ADVANCES IN CHILDHOOD ACUTE LEUKEMIAS : GENERAL OVERVIEW

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Definition of acute leukemias

- Malignant process coming from lymphoid (85 %) or myeloid (15 %) precursor cell
- Combined with dysregulation of following programs :
  - proliferation
  - differentiation
  - senescence
  - apoptosis

  ← *Acquired genetic abnormalities*
- The most frequent childhood cancer (30 %)

  420 new cases per year in France 0 – 15 yrs

  + 50 15 – 19 yrs Peak age 2 – 4 yrs ALL

  No peak age AML

Interactions between the patient and his disease (1)

Patient

1) Predisposing factors

Known
< 10 %
- Down's syndrome
- Immunodeficiencies
- Chromos. instability syndromes

Possible
- Genetic variability in xenobiotic metabolism (drugs, environment)
- DNA repair pathways
- Cell-cycle checkpoint functions

2) Subtle genetic alterations or variations affecting response to specific environmental exposures

3) Role of combination of **parents/child genotypes**

Interactions between the patient and his disease (2)

The leukemic cell + stroma

- Cytology : 3 ALL, 8 AML
- Immuno phenotype

Diagnosis, lineage, prognosis, residual disease, therap.target?

- Conventional and molecular cytogenetics
- Target molecular biology
- Global approaches : transcriptome,CGH array, SNP, proteome

Chromosom. and molecular diagnosis, MRD, leukemogenesis

⊕ Subsequent consequences of genetic abnormalities
Immunological response
Neoangiogenesis

In vitro cell cultures
Animal models

Advances (1)

- Survival rate : 1 % 1960 → 85 % (ALL), 60 % (AML)

- **Why ?**
  - (Inter)-national therapeutic trials, supportive care
  - Better knowledge of prognostic factors to stratify patients
  - Improved understanding of genetic abnormalities involved in leukemogenesis
  - The follow-up of response to CT, evaluation of minimal disease
  - Development of new target drugs aiming **to cure more and better**

Advances (2)

- **Strict organisation of pediatric oncology :**
  everywhere, the same objectives, the same rules, referent centres with appropriate technic equipment

- **More and more sophisticated biology :**
  - To classify, stratify at diagnostic and thereafter
  - To better understand leukemogenesis
  - To contribute to therapeutic innovation
  - To propose epidemiological studies and to combine them with biological criteria ?
  - To prevent the disease ?

Current risk stratification in AL

- Cytology
- Clinical variables : age, WBC
- Immunophenotype
- Detection of cytogenetic or molecular lesions
- Early response to therapy (ALL)

BPC-ALL prognostic factors at diagnosis → risk groups (1)

5 yr DFS

- Age : **< 1yr**, (> 10 yrs) — 30-50 %
- WBC > or < 50 000/mm^3
- Cytology : L1 / L2 : **No**
  L3 (Burkitt) treated differently
- Immunophenotype : BPC-ALL — 85 %
  T-ALL — 75 %

BPC-ALL prognostic factors at diagnosis → risk groups (2)

- Caryotype and molecular genetics — 5 yr DFS

  High risk, N < 10 % : unfavorable genetic criteria

• t (9;22) (q34; q11) BCR – AB	2-3 %	20 > 40 %
• IKZF1 mutation or del, without BCR – ABL		20 %
• t (4;11) (q21;q23) MLL – AF4	2-3 %	30-50 %
• hypodiploidy ≤ 44 chr	1-2 %	40-50 %
• intrachromosomal amplification on chr.21 (AML1)		29 %

BPC-ALL prognostic factors at diagnosis → risk groups (3)

- Non unfavorable genetic criteria N # 50 %

	5 yr DFS
• Hyperdiploidy > 50 chr	85 – 90 %
• t (12;21) TEL – AML1 (RUNX1)	85 – 90 %
• t (1;19) E2A – PBX1	85 – 90 %

Prognostic factors in-ALL

- WBC > 200 000/mm^3
- Immunophenotype (CD_{10}, M_y)
- Cytogenetics ±

	Incidence
t (1;14)(p33,q11)SIL-TAL	10-30 % TAL
t (10 ; 14) (q24 ; q11) or t (7 ; 14) (q35 . q24)	5 % TLX1 / HOX11
t (5;14) (q35 ; q32)	20-25 % TLX3 / HOX11

* TLX3/HOX11 expression → adverse outcome (FRALLE93)
* Negative role of cryptic changes

Minimal residual disease : a major criteria of therapeutic decisional value (ALL)

- Methods : Cytology
  Flow cytometry (10^{-4})
  Molecular biology
  - Ig/TCR rear.
    PCR 10^{-2} – 10^{-5}
  - Fusion transcrits
    RT-PCR 10^{-3} – 10^{-5}
- Blood, bone-marrow
- 2 points
  - d21 / 29
  - d35 / 42
  - Low risk MRD ≤ 10^{-4}
  - High-risk of relapses MRD > 10^{-2}
  - Intermediate MRD > 10^{-4} and < 10^{-2}
- Late monitoring =12 and = 24 mo : under study

Gene expression signatures predictive of response and outcome in high-risk children

Bhogvani D., JCO 2009

- Bone-marrow content d7 in high risk patients
- Apoptosis-facilitated genes : upregulated in rapid responders
- Multiple genes involved in cell adhesion, proliferation, antiapoptosis : upregulated in slow responders
- Analysis of gene expression profiles

  → rapid approach of biologic understanding of why clinical and laboratory variables are associated with outcome

  → to improve treatment

but no links evoked with causes

Childhood AL : a multitude of diseases

Product of alterations to the germline genetic and epigenetic code → clonal disease

- Mainly translocations → fusion transcription factors or activated signaling kinases

- Aneuploïdy, deletions in cell-cycle checkpoint genes and mutated genes (FTL3, RAS, other growth promoting pathways

In utero origin of leukemias

- Short latency of leukemias (infancy, peak age 2-4 yrs, ALL)
- Extreme developmental and cellular kinetic stres of a fœtus
- Concordance of leukemia in twins
- Archived new-born bloods : discovery of preleukemic clones

  - Rearrangts of MLL gene at 11q3 (+ chr.4,9,19) : 80 % of AML1 and 60 % of ALL (infants)
  - Rearrangts of ETV6 at chr 12 + RUNX1 on chr 21 (TEL-AML1) 25 % ALL
  - Rearrangts of RUNX1/ETO at chr 8 in 15 % AML
  - Trisomy 21 : 10-20 % AML and ALL
  - NOTCH1 mutation in TALL

- Initiation : in utero exposure to mutagen agents ?

Secondary oncogenic events : obviously needed → leukemias

- Cord blood screening ≤ 1 % positive for TEL-AML, only 1 % of them → leukemia

- Down's syndrome
  - 1st hit : trisomy 21
  - Acquired mutation of GATA1

  → Transient leukemia at birth in 5-10 % of children
  - Further mutations → M7 leukemia

- TEL-AML transloc (t12 ; 21) + partial del (12p)

- NOTCH1 mutation + SIL-TAL fusion

Role of first and 2^{ary} environmental ± genetic events?

Most remain unknown, but new technologic approaches → hypotheses concerning initiation and development of BPC-ALL (85 %) and T-ALL :

- Transfection to animal models
- In vitro long-term cultures of leukemic cells
- Use of human embryogenic stem-cells
- Sequencing of human genome, analysis of transcriptome, comparative genomics, genome sequencing of tumoral cells

TEL-AML1 leukemia fusion
ETV6 – RUNX1 t (12 ; 21)
The most common chimeric fusion gene of BCP-ALL (1)

- A preleukemic phenotype, predominantly in utero (1 %) of newborns)
- A key promotional event : an aberrant immune response to common infections (Greaves)

↓

Pre-B Cell ALL

TEL-AML1 leukemia fusion (2)

- TEL-AML1 can → a population of self-renewing human cord blood cells $CD34^{+}CD38^{-}CD19^{+}$,

  very early B cell stage (1st hit)

  to sustain a persistent preleukemia state

  → Interference with the TGFβ pathway (murine and human model systems)

TEL-AML1 leukemia fusion (3)

- TEL-AML1 expression → inhibition of response to TGFβ
  → TEL-AML1 cells proliferate slowly, but continuously, until 2nd hit → ALL

    TGFβ signaling contributes to self-renewal and differentiation + regulation of immunologic and inflammatory reactions

  → Dysregulation of TGFβ signaling (loss of sensitivity) by TEL-AML1 protein : blocks the ability of TGFβ to contribute to cell differenciation and suppress proliferation of cells.

= Argument in favor of a dysregulated immune response to infection, 2nd hit : → malignant evolution of the TEL-AML1, preleukemic clone

Pharmacogenetics : influence of polymorphisms of genes involved in several metabolic pathways

- May alter activity of drug metabolizing enzymes → efficacy and toxicity of therapy

- May influence the risk for ALL

→ Genes involved in folate metabolism pathways (DNA synthesis and repair, methylation processus)

→ Genes P450 and glutathion S-transferase enzymes

→ Multidrug resistance gene (MDR1)

→ NQO1 : protects again oxydative stress and toxic metabolites

Present needs

- Better evaluation and understanding of the heterogeneity and complexity of leukemias
  - New molecular technics, animal models, human embryonic cells
  - Role of leukemic stem cells and of stroma
  - Pharmacogenetics (pt, parents)

- New epidemiological approaches

  Taking into account the multitude of these diseases, their multi-step development

  → Large-scale studies including the analysis of geno-environment interactions.

ASN Working Group (1)

- *Between March and December 2008*

Objectives :

- To evaluate, if possible, the real risk of childhood leukemias in the vicinity of nuclear sites

- To better approach the knowledge of causal genetic and environmental factors of childhood leukemias

- To clarify the content of communication given to the population, which needs to receive neutral, transparent and updated information

ASN Working Group (2)

- It has been decided to propose under the auspices of ASN :

  • A pluralist and pluridisciplinary working group of experts (institutional and independent) in : hematology, epidemiology, nuclear industry, chemistry, infectiology, immunology…

  • A national committee in charge of follow-up of the working group and particularly its proposals with the aim to promote the most appropriate epidemiological future studies

ASN Working Group (3)

- Five meetings of the working group between December 2008 and September 2009, usually in the presence of B. GROSCHE
  - Descriptive and analytic epidemiological French studies (genetic and environmental factors)
  - Nuclear sites, and activation of a specific sub-group → listing of sites, types, classification, surrounding population, types and measures of rejet
  - Leukemic stem cells
  - German past and present studies

Childhood AL : a multitude of diseases

- A lot of potential causes → several hypotheses
  - a lot of case-control studies
  - a lot of controversial data

A multitude of epidemiological studies

Future epidemiologic studies have to be designed around the characterization of the childhood acute leukemias, with the aim to better approach the relationships between the causes and the consequences of the development of the (pre)-leukemic cell and notably the role of post-natal factors