Study of Additional Chromosomal Abnormalities in Young Adult Egyptian Chronic Myeloid Leukemia Patients

Thesis

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Presented by

Asmaa Mohammed Elsayed Eissa

(M.B., B.Ch. M.Sc.)

Under Supervision of

Prof. Dr. Mohamed Osman Azzazi

Professor of Internal Medicine and Hematology
Faculty of Medicine – Ain Shams University

Dr. Mohamed Mahmoud Moussa

Professor of Internal Medicine and Hematology
Faculty of Medicine – Ain Shams University

Dr. Mohamed Tarif Mohamed Hamza

Assistant Professor of Clinical Pathology
Faculty of Medicine – Ain Shams University

Dr. Amro Mohamed Sedky El-Ghammaz

Assistant Professor of Internal Medicine and Hematology
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
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Asmaa Mohammed Elsayed Eissa

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List of Abbreviations

ABC····· ATP Binding Cassette
ABL····· Abelson protonocogene
ACAs ····· Additional chromosomal anomalies
aCML····· Atypical CML
AGP1 ····· Acid glycoprotein-1
ALL····· Acute lymphoblastic leukemia
ALLG····· Australasian Leukemia and Lymphoma
allo SCT····· Allogeneic stem cell transplantation
AML ····· Acute myeloid leukemia
AP····· Accelerated phase
ARG ····· Abl-related gene
ASAP····· as soon as possible
ATP····· Adenosine triphosphate
AYA ····· Adolescent and young adult
Bcl-xL ····· B-cell lymphoma-extra large
BCR-ABL···· Break point cluster region - Ableson proto-oncogene
BP····· Blastic phase
CBL····· Casitas B linage c lymphoma protein
CCR····· Complete cytogenetic response
CE····· Clonal evolution
CHR····· Complete hematologic response
CMR ····· Complete Molecular Response
CML ····· Chronic myeloid leukemia
CP····· Chronic phase
CLL····· Chronic lymphocytic leukemia
CRKl····· CRK Like Proto-Oncogene, Adaptor Protein
CYP····· Cytochromes P
CTL ····· Cytotoxic T cell
DADI ····· Dasatinib Discontinuation
DAPI····· 4',6-diamidino-2-phenylindole
DCs ····· Dendritic cells
Der ····· Derivatinve
DISC····· Death-inducing signaling complex
DNA ····· Deoxyribonucleic acid
dNTPs ····· Deoxynucleotide triphosphates
DW····· Distilled water

List of Abbreviations (Cont.)

EFS····· Event free survival
ELN····· European leukemia net
EMR ····· Early molecular response
Erk ····· Extracellular signal–regulated kinases
EVI1 ····· Eco- tropic virus integration-1
FBS ····· Fetal bovine serum (FBS)..
FCS ····· fetal calf serum
FDA····· Food & drug administration
FISH ····· Flourescene insitu hybridization
FGFR ····· Fibroblast Growth Factor
Grb ····· Growth factor receptor-bound protein
GTP ····· Guanosine-5'-triphosphate
HLA····· Human leukocyte antigen
i17 ····· Isochromosome 17
IBMT ····· International Bone marrow Transplantation
ICSBP ····· interferon consensus sequence-binding protein
IRIS ····· International ranomised study of interferone
IFN∂ ····· Interferon ∂
Inv····· Inversion
Jak-STAT ···· Janus kinase/signal transducers and activators of transcription
Kb ····· Kilobase
KD ····· Kinase domain
KDa ····· Kilo Dalton
LSI ····· Locus Specific Identifier
MAPK ····· mitogen-activated protein kinase pathway
M-bcr ····· Major breakpoint cluster region
µ-bcr ····· Micro breakpoint cluster region
m-bcr····· Minor breakpoint cluster region
Mek1/Mek2 ·· Dual-specificity protein kinases
MDS/MPN ··· Myelodysplastic/myeloproliferative neoplasms
mRNA ····· Messenger Rna
M7S ····· Monosomy 7 syndrome
M MLV····· Moloney murine leukemia virus
MMR ····· Major: Molecular response
MPO ····· Myeloperoxidase
MYC····· Oncogene

List of Abbreviations (Cont.)

NCCN ····· National comprehensive cancer network
NP-40 ····· Nonyl phenoxypolyethoxylethanol
OCT-1 ····· Organic cation transporter-1
OS ····· Overall survival
PAH····· Pulmonary arterial hypertension
PBS.....Phosphate Buffered Saline
PCR ····· Polymerase chain reaction
PFS····· Progression free survival
PGDFR ····· Platelet derived growth factor receptor
Ph····· Philadelphia chromosome
PI3····· Phosphatidyl Inositol-3
PNET ····· Primitive neuroectodermal tumors
RAF····· Rapidly accelerated fibroblastoma
RAS····· Rat sarcoma
RAS-MAPK · RAS mitogen activated protein oncogene
RB1 ····· Retinoblastma protein
RBP ····· Receptor bound protein
RPMI ····· Roswell Park Memorial Institute
Rpm····· Round per minute
RT····· Reverse transcriptase
RT-PCR ····· Reverse Tanscriptase Polymerase chain reaction
SEER ····· Surveillance epidemiology end results
Shc ····· Sirohydrochlorin
Shh ····· Sonic Hedgehog
SK····· Sphingosine kinase
SPSS ····· Statistical program for social science
SRC ····· Protooncogene for sarcoma
SFKs ····· SRC family kinases
SNP ····· Single nucleotide polymorphism
SSC ····· Saline Sodium Citrate
STAT ····· Signal transducers & activators of transcription
STIM····· stopping imatinib
TFR ····· Treatment-free remission
T h····· Helper T
TKI····· Tyrosine kinase inhibitor

List of Abbreviations (Cont.)

UGT1A1 ····· Uridinediphosphateglucuronosyltransferase Family 1 Member A1
VEGF ····· Vascular Endothelial Growth Factor
WBC ····· White Blood Cell
WHO····· World Health Organization

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of Philadelphia chromosome which is the cytogenetic hallmark of chronic myeloid leukemia. It is characterized by a reciprocal translocation t (9;22) (q34;qll). The resulting molecular event is the creation of the BCR/ ABl fusion gene ***(Kaaren et al., 2009).***

CML has a worldwide incidence of 1-1.5 cases per 100.000 inhabitants. CML constitutes 15-20% of all leukemias. The median age at diagnosis is 40-60 years, and although it is rare below 20 years, all age groups can be affected, CML has a slight male predominance ***(Fletcher et al., 2011)***.

CML incidence rates in western countries vary from 0.6 to 2.0 cases per 100,000 inhabitants ***(Azzazi and Mattar, 2013).***

The disease is characterized by three phases of the disease namely chronic phase (CP), acceleration phase (AP) and blastic crisis (BC). According to the European Leukemia Net (ELN), the criteria for blastic crisis CML are percentage of blasts plus promyelocytes in peripheral blood or bone marrow $\geq$20%, progressive splenomegaly, thrombocytopenia (<100 X 10^3/uL) unrelated to therapy, and karyotypic evolution ***(Baccarani et al., 2013).***

Recent interest in additional chromosomal abnormalities (ACAs) in chronic myeloid leukemic patients is now gaining more importance particularly in progressive disease ***(Azzazi and Mattar, 2013).***

The appearance of these ACAs during treatment is commonly known as clonal evolution (CE) and seems to

play an important role in imatinib mesylate resistance; The World Health Organization (WHO) classification suggests that those patients showing ACAs emerging during treatment should be considered in accelerated phase (AP). The European Leukemia Net (ELN) recommendations suggest that the presence of ACAs at diagnosis may represent a "warning" feature, requiring careful monitoring of the patient ***(Luatti et al., 2012).***

In CML, 30-50% of resistant cases are associated with additional chromosomal abnormalities. Nonrandom, extra Ph,triosomy 8 (+8), isochromosome 17 i (17q) and triosomy 19 (19+) are the most common secondary changes [present in approximately 13-34% of cases with additional abnormalities] ***(Al-Dewik et al., 2014)***.

These changes were referred to the, "major route" of CE, whereas other less frequently observed changes such as vanishing Y chromosome, triosomy 17 (+17), monosomy 7 (-7), 21, and deletion 17 (-17) occur in less than 10% and were designated as "minor route" aberrations ***(Fabarius et al., 2011).***

Recent data suggest that there is a relation between these abnormalities and the blastic stage of chronic myeloid leukemia ***(Luatti et al., 2012).***

AIM OF THE WORK

Few studies addressing the prognostic significance of ACAs in patients treated with TKIs have been published previously. We will search in this study for ACAs, specially i (17q), (whether present at diagnosis or acquired during treatment) in young adult Egyptian chronic myeloid leukemia patients and we will try to Determine their impact on patient outcome and response to TKI therapy

CHAPTER (1)

CHRONIC MYELOID LEUKEMIA

Disease Overview

Chronic myeloid leukemia (CML) is The classic chronic myeloproliferative disorder which is a clonal stem cell disorder, characterized by acquisition of an oncogenic BCR/ABL fusion protein as a result of a reciprocal translocation (9;22) (q34;q11) [the Philadelphia (Ph) chromosome] which leads to proliferation of granulocytic elements at all stages of differentiation ***(Kaaren et al,2009)***. This was initially depicted by John Hughes Bennett in 1845 at The Royal Infirmary of Edinburgh ***(Quintás and Cortes, 2006).***

Chronic myeloid leukemia (CML) is said to be the malady of "firsts": 1) it is the first disease where the expression“leukemia” was used. 2) It is the first neoplastic disorder which was found to be connected with a recurrent chromosomal anomaly. 3) It is the first disease where targeted therapy against a fusion protein was used ***(Buyukasik et al., 2010)***.

Epidemiology:

CML frequency rates in western nations fluctuate from 0.6 to 2.0 cases for every 100,000 occupants (***Azzazi and Mattar, 2013)***. The yearly frequency of CML in the United States is 1.0 to 1.3 per 100,000 individuals, which translated to approximately 5980 new cases in 2014. CML