Diagnostic work-up of leukaemia in resource constrained countries

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National University of Singapore
National University Health System
Acute leukaemias ~40% of childhood cancers

Tan AM, et al. Singapore Children’s Cancer Registry
Indian Rim – 55% of world childhood cancer burden
Young population, vast landmass, LMIC
Survival gap
10-15%
Asia

North America

Asia

Europe

Oceania

Childhood Leukaemia in Males - Singapore
– Incidence, Mortality, Relative Survival Rates

 HM Verkooijen, GH Lim, KS Chia

**Incidence**

**5-y RSR**

**Mortality**

Year

Rate per 1,000,000

5-year RSR (%)
ASR Childhood Leukaemias for Males

0 – 4 y
P = 0.04

HM Verkooijen, GH Lim, KS Chia

HM Verkooijen, GH Lim, KS Chia
Evolution of Treatment of Childhood ALL

1960s
No cure

1970s-1980s
Cure at all cost

1990-2017
Cure at minimal cost
Frequency of Adverse Events in BFM trials
Limits of intensification of therapy

%  
35
30
25
20
15
10
5
0

81/83  86  90  95  2000  2000
Pred   Dex

Total Events
Death Relapse
Treatment Death
Incidence of Childhood cancers

- 1 in 300 children < 18 years old develops cancer
- 1 in 800 children < 18 years old develops leukemia

- Difficult to find out who develops leukemia
- Unlike adult cancers, public education/screening has little impact on childhood leukaemia.
- Malaria, dengue, TB
- High index of suspicion
Diagnosing leukaemia

- Bone marrow disease
- Fever recurrent – no source infection
- Anemia – tired, lethargy, sleeping
- Bleeding – small bruises (purpura), petechiae, bleeding nose or gums
  - Do not mix up with ITP or dengue fever or malaria
- Swollen lymph nodes
- Pain in bones – do not mix up with Juvenile Rheumatoid Arthritis (only joints affected)
  - Before Rx with steroids/prednisolone – do BM
Leukemia in children

- ALL = Acute Lymphoblastic Leukemia (85%)
- AML Acute Myeloid Leukaemia (12%)
- CML = Chronic Myeloid (2%)
- CLL does not occur in children & very rare in asian adults

FBC and peripheral blood film
Bone marrow aspiration – I do this first to reduce clots
Bone marrow trephine – cellularity, fibrosis, solid tumour spread
Posterior superior iliac spine PSIS
Anterior superior iliac spine ASIS
Sternal – not recommended
Diagnostic tests in leukemia

- FBC and morphology
- Bone marrow aspiration
- Rapid quick suction
- Clot quickly – EDTA
  - NaHep flow, cytogenetics
- Different levels to avoid hemodiluted BM

If ‘dry tap’:
Bone marrow trephine – cellularity, fibrosis, solid tumour spread
Bone marrow slides

- fixed with fresh acetone-free absolute methanol
- Romanowsky stain, such as May–Grünwald Giemsa (MGG) or Wright Giemsa.
- Leishmann stain, Wright stain – good for malaria but not nuclear morphology
- 10x eye piece, 10X/40x/ 60x/100x
Leishmann stain

MGG stain
Prognostic factors for childhood ALL

- WBC > 50,000/uL or
- Age <1yrs or > 10 yrs

- t(9;22)/BCR-ABL
- MLL rearrangements
- Hyperdiploid > 50 chr
- DNA index ≥ 1.16
- TEL-AML1

- Treatment response
- Treatment protocol
Cytogenetic abnormalities –

• laborious, technically challenging
  Different from neonatal karyotyping

Chromosomal number abnormalities

• Whole chromosomes
  – Hypodiploid <44
  – Normodiploid
  – Hyperdiploid 47-50
  – Hyperdiploid >50
  – Tetraploid

• Deletions or insertions

• Translocations

• Balanced – t(9;22), t(4;11), t(1;19)
Translocation
Mixing up the message

Mermaid  Mer-lion

Lion  Maid
Oncogene fusion
# University Malaya – multiplex PCR screening

Ariffin Hany, et al.

## Table 1. Primers used in the multiplex PCR protocol.

<table>
<thead>
<tr>
<th>Multiplex panel</th>
<th>Fusion transcript</th>
<th>1st round primers (5' to 3')</th>
<th>Fusion transcript</th>
<th>2nd round primers (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PBX1:459L18</td>
<td>GCCACGCCTTCCGCTAAC</td>
<td>PBX1:436L21</td>
<td>CATGTTGTCAGCAGCCGCATCAG</td>
</tr>
<tr>
<td></td>
<td>E2A:1075U21</td>
<td>TTCTCGTCCAGCCCTTCTACC</td>
<td>E2A:1173U19</td>
<td>CTACGACGGGGGCTTCCAC</td>
</tr>
<tr>
<td></td>
<td>TEL:871U23</td>
<td>CACTCCGTTGATTCAACAGTC</td>
<td>TEL:944U23</td>
<td>CTCACTCGGGAAGACCTGGCTTAC</td>
</tr>
<tr>
<td></td>
<td>AMLIA:1891L23</td>
<td>AGCCGAGTATTTCATCATTGC</td>
<td>AMLIA:1772L21</td>
<td>AGCACGGAGCAGAGGAAGTTG</td>
</tr>
<tr>
<td>B</td>
<td>BCR:1698U19</td>
<td>CGCTCTCCCTCCGAGAACT</td>
<td>BCR:1777U19</td>
<td>ACTGCCCCGGTTGTGGTGC</td>
</tr>
<tr>
<td></td>
<td>BCR:3060U23</td>
<td>GAGTCACTGCTGCTGCTTATGTC</td>
<td>BCR:3128U22</td>
<td>CACGTTCTGATCTCCTGCTGAC</td>
</tr>
<tr>
<td></td>
<td>ABL:661L20</td>
<td>TTTTTGTTTGGGTCTACAC</td>
<td>ABL:642L23</td>
<td>ACACATTCCTCAGTTGTATT</td>
</tr>
<tr>
<td>C</td>
<td>MLL:3730U20</td>
<td>CCGCCCTCAGCCACCTACTAC</td>
<td>MLL:3751U20</td>
<td>GGACCAGCAAAAGAAAAGT</td>
</tr>
<tr>
<td></td>
<td>MLL:3955U24</td>
<td>AGCACTCTCTCCAATGGCAATAGT</td>
<td>MLL:3996U24</td>
<td>AGCAGATGGAGTCCCAGGAGATCAG</td>
</tr>
<tr>
<td></td>
<td>AF4:1636L29</td>
<td>GAAATTTGAGTGTTTTTGAAGATGATC</td>
<td>AF4:1606L25</td>
<td>GTTTTGGTTTGGTTACAGAACT</td>
</tr>
<tr>
<td>All (ABC)</td>
<td>E2A:1075U21</td>
<td>TTCTCGTCCAGCCCTTCTACC</td>
<td>E2A:1173U19</td>
<td>CTACGACGGGGGTCTCCAC</td>
</tr>
<tr>
<td></td>
<td>E2A:1883L22</td>
<td>TTTTTCTCTTCGCGCTTTCA</td>
<td>E2A:1884L19</td>
<td>AGGTTCGGCTTCGCACCTT</td>
</tr>
</tbody>
</table>
## Hospital Level

<table>
<thead>
<tr>
<th></th>
<th>Tier 1</th>
<th>Tier 2</th>
<th>Tier 3</th>
<th>Tier 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>Regional hospital</td>
<td></td>
<td>Southern Philippines Medical Ctr</td>
<td>NUH. TMC. CMC.</td>
</tr>
<tr>
<td>Doctors</td>
<td>GP</td>
<td>General Paeds Internal Med</td>
<td>Paeds Oncology</td>
<td>Paeds Onc Subspeciality</td>
</tr>
<tr>
<td>Nurses</td>
<td>General</td>
<td>Paeds</td>
<td>Paeds Onc</td>
<td>Paeds Onco certified.</td>
</tr>
<tr>
<td>Complexity</td>
<td>ALL SR</td>
<td>ALL</td>
<td>ALL AML</td>
<td>+BMT</td>
</tr>
<tr>
<td>Lab</td>
<td>FBC. Chems</td>
<td>MTX levels</td>
<td>CsA level</td>
<td></td>
</tr>
<tr>
<td>Supportive care</td>
<td>Bld only</td>
<td>Bld, Plt</td>
<td>Bld, CS Plt</td>
<td>Stem cell Paed Surg Oncol</td>
</tr>
<tr>
<td>Crowding</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Isolation, HEPA</td>
</tr>
</tbody>
</table>
Management of adult and paediatric acute lymphoblastic leukaemia in Asia: resource-stratified guidelines from the Asian Oncology Summit 2013

Allen E J Yeoh, Daryl Tan, Chi-Kong Li, Hiroki Hori, Eric Tse, Ching-Hon Pui

Govt Health Expenditure per capita in US$

- **Low income country $10**
  - Lao, Cambodia, Myanmar, Afghanistan, Bangladesh, Pakistan
  - Tajikistan, India, Philippines
  - Sri Lanka, Vietnam, Timor-Leste, Papua New Guinea, Indonesia, Syrian Arab Republic, Mongolia, Bhutan

- **Middle income country $117**
  - China, Iran, Thailand, Mauritius, Iraq, Malaysia
  - Maldives, Russian Federation, Saudi Arabia, Oman, Singapore
  - Bahrain, Brunei, Korea, Kuwait

- **High income country $3174**
  - UAE, Qatar, Israel, Australia, Japan

World Bank
Accessed March 2013
## Country Tiers

<table>
<thead>
<tr>
<th></th>
<th>Tier 1</th>
<th>Tier 2</th>
<th>Tier 3</th>
<th>Tier 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.g.</td>
<td>Afghanistan</td>
<td>India. Pakistan</td>
<td>Malaysia. Thailand</td>
<td>Australia. Singapore</td>
</tr>
<tr>
<td>National Cancer Control Plan</td>
<td>No</td>
<td>Working on it</td>
<td>Yes</td>
<td>Yes. Include Paediatric Cancer</td>
</tr>
<tr>
<td>Cancer registry</td>
<td>No</td>
<td>Low quality</td>
<td>Adult and Paeds</td>
<td>Adult/Paeds Survival data</td>
</tr>
<tr>
<td>Universal health care</td>
<td>No</td>
<td>Moderate</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Under 5y mortality</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Access crowding</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Disparity</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Hospital level</td>
<td>Level 1</td>
<td>1,2 only</td>
<td>1,2,3</td>
<td>2,3,4</td>
</tr>
</tbody>
</table>

Diagnosis and initial management – in highest hospital level
Shared care, right siting – emails, whatsapp, phone calls for ALL Mx
Diagnostic work up and risk assignment

**Basic resources**

*Children and adults*
- Morphology with or without cytochemistry
- Chest radiography to detect mediastinal mass

*Children and adults*
- Age
- Leucocyte count
- Day 8 peripheral-blood response

EFS by Age Groups

- 1yr-9yr: 6yr EFS 84.6% n=357
- >9yr: 6yr EFS 76.1% n=99
- <1yr: 6yr EFS 65% n=21

P value=0.001
EFS by NCI Criteria

NCI Low Risk: 6yr EFS 87.1% n=284

NCI High Risk: 6yr EFS 74.8% n=192

P value=0.000
EFS by Day 8 Prednisolone Response Criteria

GPR: 6 yr EFS 83.7% n=422

PPR: 6 yr EFS 70.6% n=50

P value=0.001
Limited resources

Children

- Morphology and cytochemistry
- Immunophenotyping (restricted)
- DNA index
- RT-PCR of BCR-ABL1, MLL-AFF1, and ETV6-RUNX1

Limited resources

Children

- Age
- Leucocyte count
- Immunophenotype (T cell vs B cell)
- Blast-cell count in peripheral blood after 1 week of prednisone treatment, or percentage of leukaemic blast cells in bone marrow at day 8
- Day 15 and end-of-induction bone-marrow response
- If available, RT-PCR of BCR-ABL1, MLL-AFF1, and ETV6-RUNX1

EFS by Lineage

T-lineage ALL: 6yr EFS 87.1% n=41

B-lineage ALL: 6yr EFS 81.4% n=436

P value=0.680
EFS in subtypes of ALL

- **Pre-B ALL without BCR-ABL/t(9;22)**
- **ALL with hyperdiploid karyotype >50**
- **ALL with E2A-PBX1/t(1;19)**
- **ALL with TEL-AML1/t(12;21)**
- **T-ALL**

Survival Functions

- **t(1;19)/E2A-PBX1**
- **Hyperdip >50**
- **TEL-AML1**

- **t(9;22)/BCR-ABL1**
## Problem of Abandonment in ALL

<table>
<thead>
<tr>
<th>Hospital, Country</th>
<th>Phase</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia ALL Protocol (15 centers)</td>
<td>Treatment</td>
<td>28.5%</td>
</tr>
<tr>
<td>Yangon Children’s Hospital Myanmar</td>
<td>Treatment</td>
<td>25 %</td>
</tr>
<tr>
<td>National Hospital of Pediatrics Hanoi, Vietnam</td>
<td>Treatment</td>
<td>21.5 %</td>
</tr>
<tr>
<td>Philippine General Hospital Manila, Philippines</td>
<td>Treatment</td>
<td>34 %</td>
</tr>
<tr>
<td>Southern Philippines Medical Center Davao, Philippines</td>
<td>ALL Induction</td>
<td>38 %</td>
</tr>
<tr>
<td>Hue Central Hospital, Vietnam</td>
<td>Treatment</td>
<td>9 %</td>
</tr>
<tr>
<td>Mandalay Children’s Hospital</td>
<td>Treatment</td>
<td>20 %</td>
</tr>
<tr>
<td>University of Sto. Tomas Manila, Philippines</td>
<td>Treatment</td>
<td>27 %</td>
</tr>
</tbody>
</table>

Ko & Yetty, N, St. Juve Viva Forum 2013 Abstracts
B. N. Lan, A. Khaing, M Medina, M. Dolendo, St. Jude Viva Pre-forum 2011
Lubaton, St. Jude Viva Forum 2009 Abstracts
Ha, St. Jude Viva Forum 2013 abstracts
Puno, St. Jude Viva Forum 2010 Abstracts
Reducing abandonment

Transportation – taxi fare

Half-way house

Social worker – YOAI, Kythe

JAMA 2004, 291: 2471
Shortage of medications
Shortage of MP and MTX in Hue Province
3-drug induction is comparable to 4-drug
Start slow especially in limited resource

<table>
<thead>
<tr>
<th>MRD</th>
<th>Ma-Spore ALL 2003 – 3 drug</th>
<th>AIEOP-BFM 2000 – 4 drug</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>5-y EFS</td>
</tr>
<tr>
<td>SR</td>
<td>48</td>
<td>93.2</td>
</tr>
<tr>
<td>IR</td>
<td>45</td>
<td>83.5</td>
</tr>
<tr>
<td>HR</td>
<td>7</td>
<td>32</td>
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</tbody>
</table>
## Antibiotics

<table>
<thead>
<tr>
<th>Medication</th>
<th>Cost per Unit</th>
<th>Cost per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime 1g</td>
<td>$38</td>
<td></td>
</tr>
<tr>
<td>Amikacin 500mg</td>
<td>$6</td>
<td>$165/day</td>
</tr>
<tr>
<td>Gentamicin 80mg</td>
<td>$3</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>$65</td>
<td></td>
</tr>
<tr>
<td>Meropenem 1g</td>
<td>$100</td>
<td>Meropenem</td>
</tr>
<tr>
<td>Voriconazole 200 mg</td>
<td>$240</td>
<td>$639/day</td>
</tr>
<tr>
<td>IV antibiotics admin</td>
<td>$45</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>$80</td>
<td></td>
</tr>
<tr>
<td>Pheresed plt</td>
<td>$600</td>
<td></td>
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</tbody>
</table>
# Chemo Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone 4mg</td>
<td>10 cents</td>
</tr>
<tr>
<td>Vincristine 2 mg</td>
<td>$5</td>
</tr>
<tr>
<td>L-asparaginase</td>
<td>$60</td>
</tr>
<tr>
<td>Methotrexate IT 12mg</td>
<td>$7</td>
</tr>
<tr>
<td>Methotrexate 1000mg</td>
<td>$75</td>
</tr>
<tr>
<td>Mercaptopurine 50mg</td>
<td>$1.40</td>
</tr>
</tbody>
</table>
Supportive care
Good nursing helps reduce complications
Cross check and don’t batch drug
Minimal Residual Disease–Guided Treatment Deintensification for Children With Acute Lymphoblastic Leukemia: Results From the Malaysia-Singapore Acute Lymphoblastic Leukemia 2003 Study

Allen Eng Juh Yeoh, Hany Ariffin, Elaine Li Leng Chai, Cecilia Sze Nga Kwok, Yiong Huak Chan, Kuperan Ponnudurai, Dario Campana, Poh Lin Tan, Mei Yoke Chan, Shirley Kow Yin Kham, Lee Ai Chong, Ah Moy Tan, Hai Peng Lin, and Thuan Chong Quah

A truly Asian effort – NUS, NUH, KKH – Singaporeans, Asian children

University Malaya – Hany Ariffin, Wan Ariffin and team, Sime Darby Medical Centre, KL – Lin Hai Pheng, Chan Lee Lee
A

Cumulative Survival (proportion)

Time (years)

EFS: (n = 556) 96 events; at 5 years and 6 years: 80.6% ± 3.5%
LFS: (n = 556) 58 events; at 5 years and 6 years: 87.0% ± 3.3%
OS: (n = 556) 57 events; at 5 years: 89.2% ± 2.7%;
at 6 years: 88.4% ± 3.1%
East Asian Ancestry has Lower 6MP Tolerance

Yang et al., J Clin Oncol 2015
NUDT15 and TPMT wild type: Genetic score 0

R661 HHN Maintenance Phase Normalized 6MP dosage

(mg/m^2/day)

 NUXT15 415CC
NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity

Takaya Moriya1,2, Rina Nishii1,3,22, Virginia Perez-Andreu1,22, Wenjian Yang1, Federico Antillon Klussmann4,5, Xujie Zhao1, Ting-Nien Lin1, Keito Hoshitsuki1,6, Jacob Nersting7, Kentaro Kihira2, Ute Hofmann8,9, Yoshihiro Komada2, Motohiro Kato10, Robert McCorkle1, Lie Li1, Katsuyoshi Koh11, Cesar Rolando Najera4, Shirley Kow-Yin Kham12, Tomoya Isobe13, Zhiwei Chen12, Edwynn Kean-Hui Chiew12, Deepa Bhojwani14, Cynthia Jeffries15, Yan Lu15, Matthias Schwab8,9,16,17, Hiroto Inaba18, Ching-Hon Pui18, Mary V Relling1, Atsushi Manabe19, Hiroki Hori2, Kjeld Schmiegelow7,20, Allen E J Yeoh12,21, William E Evans1 & Jun J Yang1

Widely used as anticancer and immunosuppressive agents, thiopurines have narrow therapeutic indices owing to frequent toxicities, partly explained by TPMT genetic polymorphisms. Recent studies identified germline NUDT15 variation as another critical determinant of thiopurine intolerance, but the underlying molecular mechanisms and the clinical implications of this pharmacogenetic association remain unknown. In 270 children enrolled in clinical trials for acute lymphoblastic leukemia in Guatemala, Singapore and Japan, we identified four NUDT15 coding variants (p.Arg139Cys, p.Arg139His, p.Val18Ile and p.Val18_Val19insGlyVal) that resulted in 74.4–100% loss of nucleotide diphosphatase activity. Loss-of-function NUDT15 diplootypes were consistently associated with thiopurine intolerance across the three cohorts (P = 0.021, 2.1 × 10⁻⁵ and 0.0054, respectively; meta-analysis P = 4.45 × 10⁻⁸, allelic effect size = -1.15). Mechanistically, NUDT15 inactivated thiopurine metabolites and decreased thiopurine cytotoxicity in vitro, and patients with defective NUDT15 alleles showed excessive levels of thiopurine active metabolites and toxicity. Taken together, these results indicate that a comprehensive pharmacogenetic model integrating NUDT15 variants may inform personalized thiopurine therapy.
Combining NUDT15 and TPMT variants
Genetic score – 1 in 50 very sensitive to MP

Ma-Spore ALL 2003 (n=485)

- Score 0, 77.5%
- Score 1, 20.5%
- Score 2, 2.0%
Combined *NUDT15* and *TPMT* on MP Tolerance; Ma-Spore ALL 2003 experience

Moriyama et al. Nat Gen 2016
Resource limited countries in Indian Rim

St Jude Global-Viva Alliance

- St Jude-Viva Forum – Pre Forum for LMIC
- Viva-Asia Working Group for HIC
- Childhood Cancer registries – Diagnosis,
  - Toxic Death, Abandonment, Relapse
- Childhood ALL study – protocol based therapy
- Digital imaging – telemedicine
- Central testing and validation
- Training
- Web-meeting
Navigating seas with lab medicine
ALL is most common
Trisomy 8 AML
Del 5q
NUDT15 variants

c36_37insGGAGTC

*1

*2

p.V18_V19insGV

C415T

*3

p.R139C

G416A

*4

p.R139C

*5

p.V18I

G52A

*6

p.V18_V19insGV

c36_37insGGAGTC
Epidemiology of Leukaemia in Singapore

Tan AM, et al. SCCR.
2 sides of the coin: Leukaemia factors

B vs T ALL
T-ALL do poorer
Early preB

Cytogenetics
H>50
Hypodiploid < 44

WBC count at diagnosis

Oncogene fusions
ETV6-RUNX1
TCF3-PBX1
MLL-r
BCR-ABL1

ABL1-r
IgH-DUX4
ZNF

IKZF1 deletion
2 sides of the coin: Host factors

Age of diagnosis
  Adolescents
  Infants

Clinical status
  Immunodeficiency

Social - compliance
  Economic status
  Abandonment

Supportive care

Pharmacogenomic factors
  TPMT
  NUDT15
Childhood Leukaemia in Females - Singapore
- Incidence, Mortality, Relative Survival Rates

Incidence
5-year RSR
Mortality

5-y RSR

Mortality

5-year RSR

Year

Rate per 1,000,000

0 10 20 30 40 50

HM Verkooijen, GH Lim, KS Chia
ASR Childhood Leukaemias for Females

0 – 4 y  
P=0.02

5 – 9 y

10 – 14 y

15 – 19 y

HM Verkooijen, GH Lim, KS Chia