DUALISTIC VIEW OF MOLECULAR BIOMARKERS IN AL RISK FACTORS

PROGNOSIS

ETIOLOGY
Childhood acute lymphoblastic leukemia (ALL), one of the first malignant diseases for which large-scale therapeutic trials were conducted, has served as a paradigm for cancer research for over four decades; At present, two thirds or more of children with ALL can be cured with contemporary treatment plans;

The identification of causes and prevention/early intervention is clearly a worthwhile goal.

Mel Greaves, “The causation of childhood leukemia: a paradox of progress?”

Ching-Hon Pui, M.D. “The possibility of achieving total cure of childhood acute lymphoblastic leukemia with personalized therapy”
Bcp-ALL

- t(4;11)(q21;q23) *MLL-AFF1*
- t(9;11)(p22;q23) *MLL-AF9*
- t(9;22)(q34;q11) *BCR-ABL*
- t(12;21)(p13;q22) *ETV6-RUNX1*
- t(1;19)(q23;p13.3) *TCF3-PBX1*
- *tIGH@14q32*
- iAMP21 (*RUNX1* multiple copies)
- Numeric: hyperdiploid, hypodiploid

Submicroscopy:
- Deletions: *IKZF1, PAX5, TCF3, CDKN2A, CDKN1B, RB1*
- Críptic Translocations:
  - t(X;14)(p22;q32) ou t(Y;14)(p11;q23) fusion genes
  - *P2RY8-CRFL2*

T-ALL

- Críptic Translocations: t(5;14)(q35;q32) involving *TLX3* and *TAL1* genes
- Translocations with TCR genes
- Fusion genes: *MLL-ENL, CALM-MLLT10, ABL1-NUP214*
- Amplification *MYB* @6q23;
- Mutations: *NOTCH1, FBXW7, PTEN, PHF6, RAS, BCL11B, CDKN2A2B*
### AGE AND LEUKEMIA BIOMARKERS

<table>
<thead>
<tr>
<th>Biomarkers</th>
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<tbody>
<tr>
<td>pro-B ALL, AML</td>
</tr>
<tr>
<td>MLL/AF4, ENL, AF9, AF6, ELL;</td>
</tr>
<tr>
<td>GATA-1 mut</td>
</tr>
<tr>
<td>Hox11L2NUP98</td>
</tr>
<tr>
<td>LLA-CD10+</td>
</tr>
<tr>
<td>ETV6-RUNX1+</td>
</tr>
<tr>
<td>E2A/PBX1, HYPERDIPLOID, iAMP21</td>
</tr>
<tr>
<td>LLA-T, LMA</td>
</tr>
<tr>
<td>SIL/TAL1, NOTCH1, HOX11L2,</td>
</tr>
<tr>
<td>BCR/ABL, AML1/ETO, others</td>
</tr>
</tbody>
</table>
ALL IN EARLY CHILDHOOD

- Leukemia in early childhood, mainly I-ALL is characterized by acquired genetic alterations that contrast to ALL in children ≥10 years-old.
- *MLL-R, ETV6-RUNX1 fusion genes* in concert with few cooperating molecular aberrations might be responsible for the leukemogenesis process.
- Lessons from twins, cord blood screenings and backtracking leukemia fusion genes demonstrated that acquired mutations starts in fetal life.
B-D AND TREATMENT

TREATMENT REMAINING QUESTIONS:
• Anthracyclines in Inductions (LR ?) Dexe or Prednisone?
• What type of reinduction/intensification and maintenance?
• Who should (not) be transplanted?
• How to avoid late side effects !!

OPEN QUESTION:
Is it possible to enreveal the causality and prevent Early Age Leukemia?
• Time-frame latency for clinical onset is very short;
• Foetus is vulnerable to toxic effects of maternal intakes and environmental compounds;
• Exposure to many different substances during pregnancy through maternal circulation have been demonstrated through out placental perfusion studies.
AIMS

- To investigate maternal exposure during pregnancy associated with early childhood leukemia (EAL);
- To evaluate the gene mutations frequencies in (ALL and AML) in order to estimate the joint effect of genotypes on the risk of leukemia;
- To unravel the complex environmental and somatic gene mutations together with genetic susceptibility.
• BcP-ALL
• T-ALL
• AML

Age ≤24m

Epidemiology
• Maternal Questionnaire
• Controls selection
• Bio-samples (duos)

StUDY DESIGN

Interactions
• Somatic Biomarkers
• c- SNPs (duos)
Characterization of Acute leukemia by lineage subtypes and according to Projects

MORPHOLOGY
FCM

MOLECULAR-CYTOGENETICS
FISH-RT-PCR
• ALL and/or
• AML specific fusion genes

GENOMICS
• SNPS
• Mutations, Copy numbers
• Methylation
4 MAJOR PROJECTS*
1. Genomic study of MLL gene in infant leukemia (ALL and AML);
2. Identification of new somatic mutations in BcP-ALL and in T-ALL;
3. Epidemiological risk factor of early childhood leukemia and genetic susceptibility;
4. Molecular aberrations (type I and II) in childhood and adolescent AMLs and environmental risk factors.
Development and perspective of current Brazilian studies on the epidemiology of childhood leukemia

Maria S. Pombo de Oliveira, Sergio Koifman, Gisele M. Vasconcelos, Mariana Emerenciano, Cristiane de Oliveira Novaes, Brazilian Collaborative Study Group of Infant Acute Leukemia

Immunophenotyping-genotyping study of childhood acute leukemia

Genetic polymorphisms associated with childhood leukemia

Epidemiology and molecular studies of infant acute leukemia
I - ANÁLISE MORFOLÓGICA:
Medula óssea hipocelular com predomínio de blastos linfoide heterogeneos em relação ao tamanho, com extracelular densa e citoplasmas escassos. Compatível com LLA.

II - ANÁLISE IMUNOFENOTÍPICA:
Método: Citometria de Fluxo FACSalibur-BD;
Viabilidade da Amostra: %

II.1 - MARCAÇÃO CD45
neu, a pos. intermediário. 85% de blastos B neoplasticos

PERFIL IMUNOFENOTÍPICO:
Marcadores citoplasmáticos: CD22/CD79A, TDT/CD22 positivos; MPO, ISM negativos, associados de linhagem -
Marcadores de Membrana: CD19, CD10, CD9, CD34, CD33 positivos; HLA-DR, CD13, CD20, CD7, CD5, CD15 negativos

(*) CONCLUSÃO DA CARACTERIZAÇÃO IMUNOFENOTÍPICA:
LLA-COMUM (BII) COM ABERRACAO DE FENOTIPO

IV - GENETICA MOLECULAR

<table>
<thead>
<tr>
<th>Fusão gênica</th>
<th>Especificidade</th>
<th>RESULTADOS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR/ABL p190</td>
<td>LLA células precursoras B</td>
<td>Negativo</td>
</tr>
<tr>
<td>E2A/PBX1</td>
<td>LLA pré-B, clgm+</td>
<td>negativo</td>
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</tbody>
</table>
Epidemiological Studies

Acute Leukemia Subtypes
Age: ≤12mo; 13-23mo

MLL

Biomarkers Prognostic Factors

Epidemiology and Environment Risk Factors

Maternal Exposures

Genetic Susceptibility

- MLL-internal BCGLIAL
- del-IKZF1 in the I-ALL survival;
- FLT3, PTPN11, RAS in Bcp-ALL;
- NOTCH1, FBXW7 in T-ALL

Incidence
Survival Rate
LEUCEMIA LINFOIDE -T

- É uma neoplasia agressiva de timócitos (~15,0 % das LLAs);
- Caracterizam por alta leucometria, massa mediastinal, envolvimento do CNS;
- Mau Prognóstico (55-65% EFS);
- Classificação maturativa (EGIL) em 4 estágios de diferenciação;
- Alterações moleculares podem alterar processos regulatórios-chave (capacidade ilimitada de auto-renovação, descontrole da proliferação, bloqueio da diferenciação, e resistência a apoptose);
- Alterações somáticas mais comuns são translocações/rearranjos gênicos e mutações. Destacando-se: **SIL-TAL1, BCL11B/HOX11L2, MLL-ENL, NOTCH1, FBXW7, PTEN e KRAS.**
• ONTOGENIA DE CELULAS T e LLA-T

<table>
<thead>
<tr>
<th></th>
<th>cCD3</th>
<th>CD7</th>
<th>CD2,CD5,CD8,CD4</th>
<th>CD1a</th>
<th>CD3⁺/CD1a⁻</th>
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<tbody>
<tr>
<td>T-I</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T-II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T-III</td>
<td>+</td>
<td>+</td>
<td>+, CD4⁺/CD8⁺ ou</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD4⁺/CD8⁻</td>
<td></td>
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<tr>
<td>T-IV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Immunology Letters 2005*
Background: Molecular alterations occur frequently in T-ALL and the potential impact of those abnormalities on outcome is still controversial. The current study aimed to test whether NOTCH1 mutations and additional molecular abnormalities would impact T-ALL outcome in a series of 138 T-ALL paediatric cases.

Methods: T-ALL subtypes, status of SIL-TAL1 fusion, ectopic expression of TLX3, and mutations in FBXW7, KRAS, PTEN and NOTCH1 were assessed as overall survival (OS) and event-free survival (EFS) prognostic factors. OS and EFS were determined using the Kaplan-Meier method and compared using the log-rank test.
Testes Moleculares

**NOTCH1**

- Extração de DNA
- 6 PCRs → 5 *Nested*
- Purificação dos Produtos de PCR
- Sequenciamento
- **Status NOTCH1**

087/02 TAD c.7005_7006 insT
NOTCH1

Via sinalização crucial e grandemente usada para determinar “destinos celulares” ("cell fates") em diversos tecidos (hematopoietico);

Na linhagem linfóide a função no “cell fate” se aplica na definição do comprometimento do progenitor linfóide precoce entre às linhagens B versus T;

Nas célis T, atua na definição do tipo de receptor AB vs GD, e tipo celular CD4 vs CD8.
RESULTS: **NOTCH1, FBXW7**

- Mutações no HD 70% (42/60), 18,3% (11/60) ocorreram no PEST, e 11,7% (7/60) ambos os domínios.

**Results:** The frequencies of mutations were 43.5% for NOTCH1, while FBXW7, KRAS and PTEN exhibited frequencies of 19.1%, 9.5% and 9.4%, respectively. In 78.3% of cases, the coexistence of NOTCH1 mutations and other molecular alterations was observed. In multivariate analysis no statistical association was revealed between NOTCH1 mutations...
RESULTS

In multivariate analysis no statistical association was revealed between NOTCH1 mutations and any other variable analyzed. The mean length of the follow-up was 68.4 months and the OS was 50.7%. SIL-TAL1 was identified as an adverse prognostic factor. NOTCH1 mutation status was not associated with outcome, while the presence of NOTCH1 complex mutations (indels) were associated with a longer overall survival ($p = 0.031$) than point mutations.

Conclusion: NOTCH1 mutations alone or in combination with FBXW7 did not impact T-ALL prognosis. Nevertheless, complex NOTCH1 mutations appear to have a positive impact on OS and the SIL-TAL1 fusion was validated as a negative prognostic marker in our series of T-ALL.
T-cell lymphoblastic leukemia in early childhood presents *NOTCH1* mutations and *MLL* rearrangements

Marcela Braga Mansur\(^a\), Mariana Emerenciano\(^a\), Alessandra Splendore\(^a\), Lilian Brewer\(^a\), Rocio Hassan\(^b\), Maria S. Pombo-de-Oliveira\(^a, \ast\), Brazilian Collaborative Study Group of Infant Acute Leukemia\(^1\)

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15 T-cell leukaemia were identified in infants \( \leq 12 \) month-old;

- \( TCRg \) and \( TCRd \) rearrangements demonstrated that the clonality \( 6/9 \) casos tested - in immature cells might be initiated during fetal life;
- 10 cases (66.7\%) presented at least one molecular somatic mutation (\( MLL, NOTCH1, SIL-TAL1, HOX11L2, FLT3 \)).

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (months)</th>
<th>Gender</th>
<th>Skin color</th>
<th>WBC ( (\times 10^9/L) )</th>
<th>Outcome</th>
<th>EGIL</th>
<th>TCR ( \gamma )</th>
<th>TCR ( \delta )</th>
<th>MLL</th>
<th>FLT3</th>
<th>SIL/TAL1</th>
<th>HOX11L2</th>
<th>NOTCH1</th>
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<tbody>
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<td>6</td>
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<td>131.6</td>
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<td>Clonal</td>
<td>V81-J61/J62</td>
<td>GL</td>
<td>WT</td>
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<td>Pos</td>
<td>Mut/SNP</td>
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<td>#5</td>
<td>9</td>
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<td>80.1</td>
<td>Dead</td>
<td>T-I</td>
<td>Clonal (biallelic)</td>
<td>V62-J61</td>
<td>GL</td>
<td>WT</td>
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<td>Neg</td>
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<td>Neg</td>
<td>WT</td>
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<td>Neg</td>
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<td>GL</td>
<td>WT</td>
<td>Neg</td>
<td>Neg</td>
<td>SNP</td>
</tr>
</tbody>
</table>

ID: patient identification; F: female; M: male; WBC: white blood cells; GL: germline; R: rearranged; NA: not available; Neg: negative; Pos: positive; WT: wild type; ITD: internal tandem duplication; Mut: mutated; T-I (cCD3+, CD7+), T-II (cCD3+, CD7+, CD2+, CD5+, CD1a−, CD3− CD4+/CD8+), T-III (cCD3+, CD7+, CD2+, CD5+, CD4+ or CD8+, CD1a+), T-IV (cCD3+, CD7+, CD2+, CD5+, CD1a−, CD3+, CD4+/CD8−, or CD4−/CD8+).

* Lost follow-up.
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