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

Thesis

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Doha Elsayed Ahmed Hassnien

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List of Abbreviations

Abbr.	Full-term
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 hydridization
FITC …….. Fluorescein isothiocyanate
FLT3 ·····Fms-like tyrosine kinase 3
GAP·····GTpase activating protien
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 hydridization

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-protien, tumor suppressor

PAS·····Periodic acid Schiff

Pax-5 ·····Paired box protein-5

PB ……...Peripheral blood

PBS·····Phoaphate buffered saline

PBX1 ·····Pre B cell leukemia homebox 1

PDGF……..Platelet derived growth factor

Ph ·····Philadilphia

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 ***(Schrappe, 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-ABL1andt(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 pooroutcome 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).***