UNIVERSITI TEKNOLOGI MARA

CLONING OF THE HUMAN *THIOPURINE METHYLTRANSFERASE* (*TPMT*) GENE FRAGMENT FOR GENERATION OF COPY NUMBERS IN ABSOLUTE REAL TIME PCR.

Ahmad Firdaus Bin Mohd Azhar

2005495548

Dissertation submitted in partial fulfillment of

the requirements for Bachelor of Pharmacy (Hons.)

Faculty of Pharmacy

2008

ACKNOWLEDGEMENT

First and foremost, I would like to thank God for this opportunity given to me doing the great lab work and finished it within the timeframe required. I would like to thank especially Prof. Zaki, Dr.Teh and Dr. Rosmadi that help me a lot in understanding the concept about this lab work, helping me by guiding and supervising the work that were done. I wish to show my appreciation to both of my parents for their support. I would also thank all of the students in the lab, especially the Post Graduate students, by helping and showing me the proper way to handle and operate the machines and providing me the materials to refer to, and to my entire classmate in the lab on helping me finishing this lab work. Lastly, I would like to thank all my friends for their supports.

Thank you very much.

TABLE OF CONTENTS

ABSTRACT

Thiopurine Methyltransferase (*TPMT*) is an enzyme involved in the metabolism of thiopurine drugs. The enzyme has been found to be polymorphic resulting in wide pharmacokinetic and pharmacodynamic differences among patients. Outcome of therapy has been correlated with the expression of protein in different patients. A quantitative PCR method which measures the expression of gene is thus useful in clinical setting. Patients prescribed thiopurine should be monitored with respect to the protein expression for appropriate dosing of drugs. The aim of the study is to clone the *TPMT* gene for use in quantitative PCR process. The clone would be used for the generation of calibration curve to allow an absolute quantitative PCR method to be developed. Specific amplification of the gene of interest was performed by specific primer designed. Subsequently, the DNA encoding for the correct gene was transformed into *E-coli*. Specific primer was designed and the gene of interest was successfully amplified. The bacterial are successfully grown in the agar plate but the screening process does not show positive result in the colony selected.

CHAPTER 1

INTRODUCTION

1.1 *TPMT*

Thiopurine S-methyltransferase (*TPMT*) is an enzyme that catalyzes *S*-methylation of thiopurines drugs including 6-mercaptopurine, 6-thioguanine and azathioprine which are the drugs that are used in the treatment of acute lymphoblastic leukemia, rheumatoid arthritis and autoimmune hepatitis, as well as in organ transplantation. In order to exert their cytotoxic effects, thiopurines must be metabolised to 6-thioguanine nucleotides and TPMT enzyme lead to the formation of inactive metabolites. Outcome of therapy has been correlated with the expression of protein in different patients. The enzyme has been found to be polymorphic resulting in wide pharmacokinetic and pharmacokinetic differences among patients. Interindividual differences on the enzyme expression are high and it is estimated that 88.6% had high enzyme activity, 11.1% had intermediate activity, and 0.3% had low or undetectable activity. A quantitative PCR method which measures the expression of gene is thus useful in clinical setting. Patients prescribed thiopurine should be monitored with respect to the protein expression for appropriate dosing of drugs. Patient that have low or undetectable activity of *TPMT* are prone to the toxic effect even in the normal dose thiopurine drugs.