Curing Children with Acute Lymphoblastic Leukemia
Hyperleukocytosis
Total Therapy Studies I-IV  1962-1967

• Introduce combination chemotherapy with prednisone, vincristine, cyclophosphamide, daunorubicin, cytarabine, mercaptopurine, methotrexate

• Began Total Therapy with remission induction; intensification; central-nervous-system (CNS)-directed therapy; continuation (maintenance) therapy

• Began cranial irradiation to prevent and treat CNS leukemia
Central Nervous System Leukemia

• Symptoms include: headache, mental status changes, vision changes, seizures, coma, death
• Intracranial hemorrhage
Facial Palsy in ALL

VIIth nerve
Total Therapy V  1967-1968

Total Patients  35
Complete Remission  31

Nothing changed the fate of children with ALL (and St Jude Hospital) like Danny Thomas’s appearance on tonight show with Johnny Carson on June 26, 1972

Quote of Yaddanapudi Ravindranath
Neuroimaging Abnormalities After Cranial Irradiation

- Brain atrophy
- Encephalomalacia
- Cerebral lacunes
- Dystrophic calcification
- Leukoencephalopathy
- Necrosis/gliosis
Endocrinopathy
After Cranial Irradiation
Brain tumors after cranial irradiation

Meningioma
median onset: 20 years

Malignant astrocytoma
median onset: 8 years
Overall Survival of Childhood ALL by Treatment Era at St. Jude

I-IV (n=90) 1962-1966
21% ± 4%

V-IX (n=825) 1967-1979
84% ± 2%

X (n=428) 1979-1983
74% ± 2%

XI, XII (n=546) 1984-1991
81% ± 2%

XIIIA, XIIIB, XIV (n=465) 1991-1999
94% ± 1%

XV, XVI (n=1050) 2000-2017
84% ± 2%

Pui et al. Unpublished Data
Total Therapy XV (2000-2007): Principle of Therapy

• **Adopt effective treatment components of successful clinical trials**
  - Reinduction therapy
  - Intensive asparaginase
  - Early intensive triple intrathecal therapy

• **Personalized therapy (Precision medicine)**
  - Risk assessment based on *minimal residual disease*
  - Individualized dosage of methotrexate and mercaptopurine based on pharmacokinetics and pharmacodynamics

• **Elimination of cranial irradiation in all patients**
  - Early intensification of intrathecal therapy
Treatment Response: Most Important Prognostic Indicator

- Leukemic cell
  - Tumor burden
  - Growth potential
  - Drug resistance

- Micro-environment
  - Drug resistance

- Host
  - Age
  - Pharmacogenomics

- Therapy
  - Drug dosage
  - Drug interactions
Risk Stratification by Minimal Residual Disease Level

- **During induction (day 19)**
  - MRD $\geq 1\% \rightarrow$ intensified remission induction

- **End of induction (day 46)**
  - MRD $\geq 0.01\% \rightarrow$ intensive continuation therapy
  - MRD $\geq 1\% \rightarrow$ allogeneic transplantation

- **Continuation treatment (week 7)**
  - MRD $\geq 0.1\% \rightarrow$ allogeneic transplantation

491/492 (99.8%) patients monitored by flow and/or PCR of Ig/TCR genes; the remaining one patient with \textit{MLL-AF9} was studied with RT-PCR; hence 100% had successful studies.
Morphologic Detection of Residual Leukemia
Limited Sensitivity and Specificity

ALL

morphologic remission

$10^{10}$

$10^8$

MRD

Normal bone marrow with hematogones
St. Jude Total Therapy Study XV: Treatment Results

Overall Survival

- 93.5% ± 1.1%
- 91.7% ± 4%

Event-free Survival

- 87.5% ± 1.5%
- 85.1% ± 6.1%

N=498

3.5% ± 0.8% any CNS relapse
2.2% ± 0.7% isolated CNS relapse

Total XV: Identical Results in All Racial/Ethnic Groups

5-year Overall Survival

White n=340
94.8±2.0

Black n=79
88.3±6.2

Hispanic n=60
89.1±8.9

Asian n=19
100±0

### ALL is a Polygenic Disease by Genome-wide Analyses

<table>
<thead>
<tr>
<th>Lymphoid development</th>
<th>Tumor suppression</th>
<th>Ras signaling</th>
<th>Cytokine receptors</th>
<th>Transcriptional regulators</th>
<th>Epigenetic modifiers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX5</td>
<td>CDKN2A/B</td>
<td>NRAS</td>
<td>CRLF2</td>
<td>ERG</td>
<td>CREBBP</td>
</tr>
<tr>
<td>IKZF1/2/3</td>
<td>PTEN</td>
<td>KRAS</td>
<td>IL7R</td>
<td>TBL1XR1</td>
<td>EP300</td>
</tr>
<tr>
<td>EBF1</td>
<td>RB1</td>
<td>PTPN11</td>
<td>FLT3</td>
<td>CREBBP</td>
<td>SETD2</td>
</tr>
</tbody>
</table>

- **ALL genomes harbor ~8 to 12 alterations per case (except infant ALL)**
- **Multiple pathways affected in majority of cases**
- **Translocations early, mutations later**
- **Clonal diversity evolves during disease progression**

- DNA structural abnormalities (deletions/gains, LOH)
- Gene expression profiling
- Sequence variants (mutations, polymorphisms)

Recent Genetic Classification of ALL

Two Genomes Influence Every Cancer Patient

Germline

Host Genome
- Systemic Pharmacokinetics
- Drug Toxicity (normal tissue)

Somatic

Cancer Genome
- Cellular Pharmacodynamics
- Cellular Pharmacokinetics
- Drug Sensitivity (tumor)

Diagnosis

Relapse/Metastasis

Evans & Relling
US FDA: Pharmacogenetics in Drug Label

- **Oncology**
  - 6MP: *TPMT*
  - Irinotecan: *UGT1A1*
  - Erlotinib/gefinitib: *EGFR*
  - Imatinib: *BCR-ABL*
  - Tamoxifen: *CYP2D6*

- **Hematology**
  - Warfarin: *CYP2C9* and *VKORC1*

- **Cardiovascular**
  - Clopidogrel: *CYP2C19*

- **Antiviral**
  - Abacavir: *HLA-B*

- **Analgesics**
  - Codeine: *CYP2D6*

- **Neurology**
  - Carbamazepine: *HLA-B*

- 164 drugs with pharmacogenomic biomarkers labeling

- 18 therapeutic areas: oncology, hematology, antiviral, cardiovascular, analgesics, etc.
Priority list of pharmacogenetic tests to move from research to clinical care

- TPMT---thiopurines
- CYP2D6 --- codeine, amitriptyline, ondansetron
- G6PD---rasburicase, Septra
- CYP2C9, VKORC1---warfarin
- CYP2C19---clopidogrel, voriconazole
- DPYD---5FU
- HLA-B*5701 --- abacavir
- HLA-B*1502 --- carbamazepine (Asians ancestry)
- HLA-B*1502 --- phenytoin
- HLA-B*5801---allopurinol
- UGT1A1---irinotecan
Interactions affecting drug metabolism

• CYP450 substrates: anthracyclines, epipodophyllotoxins, vinca alkaloids, and cyclophosphamide

• CYP450 enzyme inducer
  – Anticonvulsant (e.g., phenytoin, phenobarbital, carbamazepine)
  – Increase the clearance of agents metabolized by CYP450

• CYP450 enzyme inhibitor
  – Valproic acid, azole antifungals (e.g., fluconazole, voriconazole, and posaconazole), macrolide antibiotics (clarithromycin, erythromycin, and azithromycin)
  – Increase plasma drug concentration and cause greater toxicity
Peripheral Neuropathy in Total XVI

Peripheral neuropathy (sensory and motor) and neuropathic pain

Grade 2 or above

Grade 3 or above

Dr. Sima Jeha
Vincristine Neuropathy

Hind foot valgus - collapsed longitudinal arch

Kristin Scobey and Kiri Ness
A SNP in the promoter region of *CEP72* (encoding a centrosomal protein involved in microtubule assembly) is associated with vincristine-induced neuropathy.
Risk and Severity of VCR-induced Neuropathy Related to \textit{CEP72} Genotype

(Risk allele T associated with low expression of CEP72 mRNA)

COG 0334: Combined Cohort (SJ, COG)

SJCRH T13B: Grade 3+ Only

Diouf et al. JAMA 2015;303:815-23
International Collaboration

- **1988**: Teaching, consultation, and collaboration with Taiwan Pediatric Oncology Group
- **1991**: Outreach to China: Beijing Children’s Hospital and Shanghai Children’s Medical Center
- **1995**: Founded International ALL working group joined by 15 major national study groups or institutions around the world -- international collaborative research
- **2006**: Initiated St. Jude Viva Forum in Singapore -- teaching, networking, and research in Asia
- **2014**: Formed First China National ALL Study Group
Shanghai Children’s Medical Center
Childhood Cancer in Mainland China

- 50,000 new cases per year
- 20,000 new ALL cases per year
- Before 2000, < 10% of patients with ALL were treated (increasing to ~ 30% in 2011 and >90% presently)
Estimated 5-year Survival of Children (aged 0-14 years) with Acute Lymphoblastic Leukemia in China

- 1995-99: 10.9% (95% CI, 1.5-20.2)
- 2000-04: 50.0% (95% CI, 39.7-60.2)
- 2005-09: 61.1% (95% CI, 51.3-70.8)

Data from Beijing, Changle, Cixian, Dafeng, Dalian, Donghai, Feicheng, Ganyu, Guanyun, Haimen, Haining, Jianhu, Jiashan, Jintan, Lianyungang, Linzhou, Qidong, Sihui, Taixing, Yangzhong, Zhongshan

Allemani et al. Lancet 2015;385:977-1010
China ALL Pilot Protocol

- Developed in December 2004 at St. Jude by the hematologists/oncologists from BCH and SCMC
- The first joint low- and intermediate-risk ALL protocol in China applied at the two leading pediatric centers
- Approved by St. Jude and supported by Partner in Hope foundation Limited (founded by a St. Jude board member)
- Underprivileged patients without any means to pay
- $14,400 - $16,000 per low risk patient
- $22,000 - $24,000 per intermediate patient
THIS WEEK IN THE JOURNAL

Article Summaries
Summaries | PDF

PERSPECTIVE

Marburg Hemorrhagic Fever in Angola — Fighting Fear and a Lethal Pathogen
N. Ndayimirije and M. K. Kindhauser
Extract | FREE Full Text | PDF

Saving the Children — Improving Childhood Cancer Treatment in Developing Countries
R. C. Ribeiro and C.-H. Pui
Extract | FREE Full Text | PDF

A Patient with Acute Lymphoblastic Leukemia at the Shanghai Children's Medical Center.
Children Cancer in China

• The ALL program was introduced to Chen Zhu (then Director of Shanghai Institute of Hematology) in 2005.
• Chen Zhu became Health Minister of China in 2007.
• The success (86% in continuous complete remission and affordable cost) was published in Pediatr Blood Cancer by Shanghai Children’s Medical Center (Dr. JY Tang) in 2009 and drew the attention of Chen Zhu.
• In 2010, Chen Zhu initiated New Rural Cooperative Medical Care System (新农和医疗保险), starting with childhood ALL.
Abandonment Rates in Remote Areas
Before and After NRCMCS* (新农和医疗保险)
(1151 patients 2002 – 2012 in Suzhou City)

New Rural Cooperative Medical Care System (NRCMC 新农和医疗保险)

Established in October 2014 with the full support of Shanghai Children’s Medical Center and chaired by Dr. Tang Jingyan

Included 20 major hospital/medical centers in 10 provinces, 3 direct-controlled municipalities and Hong Kong

Adapted a uniform risk-stratified treatment protocol on the basis of St. Jude Total Therapy study with some modifications according to the tolerance of Chinese patients.

Targeted to enroll 1,200 – 1,500 cases per year
123 patients from 20 provinces
China National Multi-Center Childhood ALL Study Group

VIVA –China Children’s Cancer Foundation

- Established by Jennifer Yeo (Founder of Singapore VIVA Foundation) and registered in Hong Kong in 2014 to support CCCG-ALL Group
- Budgeted RMB 3.6 millions per year with total 5-year budget RMB18 millions to support data management, diagnostic laboratory tests, education/training, etc.
Inaugural Meeting of China National Multi-Center Childhood ALL Study Group
China National Multi-Center Childhood ALL Study Group (CCCG-ALL)

October 2014 – March 2016

Total 1257 patients have been enrolled

- 675 low-risk, 552 intermediate-risk, 30 high-risk
- Age: 4 months – 16 years
- Adverse Events
  - 8 patients had resistant disease
  - 19 (1.5%) relapsed
  - 26 (2.1%) abandoned treatment mainly due to financial reason
  - 23 (1.8%) died of toxicity
  - 3 off protocol due to toxicity

Remaining 1178 (94%) patients are receiving treatment in remission.
Recommendation for Future Direction
Establish a National Child Health Center

- Education and training
  - Establish bases for continual education and training of healthcare providers
  - Establish healthcare provider qualification examination system to ensure the quality of medical care

- Enhancement of collaboration of multidisciplinary teams

- Research Priorities
  - Develop the necessary infrastructure to support clinical research
  - Apply and develop efficient mechanisms for timely application of effective new treatments
  - Prioritize emerging research initiatives
## Inherited Gene Variants Associated with the Development of Childhood ALL*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Odd Ratio 95% CI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IKZF1</strong> $^{1-3}$</td>
<td>1.69</td>
<td>Associated with B- and T-ALL</td>
</tr>
<tr>
<td><strong>ARID5B</strong> $^{1-3}$</td>
<td>1.5-1.7</td>
<td>Associated with hyperdiploid ALL: Hispanics&gt;Whites&gt;Blacks</td>
</tr>
<tr>
<td><strong>CEBPE</strong> $^{1,3}$</td>
<td>1.2-1.4</td>
<td>Associated with B- and T-ALL</td>
</tr>
<tr>
<td><strong>CDKN2A</strong> $^{4}$</td>
<td>1.1-1.5</td>
<td>Associated with B- and T-ALL</td>
</tr>
<tr>
<td><strong>BMI1-PIP4K2A</strong> $^{5,6}$</td>
<td>1.2-1.5</td>
<td>Hispanics&gt;Whites&gt;Blacks</td>
</tr>
<tr>
<td><strong>GATA3</strong> $^{6,7}$</td>
<td>1.2-5.4</td>
<td>Associated with Ph-like ALL and risk of relapse</td>
</tr>
<tr>
<td><strong>TP63, PTPRJ</strong> $^{8}$</td>
<td>0.5-0.8</td>
<td>Associated with $ETV6$-$RUNX1$ ALL</td>
</tr>
</tbody>
</table>

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Frequency of the risk allele of *ARID5B* SNP (rs10821936) increases in order for Blacks, Whites and Hispanics, consistent with the racial differences in ALL incidence.

Xu et al., J Clin Oncol 2012;30:751-7
Allele frequency at GATA3 SNP (%)
ARID5B, IKZF1, CEBPE, and BMI1-PIP4K2A variants cumulatively conferred predisposition to ALL.

Those with ≥6 risk alleles were at 9-fold higher risk than subjects with 0–1 copies.

Germline Mutations and ALL Risk

- **TP53** in 50% low-hypodiploid ALL
- **PAX5 G183S** mutations in familial ALL
  - Shah et al. Nat Genet 2013;45:1226-31
- **ETV6** mutations in familial thrombocytopenia and hematopoietic malignancy
  - Topka et al. PLOS Genet 2015;11:e1005262
Inherited TP53 mutation in a low-hypodiploid ALL kindred

Child: low hypodiploid ALL  
Father: glioblastoma multiforme

p.Gly302fs  
Homozygous in tumors  
Heterozygous skin biopsy

TP53 immunoreactivity in glioblastoma multiforme

Mike Walsh, David Ellison
96/1,120 patients (8.6%) in PCGP harbored germline mutations within 89 well characterized cancer predisposition genes

Germline Mutations of Cancer Predisposition Genes in Children with leukemia

• Whole-genome or whole-exome sequencing or both of remission bone marrow or blood sample

• 567 genes were selected for in-depth analysis based on their associated inheritance patterns, associated syndromes, penetrance, de novo mutation rate, etc.

• Germline mutations involved in 26 of 588 leukemia patients (4.4%) vs. 1.1% in 1000 Genome Project and 0.6% in Autism study

• Hypodiploid ALL has the highest rate of germline mutation (TP53); 53% among low-hypodiploid cases.

• Panel of genes associated with ALL: ANKRD26, CEBPA, DDX41, ETV6, GATA2, RUNX1, SRP72, TERC, TERT, TP53

Genome Sequencing Identified Mutations Associated with Drug Resistance in Relapsed ALL

Activating mutations in **CREBBP**: Glucocorticoid resistance

Activating mutations in **NT5C2**: Thiopurine resistance

Negative feedback-defective mutations in **PRPS1**: Thiopurine resistance

East Asians Have Poorer Mercaptopurine Tolerance

Regression coefficient estimate

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient estimate</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Asian ancestry</td>
<td>-0.14</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Yang et al., J Clin Oncol 2015;33:1235-42
Second Genome-wide Significant Locus: *NUDT15*

- Each dot is a SNP and color indicates chromosome
- Inverse log-transformed P value on the Y axis
- The taller the peak, the smaller the P value, and the stronger the association

Yang et al., J Clin Oncol 2015;33:1235-42
NUDT15 C416T Variant is Strongly Associated with Mercaptopurine Intolerance

Discovery GWAS (AALL03N1) vs Replication Cohort (St. Jude Total XV)

Yang et al., J Clin Oncol 2015;33:1235-42
NUDT15 contributes to Ancestry-related Differences in Mercaptopurine Tolerance

Yang et al., J Clin Oncol 2015;33:1235-42
Members of Leukemia Team