



# Gene expression of Forkhead box Protein 3 (FOXP3) and Wilm's Tumor 1 (WT1) in Acute Myeloid Leukemia Patients

Thesis

Submitted for the requirements of The Master Degree of Science (M.Sc.) in partial fulfillment In Biochemistry

Presented by
Radwa Alsayed Ali Mostfa
(B.Sc. Biochemistry & Nutrition, 2003)
Girl's College
Under Supervision of

Prof. Dr.

**Ahmed Osman Egiza** 

Professor of Biochemistry Faculty of Science, Biochemistry Department Ain Shams University Prof. Dr.

Magda Mahmoud Assem

professor of clinical pathology National Cancer Institute, Clinical Pathology Department Cairo University

#### Dr. Amal Fawzy Saied

Assistant professor of clinical pathology National Cancer Institute, Clinical Pathology Department Cairo University

## المالية المنظمة المنظم

# قَالُواْ سُبْحَنَكَ لَاعِلْمَ لَنَا إِلَّا مَاعَلَمْتَنَا ۚ إِنَّكَ أَنتَ ٱلْعَلِيمُ الْحَكِيمُ اللَّهُ الْعَلِيمُ الْحَكِيمُ اللَّهِ الْعَلِيمُ اللَّهُ الْعَلِيمُ اللَّهُ الْعَلِيمُ اللَّهُ اللَّهُ الْعَلِيمُ اللَّهُ الللَّهُ اللَّهُ اللَّا اللَّهُ اللّ

صَّنَاكَ وَ السَّنَ الْعُظَمِينَ، سورة البقرة- آية: ٣٢

### Acknowledgments

First, foremost, and all thanks to Allah by whose grace this work had been completed and by whose grace all my life is arranged in the best. Nobody can imagine this way that had been drawn by the mercifulness of the God.

After thanking Allah, I would like to express my deepest respect and sincere gratitude to *Prof. Dr.* Ahmed Osman Egiza, professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for his keen supervision, help, skillful cooperation and sincere guidance during the work.

I am greatly indebted and would like to express my sincere appreciation toward *Prof. Dr.* Magda Mahmoud Assem, Professor of Clinical Pathology, Clinical Pathology Department, National Cancer Institute, Cairo University For her generous and precious supervision. She always advises me in all things. *Dr.* Amal Fawzy Saied, Assistant Professor of Clinical Pathology, Clinical Pathology Department, National Cancer Institute, Cairo University for her generous supervision.

Also, I cannot forget to thank Haematology Unit of Clinical Pathology Department National Cancer Institute Cairo University teamwork and my dear friend **Nehal Hussien** lab chemist for supporting me during practical work, Cancer Biology Department, National Cancer Institute, Cairo University.

I will not forget to thank all my real friends for their encouragement to exceed all barriers in my way and during the hard times.

**Thanks** 

Radwa Alsayed Ali

## Dedication

T dedicate this work with all my love

To my Mother and to the spirit of my Sather

To my sisters Thoria, Hermeen, and Rasha

To my brothers Ahmos, Tahseen, and Hazem

And to all people 🔊 love

## Declaration

This thesis has not been submitted for a degree at this or any other university.

Radwa Alsayed Ali Mostfa

## Biography

Name : Radwa Alsayed Ali Mostfa

Date of Graduation: May 2003, Faculty of Girl's college,

Biochemistry and Nutrition Department,

Ain Shams University.

Degree of Awarded : B.Sc. in Biochemistry and Nutrition,

Very Good.

#### **Table of Contents**

Table of Contents		
List of tables		
List of figures		
List of abbreviations	X	
Abstract	ΧV	
Introduction	1	
Aim of work	4	
Review of literature	5	
Acute Myeloid Leukemia (AML)	5	
<ul> <li>Pathophysiology</li> </ul>	6	
<ul> <li>Epidemiology</li> </ul>	7	
General incidence	8	
<ul><li>○ AML in Egypt</li></ul>	8	
Age incidence	9	
Sex incidence	9	
○ Geographical variations	9	
o Etiology	10	
<ul> <li>Routinely Diagnostic workup</li> </ul>	13	
<ul> <li>Morphology</li> </ul>	13	
❖ Peripheral blood	13	
❖ Bone marrow examination	14	
<ul> <li>Cytochemistry</li> </ul>	15	
Chemistry profile	17	
<ul> <li>Immunophenotyping</li> </ul>	18	
Cytogenetics and molecular genetics	20	
o Classification	22	
o Prognosis	26	
Fetal Liver Tyrosin Kinase 3 (FLT3) gene	29	
❖ Structure and expression of FLT3	29	
Forkhead box protein 3 (FOXP3) gene	31	
<ul> <li>FOXP3 gene Structure and Function</li> </ul>	31	

#### **Table of contents**

0	Mutation of FOXP3 gene	33
0	Expression of FOXP3	34
0	FOXP3 and Protein Interactions	37
0	The role of FOXP3 in the Development of Treg	38
	Cells	
0	Tregs in the immunosuppressive microinvironment of AML	40
0	The role of FOXP3 in nonhematopoietic Cells	43
0	FoxP3 involvement in Cancer	43
- W	ILMS' TUMOR GENE 1 ( <i>WT1</i> )	47
0	WT1 gene expression and Structure	48
0	WT1 gene and apoptosis	51
0	WT1 gene and hematopoiesis	52
0	Functions of WT1	54
0	WT1 as a transcription factor	55
0	WT1 and its Interacting Partners	57
0	WT1 as a tumor suppressor gene	58
0	WT1 gene expression in human cancers	58
0	Expression of WT1 gene in leukemia	61
0	WT1 gene Expression levels and clinical	63
0	prognosis of leukemia. WT1 as a target for immunotherapy	65
	Will as a target for immunotherapy	00
Subjects	and Methods	67
0	Subjects	67
0	Treatment Protocols	68
0	Endpoints	70
0	Samples	70
0	Scheme of the practical plan	71
0	Methods	71
0	RNA extraction	72
0	Determination of concentration, yield and purity	77
	of RNA	

#### **Table of contents**

o cDNA synthesis	78		
<ul> <li>Determination of FOXP3 and WT1 Gene</li> </ul>	81		
Expression by Quantitative RT-PCR			
Statistical Analysis	86		
Results	87		
Results	01		
Discussion			
Summary and conclusion			
References			
Arabic summary			