

Detection of Minimal Residual Disease B-Lineage Childhood Acute Lymphoblastic Leukemia by FISH and PCR

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

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List of Abbreviations

<i>Abbr.</i>	<i>Full-term</i>
Ab	Antibody
ABL	Abelson murine leukemia viral oncogene homolog 1
AF4	Asymmetric flow field flow fractionation
AFF1	AF4/FMR2 Family member 1
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
BCR	Break point cluster region protein
BDCA4	Blood dendritic cell antigen 4
BM	Bone marrow
CALLA	Common acute lymphoblastic leukemia-associated antigen
CBC	Complete blood count
CD	Cluster of differentiation
CDKN2A/B	Cyclin dependant kinase
CNS	Central nervous system
CR	Complete response
CRLF2	Cytokine receptor-like factor 2
CSF	Cerebrospinal fluid
CSF1R	Colony stimulating factor 1receptor
Cy or c	cytoplasmic
DIC	Disseminated intravascular coagulation
DNA	Deoxy ribose nucleic acid
E2A (TCF3)	Transcription factor 3
EBV	Epstein-Barr virus
EDTA	Ethylene diamine tetra-acetic
EGIL	European Group for the Immunological Characterization of Leukemias

EPOR	Erythropoietin receptor
F	Female
FAB	French-American-British
FCM	Flow cytometry
FISH	Fluorescence in situ hybridization
FITC	Fluorescein isothiocyanate
FLT3	Fms-like tyrosine kinase 3
GAP	GTPase activating protein
G-CSF	Granulocyte colony-stimulating factor
GIT	Gastrointestinal tract
GTPase	Guanosine triphosphatase
GUT	Genitourinary tract
Hb	Hemoglobin
HCL	Hydrochloric acid
HLA-DR	Human leukocyte antigen-DR
HLF	Hepatic leukemia factor
HS	Highly significant
HSCT	Hematopoietic stem cell transplantation
HTLV	Human T-cell leukemia/lymphoma virus
Ig	Immunoglobulin
IHC	Immunohistochemistry
IKZF1	Ikaros family zinc finger protein 1
IPT	Immunophenotype, immunophenotyping
ISS	International Staging System
ITP	Immune (idiopathic) thrombocytopenic
IV	Intravenous
JAK2	Janus kinase 2
kb	Kilo base
KCL	Potassium chloride
kd, kDa	Kilo dalton

LAP	Leukemia associated phenotype
LN	Lymph nodes
M	Male
MALT	Mucosa associated lymphoid tissue
M-bcr	Major break cluster region
m-bcr	Minor break cluster region
MCA	Monoclonal antibody
M-FISH	Multicolor fluorescence in situ hybridization
MLL	Myeloid lymphoid leukemia, mixed lineage leukemia
MPO	Myeloperoxidase
MRD	Minimal residual disease
mRNA	Messenger ribonucleic acid
NaOH	Sodium bicarbonate
NIP	Neuropilin interacting protein-1
NK	Natural killer
No, n	Number
NOS	Not otherwise specified
NS	Non-significant
NSE	Non specific esterase
NTRK3	Neurotrophic receptor tyrosine kinase 3
P53	Cellular tumor antigen, phosphor-protein, tumor suppressor
PAS	Periodic acid Schiff
Pax-5	Paired box protein-5
PB	Peripheral blood
PBS	Phosphate buffered saline
PBX1	Pre B cell leukemia homebox 1
PDGF	Platelet derived growth factor
Ph	Philadelphia
PLT	Platelet

RQ-PCR	Real time quantitative reverse transcriptase polymerase chain reaction
RT-PCR	Reverse transcriptase-polymerase chain reaction
RUNX	Runt related transcription factor 1
S	Significant
SBB	Sudan black B
SCLC	Small cell lung cancer
SD	Standard deviation
SEMA	Semaphorin
Sig	Significance
sIg/smIg	Surface immunoglobulin
SKY	Spectral karyotyping
t	Student's t test
TBX1	T-box transcription factor 1
TCF3	Transcription factor 3
TCR	T-cell receptor
TdT	Terminal deoxynucleotidyl transferase
TLC	Total leucocytic count
TM	Transmembrane
TP53	Tumor protein 53
TSLPR	Thymocyte stromal lymphopoietin receptor
TYK2	Tyrosine kinase 2
USA	United States of America
VDJ	Variable diversity joining regions
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factor
WBCs	White blood cells
WHO	World Health Organization
-ve	Negative
+ve	Positive

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Introduction

The outcome for childhood acute lymphoblastic leukemia (ALL) has dramatically improved over the last 50 years with current cure rates approaching 90% and this is attributable to the introduction and gradual intensification of combination chemotherapy, with contemporary regimens involving the use of 7-8 drugs, along with improvement of prognostic factors (*Irving et al., 2009*).

However, these data suggest that a proportion of children are likely to be over treated with current therapeutic regimens and conversely a proportion may benefit from more intensive therapy. Thus, the challenges now remaining are to further increase cure rates and to achieve this cure with the minimal chemotherapy to avoid unnecessary toxicities. This goal may be achieved by tailoring therapy to each individual patient's risk of relapse (*Hang Cheng et al., 2013*).

Several studies have shown that minimal residual disease (MRD) status during the early stages of therapy provides prognostic information which is independent of more classic prognostic markers such as presenting white blood cell count, age, cytogenetic analyses and immunophenotype. In many ALL protocols, days 8 and 15 of induction therapy are considered the first checkpoints to test the in vivo sensitivity of the leukemia in the individual patient;thus, enabling risk-

directed therapy, i.e. more intensive therapy for MRD positive, high-risk patients and dose reduction for good responders (*Schrappé, 2012*).

The MRD can be assessed by numerous methods. Leukemic cells can be distinguished from normal hematopoietic cells on the basis of chromosomal or molecular abnormalities, antigen receptor gene rearrangements and immunophenotype (*Fiser et al., 2012*).

In ALL, individual chromosomal abnormalities remain strong independent indicators of outcome, especially to indicate risk of relapse (*Moorman et al., 2010*). Among these abnormalities, those with the most significant impact for risk stratification for treatment are t(9;22)(q34;q11)/BCR-ABL1 and t(1;19)(q23;p13.3)/TCF3-PBX1 fusion (*Kager et al., 2007*).

The Philadelphia chromosome t(9;22) was associated with an extremely poor prognosis (especially in those who presented with a high WBC count or had a slow early response to initial therapy), and its presence had been considered an indication for allogeneic hematopoietic stem cell transplantation (HSCT) in patients in first remission (*Arico et al., 2010*).

The t(1;19) translocation had been associated with inferior outcome in the context of antimetabolite-based therapy, but the adverse prognostic significance was largely negated by more aggressive multiagent therapies (**Andersen et al., 2011**). Patients with the t(1;19) translocation had an overall poor outcome comparable to children lacking this translocation, with a higher risk of CNS relapse and a lower rate of bone marrow relapse, suggesting that more intensive CNS therapy may be needed for these patients (**Jeha et al., 2009**).

Molecular characterization of the genetic changes has yielded a wealth of information on the mechanism of leukemogenesis. Those findings have also allowed the development of sensitive techniques such as fluorescence in situ hybridization (FISH) for identification of underlying molecular defects, which can be applied to evaluate disease prognosis, monitor response to treatment and predict minimal residual disease. Studies have demonstrated the excellent diagnostic sensitivity of FISH for detecting translocations. FISH also provides the unique benefit of clarifying complex karyotypes with identification of derivative chromosomes, ring chromosomes, and complex translocations involving more than 2 chromosomes (**Nordkamp et al., 2009**).