ONCOGENES AND FLOW CYTOMETRIC STUDIES IN BREAST CANCER

Thesis

Submitted For Partial Fulfilment Of
Ph.D Degree In Biochemistry

By

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M.Sc. Biochemistry

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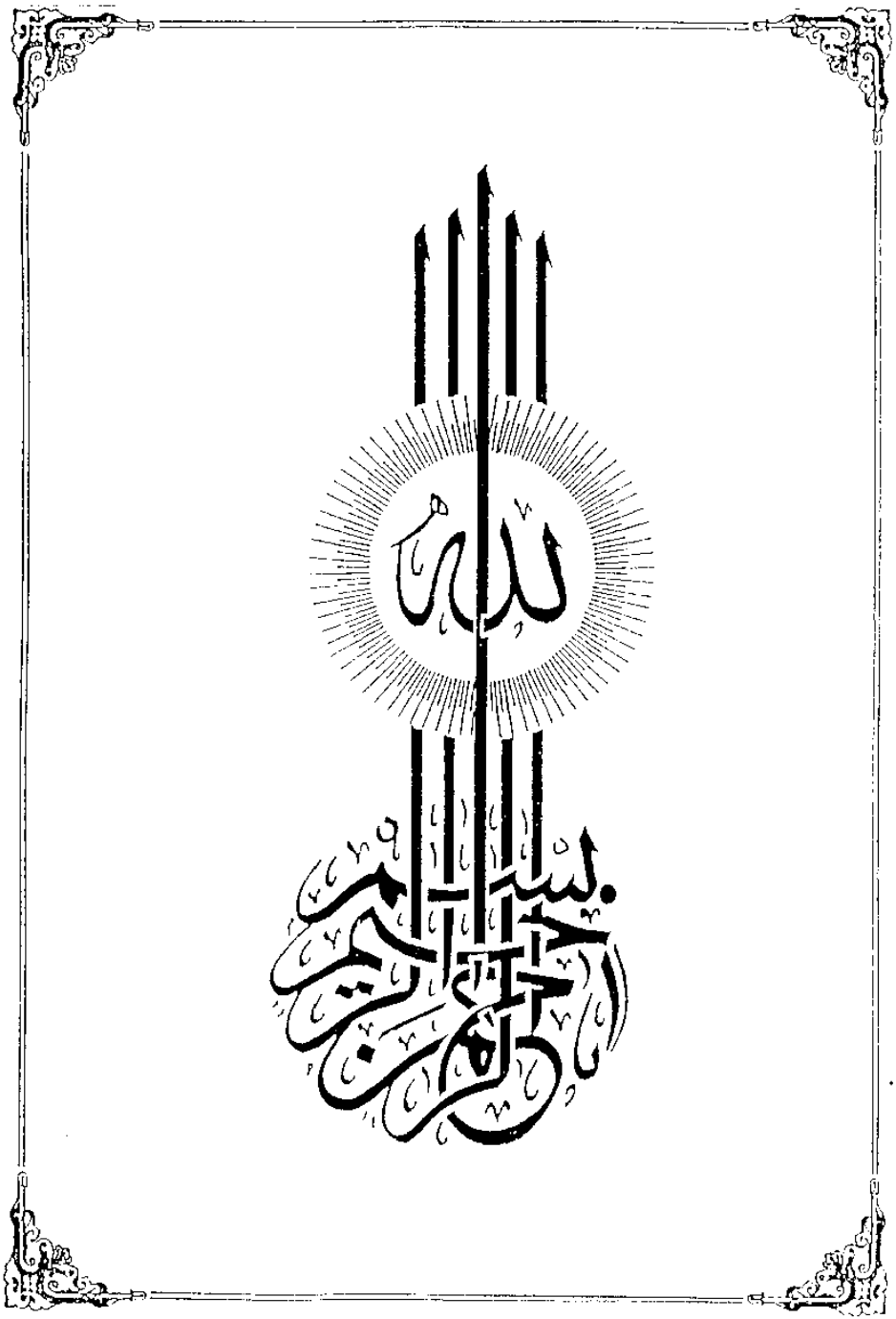

Acknowledgement

*"First of all thanks **GOD** who supplies me power to overcome problems".*

*I would like to express my gratitude and esteem to **Prof. Dr. Ali Khalifa Ali;** Professor of Biochemistry and Head of Oncology Diagnostic Unit, faculty of medicine, Ain Shams University and **Prof Dr, Robert C. Bast, Jr.**; Head, Division of Medicine, M.D Anderson cancer center not only for guiding the choice of the subject and laid out the work plan but also they continually gave their productive advice and sincere encouragement. Thanks to their effort, this thesis came to a conclusion.*

*I am honored to have **Dr Jeffery R. Marks**, Assistant Professor. Department of Surgery Duke University Medical Center, and I am indebted to him for his valuable advice and continuous guidance without which this thesis would have not been delivered in this form.*

DUKE COMPREHENSIVE CANCER CENTER

f the Director

March 24, 1994

RE: AZZA ABOU-GHALIA

To Whom It May Concern:

Dr. Azza Abou-Ghalia has performed post-doctoral studies in my laboratory between September 1992 and March of 1994. During this time she developed and compared different methods for detecting breast cancer micrometastases in bone marrow. A two-color immunofluorescent technique was developed that permitted detection of breast cancer cells by flow cytometry with greater precision than could be achieved with single color immunofluorescence. She had explored the possibility of using ligase chain reactions to detect cells with mutated p53 in bone marrow. She also had undertaken studies to compare mutations of p53 in primary breast cancers with mutations in p53 of cells sorted from marrow using monoclonal antibodies. In these studies she demonstrated the ability to learn new techniques and to develop her own experiments. Dr. Abou-Ghalia has shown great persistence in carrying out her studies and in writing a thesis. She clearly grew in her knowledge of science during the year and a half that she had spent with our group. I can recommend her to you with great enthusiasm as a physician/scientist with a sincere interest in translational research.

Sincerely,

Robert C. Bast Jr., M.D.
R. Wayne Rundles Professor of Medicine
Director, Duke Comprehensive Cancer Center

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24 March 1994

To whom it may concern,

Dr. Azza Abou Ghalia has worked in my laboratory during the past 15 months, from the beginning of 1993 through March 1994. She has been under my direct supervision during this entire period and I am therefore in a good position to judge her abilities, knowledge, determination, and suitability as a scientist. Dr. Ghalia began her work on a new molecular technique to detect low levels of mutant genes amidst many copies of a wild-type gene. The purpose of these studies was to confirm and quantitate the presence of malignant cells in the bone marrow of breast and ovarian cancer patients undergoing autologous bone marrow transplantation by detecting p53 mutations. The technique employed was the ligase chain reaction (LCR), a recently developed and highly experimental method similar in concept to the polymerase chain reaction. Our laboratory had no previous experience with this technique before Azza began. She was able to develop LCR to a point where it functioned reproducibly. The assay had many inherent technical problems and through her diligence, Azza was able to overcome all of them. The final outcome of these experiments, however, was the realization that the sensitivity and specificity of the LCR was insufficient for detecting rare malignant cells. This conclusion was not made lightly. Azza attempted many permutations of the original protocol in order to overcome these barriers. Only after this arduous process did we unequivocally conclude that the technique, as currently configured, was functional but not for the original purpose. I feel confident that the LCR can now be used in my laboratory for other purposes, only as a result of Azza's efforts.

Azza did other work that was related to developing the LCR assay and in all instances, carried it out with patience, care, and diligence. We had many long discussions over experiments and experimental details. She was able to pick up protocols and techniques very quickly and understood the nuances involved. Azza was always a pleasure to work with and interacted well with everyone in the laboratory. She was an asset in my laboratory and will certainly be a greater asset in the future with her added experience gained at Duke University. She has the makings of a skilled and dedicated medical scientist and brings a rare dedication to her work. I wish Dr. Ghalia well in all her future endeavors.

Sincerely,

Jeffrey R. Marks, Ph.D.

THIS WORK IS DEDICATED TO MY SON

LIST OF FIGURES

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LIST OF ABBREVIATIONS.

ATP	: Adenosine triphosphate.
DNA	: Deoxy ribonucleic acid.
dNTP	: Deoxy nucleoside triphosphate.
FACS	: Fluorescent activated cell sorter.
FBS	: Foetal bovine serum.
FCM	: Flow cytometry.
FCS	: Flow cytometric cell sorting.
FITC	: Flourescine isothiothyanate.
HMEC	: Human mammary epithelial cell line.
LCR	: Ligase chain reaction.
MOAB	: Monoclonal antibody.
MERS	: Oligomers.
PCR	: Polymerase chain reaction.
PBS	: Phosphate buffer saline.
RNA	: Ribonucleic acid.
S phase	: Synthetic phase.
TAQ polymerase	: Thermus aquaticus polymerase.
TEA	: Tris edeta acetate.
TLC	: Thin layer chromatography.
Tm	: Melting temperature.

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