UNIVERSITI TEKNOLOGI MARA

# DEVELOPMENT OF A PCR METHOD FOR DETECTION OF *UGT1A1* POLYMORPHISM

KHAIRUL MUHAIMIN BIN OTHMAN

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# ABSTRACT

Irinotecan is a camptothecin analog with potent antitumor activity resulting from the inhibition of topoisomerase. Dose-limiting toxicity of irinotecan includes severe leukopenia, neutropenia and diarrhea. Genetic polymorphisms of UDP-glucuronosyltransferase (UGT) 1A1, a key metabolizing enzyme of irinotecan, are important determinants of individual variations in susceptibility to toxicity. Pharmacogenomic studies of irinotecan toxicity have therefore focused on genetic polymorphisms of the *UGT1A1* gene. This project aimed to develop a simple PCR test to identify genetic variants of *UGT1A1* and mainly focus on *UGT1A1*6* and *UGT1A1*27*, single nucleotide polymorphisms in exon 1 of the *UGT1A1* gene that are found mainly in Asian. The allele specific PCR amplification was chosen to be studied because it is suitable for the detection of single nucleotide polymorphism within the gene. In this study, method was optimized only for detection of **27* allele but not for **6* allele. Problem with the primer designed for amplification of *6 allele was thought to be the possible reason for not able to optimize the method for *6 allele. Pipetting error also could be one of the reason for unsuccessful amplification process. Further study should be carried out to continue this beneficial research. This PCR method can also be commercialized as a tool for individualization of pharmacotherapy especially anti-cancer therapy once the method is fully optimized and validated as it is cost and time saving in patient management due to reduced effort spent in trial and error procedure.

# CHAPTER 1

# INTRODUCTION

Irinotecan is a camptothecin analog with potent antitumor activity resulting from the inhibition of topoisomerase. This anticancer drug is now used widely to treat colorectal, lung and other types of cancers. Dose-limiting toxicity of irinotecan includes severe leukopenia, neutropenia and diarrhea. Genetic polymorphisms of uridine-diphosphate (UDP) glucuronosyltransferase (UGT) 1A1, a key metabolizing enzyme of irinotecan, are important determinants of individual variations in susceptibility to toxicity. Irinotecan is a prodrug that is metabolized by carboxylesterase to its principal active metabolite, SN-38. SN-38 is subsequently conjugated mainly by UGT1A1 to a more polar, inactive glucuronide (SN-38G). Severe toxicity is attributed, at least in part, to increased exposure to SN-38 caused by decreased UGT1A1 activity due to genetic polymorphisms (Ando, 1998).

Pharmacogenomic studies of irinotecan toxicity have therefore focused on genetic polymorphisms of the *UGT1A1* gene, especially *UGT1A1*28*, a variant sequence in the promoter region. But this research is mainly focus on *UGT1A1*6* and *UGT1A1*27*, single nucleotide polymorphisms in exon 1 of the *UGT1A1* gene. These variants are found mainly in Asians (Araki, 2006). This research aimed to develop a simple PCR test to identify genetic variants of *UGT1A1*. The allele specific PCR amplification was