Minimal Residual Disease in Chronic Myeloid Leukaemia After Imatinib Treatment

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Abstract

Around 50% of chronic myeloid leukaemia (CML) patients who remain on imatinib treatment for more than 5 years will achieve a complete molecular response (CMR), defined by undetectable *BCR-ABL* mRNA in a sensitive reverse transcriptase real-time quantitative PCR (RQ-PCR) assay. Given the increasing importance of CMR on imatinib therapy the primary aim of this study was to improve the accuracy and sensitivity of MRD detection to allow a more accurate estimation of relapse risk when therapy is withdrawn. Firstly, we investigated ways of improving the sensitivity of RT-PCR methods for the detection of *BCR-ABL* mRNA. Secondly, we investigated the use of the patient-specific *BCR-ABL* gene for the detection of MRD. Thirdly, we conducted a multi-centre clinical trial of imatinib withdrawal in selected CML patients in a stable CMR. This clinical trial provided patient samples that could be used to test our optimized MRD assays, and provided clinical data on the risk and patterns of relapse after withdrawal of imatinib therapy. The trial is ongoing, but an interim analysis of the study data was performed. In 22 patients the estimated probability of molecular relapse after imatinib withdrawal was 54%, and 60% of relapses occurred within the first 4 months.

The average detection limit of *BCR-ABL* mRNA by RQ-PCR is estimated at around 4.5 log below the level of *BCR-ABL* prior to commencing treatment. The number of leukaemic cells at diagnosis is around 10^{12}, so the number of residual leukaemic cells in CMR might vary from zero to over a million. We hypothesized that the amount of residual leukaemia in CMR is variable between patients, and that this heterogeneity is a determinant of the risk of relapse when treatment is withdrawn. We developed more sensitive methods for the detection of *BCR-ABL* and tested these methods in samples from our study patients.

We showed that random pentadecamer (15-mer) primers improved the efficiency of reverse transcriptase PCR (RT-PCR), and resulted in a lower detection limit of *BCR-ABL* mRNA. We also developed a novel nested RT-PCR method using real-time PCR for the second round of the reaction, and this resulted in a lower detection limit of *BCR-ABL* in patient samples. The utility of this nested RT-PCR method was limited by a false positive rate of 2-3% in the HeLa cell line that we used as our negative control. Consequently, we examined the detection of the patient-specific genomic *BCR-ABL* sequence as an alternative to RT-PCR.

Breakpoints in *BCR* and *ABL1* in CML patients are widely dispersed over 3 kb and 150 kb, respectively. Therefore, the *BCR-ABL* genomic sequence is essentially unique to each patient. We sequenced the genomic breakpoints of 43 CML patients. We showed that the distribution of breakpoints in *BCR* and *ABL1* was non-random, but we were unable to identify any genomic feature that determined the specific location of individual breakpoints. We developed a novel *BCR-ABL* DNA Q-PCR method for 12 of the study patients, and in 11 of the patients *BCR-ABL* DNA was detected when the patient was in a CMR, confirming that this method was more sensitive than RQ-PCR.

Contrary to our hypothesis, the detection of *BCR-ABL* DNA was not predictive of relapse. In most patients who relapsed there was a significant increase in *BCR-ABL* DNA prior to mRNA relapse. Two patients had stable levels of *BCR-ABL* DNA measurable on multiple occasions, but remained in remission after 6 months and 15 months, respectively. We have shown that a stable CMR after the withdrawal of imatinib therapy does not necessarily indicate the eradication of leukaemia.

Declaration of Originality

This work contains no material which has been accepted for the award to me of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

%	*per centum*
ABL1	Abelson murine leukaemia virus human homologue 1 gene
aDNA	Amplified DNA
ALLG	Australasian Leukaemia & Lymphoma Group
AP	Accelerated Phase
BC	Blast Crisis
BCR	Breakpoint Cluster Region gene
BCR-ABL	*BCR-ABL1* fusion oncogene
BM	Bone Marrow
bp	Nucleotide base pairs
C	Degrees Celsius
CCR	Complete Cytogenetic Response
cDNA	Complementary DNA
CHR	Complete Haematological Response
CML	Chronic Myeloid Leukaemia
CMR	Complete Molecular Response
CP	Chronic Phase
Ct	Fluorescence threshold cycle number
CTL	Cytotoxic T Lymphocyte
DNA	Deoxyribonucleic Acid
EAC	Europe Against Cancer collaborative group
EDTA	Ethylenediaminetetra-acetic acid
e.g.	*exempli gratia*
et al.	*et alia*
g	Grams
g	Gravity
gDNA	Genomic DNA
GUSB	β glucuronidase gene
h	Hours
i.e.	*id est*
IFN	Interferon-α
IM	Imatinib mesylate
IMVS	Institute of Medical & Veterinary Science
IS	International Standard (*BCR-ABL* mRNA level)
Kb	Kilo base pairs
L	Litres
log	Logarithm_{10}
m	Milli (10^{-3})
MCR	Major Cytogenetic Response
min	Minutes
MMR	Major Molecular Response
MRD	Minimal Residual Disease
mRNA	Messenger RNA
µ	Micro (10^{-6})
n	Nano (10^{-9})
NHEJ	Non-homologous end-joining
nt	Nucleotide
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
Ph	Philadelphia

p	Pico (10^{-12})
Q-PCR	Quantitative PCR
R6	Random hexamer oligonucleotide
R15	Random pentadecamer oligonucleotide
RNA	Ribonucleic Acid
rpm	Revolutions per minute
RT-PCR	Reverse Transcriptase PCR
s	Seconds
UV	Ultraviolet
V	Volts
vs	*versus*