAL NOS-Rare
  B/T
  Myeloid/B/T
## WHO 2008 Frequency

<table>
<thead>
<tr>
<th>WHO 2008 Categorization</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPAL with t(9;22)(q34;q11.2) BCR-ABL1</td>
<td>15-20%</td>
</tr>
<tr>
<td>MPAL with t(v;11q23) MLL</td>
<td>4.3-8%</td>
</tr>
<tr>
<td>MPAL B/Myeloid NOS</td>
<td>58%</td>
</tr>
<tr>
<td>MPAL T/Myeloid NOS</td>
<td>36%</td>
</tr>
<tr>
<td>MPAL NOS-Rare: B/T</td>
<td>4%</td>
</tr>
<tr>
<td>MPAL NOS-RARE: Myeloid/B/T</td>
<td>2%</td>
</tr>
</tbody>
</table>
## Prevalence: EGIL vs. WHO

<table>
<thead>
<tr>
<th>Study</th>
<th>Total Number of Acute Leukemia Cases</th>
<th>Biphenotypic AL Diagnosed Using EGIL</th>
<th>MPAL Diagnosed Using WHO 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weinberg &amp; Arber, 2010</td>
<td>7627</td>
<td>213 (2.8%)</td>
<td>119 (1.6%)</td>
</tr>
<tr>
<td>van den Ancker et al., 2010</td>
<td>517</td>
<td>30 (5.8%)</td>
<td>8 (1.5%)</td>
</tr>
</tbody>
</table>
Long-term self-renewing stem cell → Short-term self-renewing stem cell → Multipotent progenitor hematopoietic stem cell

- SCF, IL-1, 3, 6
- FL, SCF, GM-CSF
- IL-1, 3, 6, 11
- FL, SCF

Common myeloid progenitor

- FL, SCF, GM-CSF
- IL-3

Granulocyte-monocyte progenitor
- G-CSF
- Myeloblast
- Monocyte
- Neutrophil
- Macrophage

Eosinophil-basophil progenitor
- M-CSF
- Myeloblast
- Eosinophil
- Basophil
- Mast cell

Common lymphoid progenitor

- SCF, GM-CSF
- IL-3

Megakaryocyte-erythrocyte progenitor
- EPO
- TPO
- Pronormoblast
- Megakaryoblast
- Erythrocyte
- Megakaryocyte
- Platelets
- Plasma cell

Dendritic cell
- Pre-B
- Pre-T
- Natural killer cell


(Keohane, et al., 2015)
Genetic Alterations

• 24-32% have complex karyotypes
  – Del 6q; or abnormalities in 7q or 5q
• 13% Normal karyotypes
t(9;22)(q34;q11.2) \( BCR-ABL1 \)

- Poor prognostic indicator
  - Higher WBC count
- Lower incidence in children
- B+M phenotype most common
- Other cytogenetic abnormalities in 30% of patients
- Imatinib
BCR-ABL1 Gene

Chromosome 22
Normal BCR1 Gene

Chromosome 9
Normal ABL Gene

Panel A

Four Common Fusion Genes Produce Three Fusion Proteins

Panel B

Two Most Common Fusion Genes Involve the Major BCR and Form One Fusion Protein

Panel C

Less Common Fusion Gene Involves the Minor BCR and Forms One Fusion Protein

Panel D

Least Common Fusion Gene Involves the Micro BCR and Forms One Fusion Protein

bcr = breakpoint cluster region, abl = Abelson oncogene

(Keohane, et al., 2015)
t(v;11q23) MLL rearranged

• Poor Prognostic Indicator
• Numerous rearrangements in literature
  – Most common: t(4;11)(q21;q23) $MLL-MLLT2$
  – t(11;19)(q23;p13) $MLL-MLLT1$
• Slightly higher incidence in children
• B+M phenotype is most common
• High WBC
A histone H3 lysine methylation, Pol II phosphorylation

B histone acetylation

C histone H4 arginine methylation

D dimerization

Slany, 2009
• Summary: MLL fusion genes promote expression of target genes (Hox genes) that arrest development and promote self renewal

Slany, 2009
Other Mutations

• DNMT3A
• EZH2
• IDHI/2
• TET1
• TET3
• ASXL1
• NOTCH1
DNA Methyltransferase 3A (DNMT3A)

• Most common MPAL epigenetic modification
  – Methylation of DNA sequences
• 56% of MPAL M/T have DNMT3A mutation
• AML: global hypomethylation
• T-ALL: hypermethylation especially at CpG islands
• In AML & ALL no effect on CR but lower OS
• Treatment: 5-azacytidine or decitabine
Enhancer of Zeste Homolog 2 (EZH2)

- Polycomb group (PcG) protein that forms a multi-subunit polycomb repressive complex, PRC-2
- Methylates the histone (H3K27me3) which represses DNA transcription
microRNA (miRNA)

- SS RNA molecules that post-transcriptionally regulate gene expression by modifying mRNA stability or translational initiation/elongation
- miRNA profiles characteristic of different cancers
- Analysis of 17 patients with MPAL suggests that MPAL may not be a unique entity
## Outcomes & Disease Course

- **Median survival: 18 months**

<table>
<thead>
<tr>
<th>Authors year of publication</th>
<th>Number of patients</th>
<th>Age median (years)</th>
<th>Received HSCT</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killick et al. 1999</td>
<td>25</td>
<td>≈28</td>
<td>36.00%</td>
<td>≈7 months</td>
</tr>
<tr>
<td>Aribi et al. 2007</td>
<td>31</td>
<td>47</td>
<td>12.90%</td>
<td>≈2.4 years</td>
</tr>
<tr>
<td>Rubnitz et al. 2009</td>
<td>35</td>
<td>10</td>
<td>34.30%</td>
<td>≈3 years</td>
</tr>
<tr>
<td>Al-Seraihy et al. 2009</td>
<td>24</td>
<td>8.7</td>
<td>45.80%</td>
<td>&gt;6 years</td>
</tr>
<tr>
<td>Gerr et al. 2010</td>
<td>92</td>
<td>8.9</td>
<td>35.90%</td>
<td>≈4 years</td>
</tr>
<tr>
<td>Matutes et al. 2011</td>
<td>100</td>
<td>28 pts ≤ 15</td>
<td>20.00%</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68 pts &gt; 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yan et al. 2012</td>
<td>117</td>
<td>35</td>
<td>6.80%</td>
<td>≈9–10 months</td>
</tr>
<tr>
<td>Heesch et al. 2013</td>
<td>26</td>
<td>57</td>
<td>34.80%</td>
<td>≈21 months</td>
</tr>
<tr>
<td>Deffis-Court et al. 2014</td>
<td>27</td>
<td>33</td>
<td>11.10%</td>
<td>14.8 months</td>
</tr>
<tr>
<td>Weinberg et al. 2014</td>
<td>61 (only 31 with follow-up)</td>
<td>32</td>
<td>19.00%</td>
<td>≈3.5 years</td>
</tr>
<tr>
<td>Shimizu et al. 2014</td>
<td>13</td>
<td>52</td>
<td>61.50%</td>
<td>≈35 months</td>
</tr>
</tbody>
</table>
Prognostic Factors

- Ph+ (Ph+ 8 months vs Ph- 139 vs Other 28 months)
- MLL rearrangement
- Age (139 mo [children] vs 11 mo [adults])
- WBC Count
- Creatinine and Uric Acid
- Extramedullary Involvement
MPAL Survival Probability by Age

Product-Limit Survival Estimates
With Number of Subjects at Risk

Logrank p < .0001

(Shi & Munker, 2015)
Current Treatment

• Poor prognosis when compared respective ALL entities. Similar to AML?
  – Cytogenetic Abnormalities
  – Efflux Pump Expression

• ALL, AML, and targeted therapies

• HSCT
Acute Leukemia Survival Probability

Product-Limit Survival Estimates
With Number of Subjects at Risk

Trend: Logrank p < .0001

Survival Probability
Survival in Years

Site
1: Acute Lymphocytic Leukemia
2: Acute Myeloid Leukemia
3: Mixed Phenotype Acute Leukemia
4: Other Acute Leukemia

(Shi & Munker, 2015)
Acute Leukemia Survival Probability

(Shi & Munker, 2015)
MPAL Survival Probability

Product-Limit Survival Estimates
With Number of Subjects at Risk

Logrank p=0.0003

Survival Probability

Survival in Years

Period 2001-2005 2006-2011
2001-2005 135 43
2006-2011 178 17

(Shi & Munker, 2015)
# AML vs ALL Treatment for MPAL

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML Therapy</td>
<td>41% (ped 52%)</td>
</tr>
<tr>
<td>ALL Therapy</td>
<td>85% (ped 83%)</td>
</tr>
</tbody>
</table>
Targeted Therapies-MLL

- Histone-modifying enzyme inhibitor
- Bromodomain inhibitor
- Glycogen synthase kinase 3 or Beta-catenin inhibitors
Targeted Therapies- Ph+

• Age appropriate ALL therapy with TKI?
  – Pediatric versus legacy therapies?

• AML therapy with TKI?
ALL w/Imatinib vs MPAL w/Imatinib

Shimizu et al., 2014

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
<th>5yr OS</th>
<th>5 yr DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph+ B-ALL</td>
<td>85%</td>
<td>33%</td>
<td>42%</td>
</tr>
<tr>
<td>Ph+ MPAL</td>
<td>100%</td>
<td>55%</td>
<td>46%</td>
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</tbody>
</table>

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MPAL with AML or ALL Therapy (both with Imatinib)

Shimizu et al., 2014

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
<th>5yr OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph+ MPAL with AML Therapy</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Ph+ MPAL with ALL Therapy</td>
<td>100%</td>
<td>63%</td>
</tr>
</tbody>
</table>
HSCT

• Every study suggests that alloSCT after CR in adults improves outcomes
• Pediatric results are more variable

<table>
<thead>
<tr>
<th></th>
<th>5 year OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAL with alloSCT</td>
<td>70%</td>
</tr>
<tr>
<td>ALAL without alloSCT</td>
<td>19%</td>
</tr>
</tbody>
</table>
Other Treatments

• Mixed AML and ALL therapies
Case Resolution

• Patient was placed on leukapheresis to reduce WBC
• TLS prophylaxis
• 7+3 induction therapy with 14 days of imatinib
• Supportive RBC and platelets
• 6 months later, distinct blast population recognized in bone marrow
Case 2

- 6 month old female presenting with vomiting
- Splenomegaly
# Selected Patient Results

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>$41.8 \times 10^9/L$</td>
<td>$6-18 \times 10^9/L$</td>
</tr>
<tr>
<td>RBC</td>
<td>$3.32 \times 10^{12}/L$</td>
<td>$3.6-5.2 \times 10^{12}/L$</td>
</tr>
<tr>
<td>HGB</td>
<td>9.5 g/dl</td>
<td>10.4-15.6 g/dl</td>
</tr>
<tr>
<td>HCT</td>
<td>28.5%</td>
<td>35-51%</td>
</tr>
<tr>
<td>Platelet</td>
<td>$99 \times 10^9/L$</td>
<td>150-450 $\times 10^9/L$</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>8.3 mg/dl</td>
<td>2-7 mg/dl</td>
</tr>
</tbody>
</table>
# Patient WBC Differential

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelocytes</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>Bands</td>
<td>3%</td>
<td>0-5%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>52%</td>
<td>48-78%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>11%</td>
<td>2-11%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2%</td>
<td>0-4%</td>
</tr>
</tbody>
</table>
Patient Bone Marrow

- Myelopoiesis: Increased with maturation
- Erythropoiesis: decreased
- Megakaryopoiesis: Decreased
- FISH- Negative for ALL, AML, MDS, & BCR-ABL
- Flow cytometry- no evidence of lymphoma, acute leukemia, or other conditions with increased blasts
Juvenile Myelomonocytic Leukemia (JMML)

- Rare myeloid malignancy occurring in young children
  - 1.3 cases per million/ 2.4% of all childhood leukemias
- Proliferation of granulocytic and monocytic lineages
Typical Presentation

- Fever
- Respiratory Symptoms
- Hepatosplenomegaly
- Skin Rash
- Elevated WBC with increased monocytosis, immature granulocytes, anemia, and thrombocytopenia
Incidence

- <2 years old
- 2x more likely in males
- Genetic syndromes
  - Neurofibromatosis-1 (NF1)
  - Noonan syndrome (NS)
A. Normal Myeloid Differentiation

- HSC → CMP → GMP → Myeloblast → Myelocyte → Neutrophil
- Monoblast → Promonocyte → Monocyte

B. Acute Myeloid Leukemia

- HSC → CMP → GMP → Myeloblast → Myelocyte → Neutrophil
- Monoblast → Promonocyte → Monocyte

C. Juvenile Myelomonocytic Leukemia

- HSC → CMP → GMP → Myeloblast → Myelocyte → Neutrophil
- Monoblast → Promonocyte → Monocyte

Chan et al., 2009
WHO Criteria

- Peripheral blood monocytosis >1x10⁹/L
- Blasts (including promonocytes) are <20% of leukocytes in the pb and bm
- BCR-ABL1 negative
- Plus two or more of the following
  - Increased HGB F
  - Immature granulocytes in PB
  - WBC count >10x10⁹/L
  - Clonal Chromosomal abnormality
  - GM-CSF hypersensitivity of myeloid progenitors in vitro
New Updated Criteria

• Clinical and hematologic findings (all 4)
  – Peripheral blood monocyte count >1 x10^9/L
  – Blast percentage in pb and bm <20%
  – Splenomegaly
  – Absence of BCR/ABL rearrangement
New Updated Criteria

• Oncogenetic studies (pick 1)
  – Somatic mutation in PTPN11, K-RAS, or N-RAS
  – Clinical diagnosis of NF-1 or germline NF1 mutation
  – Germline CBL mutation and loss of heterozygosity of CBL
New Updated Criteria

• For 10% of patients without oncogenetic criteria (pick 2)
  – Monosomy 7 or any other chromosomal abnormality
  – Hgb F increased for age
  – Myeloid precursors in pb
  – Spontaneous growth or GM-CSF hypersensitivity in colony assay
  – Hyperphosphorylation of STAT5
Cytogenetics

- 65% of patients have normal karyotype
- Monosomy 7
  - 25% of patients
  - Present with lower WBC
  - Negative prognostic factor?
- Other rare abnormalities include del 5q & t(1;5)
Germline Mutations “RASopathies”

• Neuro-cardio-facio cutaneous syndromes
• Noonan Syndrome
  – 1 in 1,000-2,500 births
  – Mutations usually occurring in PTPN11
• Neurofibromatosis 1
  – Mutation in gene NF-1 (GAP)
PTPN11 Mutation in JMML

- Encodes SHP-2 tyrosine phosphatase
- Mutation in 50% of patients with Noonan Syndrome (NS)
- 35% of JMML patients have PTPN11 mutation
- All mutations in the SH-2 or PTP surfaces
- Mutations prevent autoinhibition
RAS Mutations in JMML

- 20-25% of JMML patients are heterozygous for RAS mutations
- Locks RAS-GTP on
  - Resistance to GAPs (GTPase activating proteins)
- K-RAS & N-RAS typically have single glycine substitution
  - K-RAS: particularly aggressive
  - N-RAS: Aggressive but periodic spontaneous remissions noted
CBL

• Ubiquitin ligase

• 12% of JMML patients

• Binds to GRB2 preventing it from binding SOS
  – SOS is a guanine nucleotide exchange factor (GEF) for the RAS Pathway

• Spontaneous remission
Other Mutations

- SETBP1
- JAK3
Poor Prognostic Factors

- Low PLT
- >2 years old
- Increased Hgb F
- AML like profile
- Extent of methylation
Disease Free Survival by Age

Children younger than 1.4 years,
5-year DFS: 53%

Children older than 1.4 years
5-year DFS: 30%

p = 0.012

Locatelli et al., 2013
Bresolin et al., 2010
DNA Methylation

• Hypermethylation of CpG islands have a poor prognosis
• Treatment with Azacitididine
Treatment: HSCT

- Recommended for most patients with JMML with NF-1; somatic PTPN11 mutations, somatic KRAS mutations, and most somatic NRAS mutations
- Not recommended in most CBL mutations
Overall Survival, Event-Free Survival, Relapse Incidence and Transplant-Related Mortality

SURV:  N = 100; E = 34
EFS:    N = 100; E = 47
RI:     N = 100; E = 34
TRM:    N = 100; E = 13

SURV = 64% (54-74)
EFS = 52% (42-62)
RI = 35% (27-46)
TRM = 13% (8-22)

No. of cases at risk:

<table>
<thead>
<tr>
<th>Year</th>
<th>100</th>
<th>72</th>
<th>54</th>
<th>43</th>
<th>29</th>
<th>17</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRM</td>
<td>100</td>
<td>55</td>
<td>45</td>
<td>35</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Locatelli et al., 2005

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Cord Blood

- Easier and quicker to obtain
- Patients have low body weight
- Lower incidence & severity of Graft versus Host Disease (GVHD)
- Similar relapse rates and overall survival
Cord Blood

5-year OS: 52%
Graft vs Leukemia

• Immediate withdrawal of immunosuppressive therapy may facilitate graft immunologic response to re-emerging JMML cells
• Decreased relapse in patients with Grade II-III GVHD
• Donor lymphocyte infusion not successful
Conventional Chemotherapy

- Intensive preparatory regimes before HSCT may not help
- Many regimes have been tested with no real improvements
Splenectomy

Event-Free Survival by Spleen Size at HSCT

No. of cases at risk:

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 cm</td>
<td>34</td>
<td>22</td>
<td>17</td>
<td>14</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>36</td>
<td>17</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>24</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

< 5 cm = 61% (44-78)

SPLENECTOMIZED = 48% (28-69)

> 5 cm = 44% (26-62)

P = N.S.

Locatelli et al., 2005
Recent & Future Treatments

• Farnesyl transferase- no effect on EFS
• Retroviral mutation of NRAS- successful in mice
• Pharmacological inhibition of MEK kinase
  – PD0325901-halted
  – AZD6244- being tested in non-JMML models
  – Trametinib- starting trials in JMML models
Case 2 Resolution

• Negative for NS and NF-1
• Unrelated cord blood transplant
• Transfusion dependent
• 1 month after transplant
  – No evidence of GVHD
  – Respiratory failure of unknown etiology
References

Available as a separate handout