

STUDY OF IMMUNOGLOBULIN LIKE TRANSCRIPT 3 (ILT3) EXPRESSION IN ACUTE MYELOID LEUKEMIA

Thesis

**For Partial Fulfillment of Master Degree of Clinical and
Chemical Pathology**

By

Reham Abd Al-Fattah Moussa

M.B., B.Ch

Faculty of Medicine - Cairo University

Under Supervision Of

Prof. Nevine Ahmed Kassim

Professor of Clinical and Chemical Pathology

Faculty of Medicine - Ain Shams University

Prof. Soha Raouf Youssef

Professor of Clinical and Chemical Pathology

Faculty of Medicine - Ain Shams University

Dr. Deena Samir Mohamed Eissa

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine - Ain Shams University

*Faculty of Medicine
Ain Shams University*

2014

List of Contents

<i>Title</i>	<i>Page No.</i>
List of Abbreviations.....	ii
List of Tables	v
List of Figures	viii
Introduction	1
Aim of the work.....	3
<u>Review of Literature</u>	
• Chapter (1): Acute Myeloid Leukemia	4
• Chapter (2): ILT3 (CD85K)	41
Subjects and Methods	54
Results	63
Discussion	98
Summary	107
Recommendations	112
References	113
Arabic Summary	--

List of Abbreviations

AML	Acute myeloid leukemia
APCs	Non-professional antigen presenting cells
APL	Acute promyelocytic leukemia
ATRA	All-trans retinoic acid
B19V	Parvovirus B19
BM	Bone marrow
CBC	Complete blood count
CBF	Core binding factor
CR	Complete remission
CTL	Cytotoxic T-lymphocyte
DCs	Dendritic cells
DIC	Disseminated intravascular coagulopathy
FAB	French-American-British
FCM	Flow cytometry
FcγRIII	Fc portion of IgG
FITC	Fluorescein isothiocyanate
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
GVL	Graft-versus leukemia
HLA	Human leukocytic antigen
HSC	Hematopoietic stem cells
IFN-γ	Interferon-γ
IHC	Immunohistochemistry
ILT3	Immunoglobulin Like Transcript 3

List of Abbreviations (Cont...)

ILTs	Immunoglobulin-like transcripts
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motifs
K	Kappa index
MDCs	Monocytoid dendritic cells
MDS	Myelodysplastic syndrome
MIC	Morphologic-Immunologic Cytogenetic
MoAbs	Monoclonal antibodies
MPO	Myeloperoxidase
MRD	Minimal Residual Disease
NSE	Non-specific esterase
PAS	PeriodicAcidSchiff
PNH	paroxysmal nocturnal hemoglobinuria
r	Pearson's correlation coefficient
RAR	Retinoic acid receptor
RBC	Red blood cells
ROC	Receiver operator characteristics
rs	Spearman's rank correlation coefficient
RT-PCR	Reverse transcriptase-polymerase chain reaction
SBB	Sudan Black B
SPSS	Statistical Program for Social Science
t-AML	AML transformation
TdT)	Terminal deoxynucleotidyltransferase
TLC	The total leucocytic count

List of Abbreviations (Cont...)

TNFs	Tumor necrosis factors
WBCs	White blood cells
WHO	World Health Organization
x²	Chi-square

List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (1):	Morphologic (FAB) classification of AML.	16
Table (2):	MIC classification of AML: showing the association of morphology (FAB) with cyto-genetics and immune-phenotyping.	18
Table (3):	WHO Classification of AML and Related Neoplasms).	19
Table (4):	Shows the cytochemistry for AML.	27
Table (5):	Panel of MoAbs to differentiate AML and ALL	28
Table (6):	Immunophenotypic markers of AML.....	29
Table (7):	Clinical correlates of frequent cytogenetic abnormalities observed in AML.	31
Table (8):	Prognostic factors in acute myeloid leukemia.	35
Table (9):	Comparison between patients and control as regard demographic data.	63
Table. (10):	Distribution of the patients according to different clinical sub-groups.	64
Table (11):	Comparison between patients and control as regard CBC and BM findings.....	66
Table (12):	Cytochemical study of the patients' group by non specific esterase stain.	69
Table (13):	Percent of blast cells expressing CD14 among patients group.	71
Table (14):	Percent of blast cells expressing ILT3 among patients group.	71
Table (15):	Descriptive immunophenotype data of the patients group.	72
Fig. (16):	ILT3 expression in patients and controls on different cellular populations.	73
Fig. (17):	Comparison between patients and control as regards expression of ILT3 on blast cells (percentage, absolute count and Mn Ix).	74

List of Tables (Cont...)

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (18):	Comparison between patients and control as regards expression of ILT3 on monocyte (percentage, absolute count and Mn Ix).....	76
Fig. (19):	Comparison between patients and control as regards expression of ILT3 on granulocytes (percente, abselute count and Mn Ix).....	78
Table (20):	Correlation Study between percent of Blast cells expressing ILT3 and other diagnostic parameters in the patients' groups Using Pearson Correlation Coefficient Test.....	81
Table (21):	Correlation Study between absolute count and mean intensity of Blast cells expressing ILT3 and other diagnostic parameters in the patients' groups Using Pearson Correlation Coefficient Test.....	84
Table (22):	Correlation Study between ILT3 expression on BM monocytes (percent,absolute count and MnIx) to other diagnostic parameters in the patients' groups Using Pearson Correlation Coefficient Test.	87
Table (23):	Correlation Study between percent of Blast cells expressing CD14 to other diagnostic parameters in the patients' groups Using Pearson Correlation Coefficient Test.....	89
Table (24):	Correlation Study between CD14 expression on BM monocytes (percent, absolute count and MnIx) in the patients' groups Using Pearson Correlation Coefficient Test.....	91
Table (25):	Diagnostic Performance of percent of blast cells expressing ILT3 in discrimination between patients and controls.....	92
Table (26):	Diagnostic Performance of absolute countof blast cells expressing ILT3 in discrimination between patients and controls.....	93
Table (27):	Diagnostic Performance of mean intensity ILT3 expression on blast cells in discrimination between patients and controls.....	94

List of Tables (Cont...)

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (28):	Diagnostic performance of percent of ILT3 +ve Blast cells in discriminating monocytic leukemia from non monocytic leukemia.	95
Table (29):	ComparingtheDiagnostic Performance of percent of ILT3 +ve and CD14 +ve in Discrimination between monocytic and non monocytic leukemia.	96
Table (30):	Kappa test between ILT3 expression on blast cells and other diagnostic parameters.....	97

List of Figures

<i>Fig. No.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (1):	Several mutations are necessary for the development of acute myeloid leukemia (AML)	12
Fig. (2):	Morphology of blast cells in different AML subtypes in BM aspirate smears.....	26
Fig. (3):	Treatment options in AML	36
Fig. (4):	ILT/LIR/MIR receptor family.....	42
Fig. (5):	Cellular distribution of ILT/LIR/MIR isotypes. Distinct isotypes are differentially expressed on lymphocytes.....	44
Fig. (6):	Mechanisms underlying CD8 ⁺ Treg suppression.....	46
Fig. (7):	Immunohistochemical staining of macrophages with anti-ILT3 antibodies in a lymph node from a patient with metastatic pancreas carcinoma	52
Fig. (8):	Distribution of the patients according to different clinical sub-groups.....	65
Fig. (9):	Comparison between patients and control as regard blast in BM.....	67
Fig. (10):	Distribution of patients into monocytic leukemia and non monocytic leukemia.	68
Fig. (11):	Results of Non Specific esterase staining among the patients' group.	69
Fig. (12):	Percent of blast cells expressing ILT3 in patients and controls (Boxplot).	75
Fig. (13):	Absolute count of blastcells expressing ILT3 in patients and controls.....	75
Fig. (14):	Mean intensity of ILT3 expression on Blast cells in patients and controls (Boxplot).	75
Fig. (15):	Percent of monocytes expressing ILT3 in patients and controls (Boxplot).	77
Fig. (16):	Absolute count of monocytesexpressing ILT3 in patients and controls.	77

List of Figures

<i>Fig. No.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (17):	Mean intensity of ILT3 expression on monocytes in patients and controls (Boxplot).	77
Fig. (18):	Percent of granulocytes expressing ILT3 in patients and controls (Boxplot).	79
Fig. (19):	Absolute count of granulocytes expressing ILT3 in patients and controls.	79
Fig. (20):	Mean intensity of ILT3 expression on granulocytes in patients and controls. (Boxplot).	79
Fig. (21):	Significant positive correlation between percent of Blast cells expressing ILT3 and monocytes in PB.....	82
Fig. (22):	Significant negative correlation between percent of Blast cells expressing ILT3 and percent of Blast cells expressing CD13.	82
Fig. (23):	Significant negative correlation between percent of Blast cells expressing ILT3 and percent of Blast cells expressing CD117.	82
Fig. (24):	Significant negative correlation between percent of Blast cells expressing ILT3 and percent of Blast cells expressing CD34.	82
Fig. (25):	A scatter diagram showing significant positive correlation between Blast cells expressing ILT3 and Blast cells expressing CD14 among patients.....	82
Fig. (26):	A scatter diagram showing significant positive correlation between absolute count of Blast cells expressing ILT3 and monocytes in PB.	84
Fig. (27):	A scatter diagram showing significant positive correlation between MnIx of ILT3 expression on blast cells and percent of monocyte in peripheral blood in patients group.	84
Fig. (28):	A scatter diagram showing significant negative correlation between MnIx of ILT3 expression on blast cells and percent of cells expressing CD13 in patients group.	85
Fig. (29):	A scatter diagram showing significant positive correlation between Mn In of ILT3 expression on Blast cells and percent of blast cells expressing CD14 among patient groups.	85

List of Figures

<i>Fig. No.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (30):	A scatter diagram showing significant negative correlation between MnIx of ILT3 expression on blast cells and percent of cells expressing CD117 in patients group.	85
Fig. (31):	A scatter diagram showing significant negative correlation between MnIx of ILT3 expression on blast cells and percent of cells expressing CD34 in patients group.	85
Fig. (32):	A scatter diagram showing significant positive correlation between Blast cells expressing CD14 and percent of monocytes in peripheral blood among patients,	89
Fig. (33):	A scatter diagram showing significant negative correlation between Blast cells expressing CD14 and percent of cells expressing CD117 in peripheral blood among patients.	89
Fig. (34):	A scatter diagram showing significant positive correlation between absolute count of CD14 expressing on BM monocytes and percent of monocytes in PB among patients.	91
Fig. (35):	Correlation Study between mean intensity of CD14 expressing on BM monocytes and percent of monocytes in peripheral blood of AML patient.	91
Photo (1):	Staining of AML cases with non specific esterase.	70

INTRODUCTION

Acute myeloid leukemia (AML) is one of the most common types of leukemias in adults (*Arber et al., 2008; Foran, 2010*). Despite major advances in our understanding of the biology of AML, the five- year survival of AML patients is only 20-40%. It has been proposed that AML originates from self renewing hematopoietic stem cells (HSC)/progenitors that have acquired multiple genetic and/or epigenetic changes (*Becher, 2011*). The heterogeneity of AML is evident from the wide variety of clinical manifestations, phenotypic features and response to therapy (*Estey and Dohner, 2006; Schlenk et al., 2008; Marcucci et al., 2011*).

In clinical practice, the accurate diagnosis of AML subtypes is essential for risk stratification and treatment planning. The World Health Organization (WHO) and the French-American-British (FAB) classification systems are most commonly used to subtype AML (*Arber et al., 2008*).

AML with monocytic differentiation includes FAB M4, M5a and M5b subtypes and shows distinct clinical features (*Tallman, 2004; Dohner et al., 2008*). Although the expression of some markers by leukemic cells is helpful for lineage assignment. The diagnosis of AML with monocytic differentiation remains challenging (*Klco et al., 2011*).

Flow cytometry (FCM), is a powerful tool for the investigation of normal and neoplastic cells, and their classification at every level. Therefore, it has great prognostic and therapeutic implications. Its ability to measure multiple parameters (chemical or physical characteristics) on individual cells, in a suspension at high speed, is ideal for the study of leukemic cells. It identifies cell markers by applying monoclonal antibodies against them (*Pervez et al., 2005*).

The inhibitory receptor Immunoglobulin Like Transcript 3 (ILT3) is a member of immunoglobulin –like transcript (ILT, LIR, or LILR) family and is expressed by dendritic cells, monocytes, endothelial cells and osteoclasts, but not by lymphocytes(*Cella et al., 1997; Kim et al., 2006*). Dendritic cells expressing high levels of inhibitory receptors ILT3 and ILT4 were shown to induce anergy of CD4⁺ T helper cells and differentiation of CD8⁺ T suppressor cells. (*Anderson and Allen, 2009; Chang et al., 2009*).

Recently ILT3 expression was reported to be a reliable marker that could distinguish AML with monocytic differentiation from other types of AML (*Dobrowolska et al., 2013*).

AIM OF THE WORK

This study aims to study the pattern of ILT3 expression by leukemic myeloid precursors in AML cases with respect to its different subtypes & clinical relevance.

Chapter 1

ACUTE MYELOID LEUKEMIA

Definition

AML is a malignancy originating from a multipotential hematopoietic cell characterized by clonal proliferation of abnormal blast cells in the marrow and impaired production of normal blood cells resulting in anemia, thrombocytopenia and low, normal, or high white cell counts depending on the number of leukemic cells in the blood. It occurs in nine morphologic variants, each with characteristic cytologic, genetic, and sometimes clinical features (*Lichtman et al., 2011*).

Epidemiology

AMLs are infrequent, yet highly malignant neoplasms responsible for a large number of cancer-related deaths (*Deschler and Lübbert, 2006*).

1. Incidence

It accounts for about 80% of all leukemias in adults and 15%-20% in children (*Lichtman et al., 2011*).

2. Age

Acute myeloid leukemia is generally a disease of older people and is uncommon before the age of 45. The average