MOLECULAR SCREENING FOR SOME COMMON *BCR/ABL* GENE MUTATIONS IN CHRONIC MYELOID LEUKEMIA PATIENTS RESISTANT TO IMATINIB MESYLATE

A thesis submitted for Ph.D. degree in Science in Biochemistry

Presented by

Mohamed Ahmed Mohamed Ali

(M.Sc. in Biochemistry, 2006)

Under supervision of

Prof. Dr. Fahmy T. Ali
Prof. of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr. Wafaa H. El-Metenawy
Prof. of Hematology-Cancer Biology
Faculty of Medicine
Cairo University

Dr. Mohamed R. Mohamed
Ass. Prof. of Biochemistry
Faculty of Science
Ain Shams University

Dr. Yasser I.H. El-Nahass
Ass. Prof. of clinical Pathology
National Cancer Institute
Cairo University

Dr. Mahmoud M. Said
Lecturer of Biochemistry
Faculty of Science
Ain Shams University

Faculty of Science
Ain Shams University
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مسح جزيئي لبعض الطفرات الشائعة في جين *BCR/ABL* في مرضى سرطان الدم النخاعى المزمن المقاومين لعقار ميسيلات الإماتينيب

رسالة للحصول على درجة دكتوراه الفلسفة في العلوم في الكيمياء الحيوية

مقدمة من

محمد أحمد محمد علي

(ماجستير في الكيمياء الحيوية ٢٠٠٦)

تحت إشراف

أ.د. فهمي توفيق على
أستاذ الكيمياء الحيوية
كلية العلوم- جامعة عين شمس

أ.د. وفاء حسن المتناوى
أستاذ أمراض الدم وبيولوجيا الأورام
كلية الطب- جامعة القاهرة

د. محمد رجاء محمد
أستاذ مساعد الكيمياء الحيوية
كلية العلوم- جامعة عين شمس

د. ياسر إبراهيم حسن النحاس
أستاذ مساعد الباثولوجيا الإكلينيكية
معهد الأورام القومي- جامعة القاهرة

د. محمود محمد سعيد
مدرس الكيمياء الحيوية
كلية العلوم- جامعة عين شمس

كلية العلوم
جامعة عين شمس
٢٠١٠

Abstract

The current study investigated the mechanism of resistance to imatinib in chronic myeloid leukemia (CML) patients through screening for point mutations in the *BCR-ABL* kinase domain.

Examination of serial measurements of Abelson-breakpoint cluster region (*BCR-ABL*) mRNA in 100 CML patients treated with imatinib using real time quantitative - polymerase chain reaction (RQ-PCR) revealed that 19 patients achieved a complete molecular response (CMR), 53 patients achieved a major molecular response (MMR) and 12 patients achieved a suboptimal response to imatinib, whereas 16 patients showed resistance to imatinib.

The frequency of mutations in patients with increasing *BCR-ABL* transcript levels (n=16), and those with stable or decreasing levels (n=32) was determined using allele specific oligonucleotide - polymerase chain reaction (ASO-PCR). Fourteen out of the sixteen patients (87.5%) with > 2-fold rise in the *BCR-ABL* transcript levels had detectable mutations, whereas none of the 32 patients with stable or decreasing *BCR-ABL* transcript levels had any detectable mutation ($P < 0.001$).

The presence of a mutation was significantly associated with a greater likelihood of subsequent progression to accelerated phase / blast crisis ($P < 0.001$) and shorter survival ($P < 0.001$). Patients harboring P-loop mutations showed poor overall survival ($P = 0.012$) and progression free survival ($P = 0.02$) compared with patients harboring non-P-loop mutations. Patients carrying T315I mutation seemed to have a particularly poor outcome in terms of

survival (P = 0.014), but not in terms of time to progression (P = 0.450) compared with patients harboring non-P-loop mutations.

These data suggest that a rise in *BCR-ABL* transcript levels of > 2-fold can be used as a primary indicator to test patients for *BCR-ABL* kinase domain mutations and that ASO-PCR is a valuable tool allowing a timely detection of mutations. Moreover, early detection of *BCR-ABL* mutations may play a role in identifying patients who are likely to become resistant to imatinib therapy, for whom alternative therapeutic options should be considered.

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Mohamed Ahmed Mohamed Ali

This thesis has not been submitted
to this or any other university

Mohamed Ahmed Mohamed Ali

Aim of the work

Point mutations within the Abelson-breakpoint cluster region (*BCR-ABL*) kinase domain of the *BCR-ABL* gene in imatinib-treated chronic myeloid leukemia (CML) patients are the most commonly identified mechanism of resistance to imatinib. As alternative therapies are available for imatinib-resistant CML patients, early detection of mutations may provide clinical benefit by allowing early intervention. Because the emergence of a detectable mutant clone leads to a rise in the *BCR-ABL* transcript levels measured by real time quantitative - polymerase chain reaction (RQ-PCR), this rise can therefore be used as a sensitive trigger to screen for mutations.

The present study was carried out to shed further light on the frequency, distribution, and prognostic significance of *BCR-ABL* mutations in imatinib-resistant CML patients.

Contents

Abbreviations

A	:	Alanine (Ala).
AB	:	Actin binding domain.
ABC	:	ATP binding cassette.
ABC	:	Acute blast crisis.
ABL	:	Abelson.
AGP	:	α1- acid glycoprotein.
AKT	:	Murine thymoma serine/threonine kinase oncogene homolog.
ALL	:	Acute lymphoblastic leukemia.
Allo-HSCT	:	Allogeneic - hematopoietic stem cell transplantation.
A-loop	:	Activation loop.
ALT	:	Alanine aminotransferase.
AML	:	Acute myeloid leukemia.
AP	:	Accelerated phase.
Ara-C	:	Arabinoside cytosine (cytarabine).
ASO-PCR	:	Allele specific oligonucleotide -polymerase chain reaction.
AST	:	Aspartate aminotransferase.
ATP	:	Adenosine 5'-triphosphate.
BAD	:	BCL2-associated antagonist of cell death.
BC	:	Blast crisis.
BCL2	:	B-Cell leukemia/Lymphoma 2.
BCL-X	:	A member of the BCL2 family of proteins.
BCR	:	Breakpoint cluster region serine/threonine kinase.
BCR-ABL	:	Breakpoint cluster region-Abelson.
Bp	:	Base pair.
BT	:	Blastic transformation.
C	:	Cysteine (Cys).
CBL	:	Casitas B-lineage lymphoma.
CcyR	:	Complete cytogenetic response.

CD34	:	Cluster of differentiation 34.
CDKs	:	Cyclin-dependent kinases.
CG	:	Control gene.
CHR	:	Complete hematologic response.
C-loop	:	Catalytic loop.
CML	:	Chronic myeloid leukemia.
CMR	:	Complete molecular response.
CP	:	Chronic phase.
CP-CML	:	Chronic phase - chronic myeloid leukemia.
CR	:	Common reverse.
CRK	:	CT 10 sarcoma oncogene homolog.
CRKL		CT 10 sarcoma oncogene homolog-like protein.
Ct	:	Cycle threshold.
D	:	Aspartic acid (Asp).
DB	:	DNA binding domain.
DEAE	:	Diethylaminoethyl.
DEPC	:	Diethyl pyrocarbonate.
D-HPLC	:	Denaturing - high performance liquid chromatography.
dNTP	:	Deoxynucleotide triphosphate.
DSBs	:	Double strand breaks.
DTT	:	Dithiothreitol.
E	:	Glutamic acid (Glu).
E2F	:	E2F transcription factor.
EAC	:	Europe against cancer.
EDTA	:	Ethylenediaminetetraacetic acid.
ERK	:	Extracellular signal-regulated serine/ threonine kinase.
F	:	Phenylalanine (Phe).
FAM	:	6-carboxyfluorescein.
FDA	:	Food and drug administration.
FG	:	Fusion gene.
FISH	:	Fluorescence in situ hybridization.