Prognostic Markers Associated With Tyrosine Kinase Inhibitor Treatment Response and Maintenance of Treatment Free Remission in Chronic Myeloid Leukaemia

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Abstract

Treatment outcomes in Chronic Phase Chronic Myeloid Leukaemia (CP-CML) have dramatically improved with the introduction of highly active tyrosine kinase inhibitors (TKIs). However, treatment responses are highly heterogeneous. The aim of this thesis is to identify prognostic markers that may help individualise treatment and optimise outcome by stratifying patients into risk groups. Selective treatment intensification is important - more potent treatment may be associated with increased toxicity, and universal adoption of the most potent treatment does not optimally balance risk versus benefit, nor is such a strategy cost effective.

This thesis will summarise factors with prognostic significance in CP-CML. Such predictive factors include the metric called the "halving time", which measures the velocity of *BCR-ABL1* decline with initial TKI treatment. This correlates well with future treatment response and risk of disease progression. Conversely, the treatment resistance can be measured by the speed at which the *BCR-ABL1* rises, in a similar metric called the "doubling time". A patient's Killer Immunoglobulin-like Receptor (KIR) genotype is also correlated with survival as well as molecular outcomes. Whilst the biological basis for this interaction is poorly understand, it is believed to be underpinned by the innate immune system's role in tumour surveillance and suppression. In the setting of disease resistance mediated by *BCR-ABL1* kinase domain mutations, demonstrating an increased number of low level mutants via the use of ultra sensitive mutation detection techniques may help prognosticate patients with a history of the T315I mutation.

Early molecular response (EMR, *BCR-ABL1* ≤10% at 3 months after starting treatment) is currently acknowledged as one of the strongest prognostic markers, and salvage strategies targeting patients failing to achieve time dependent molecular targets may be an optimal point of intervention. The TIDEL-II study examined such a strategy, firstly by imatinib dose escalation, followed by switching to nilotinib, in patients who fail to achieve time dependent molecular responses. This study also examined the effectiveness of increasing imatinib dose in patients with serum trough levels <1000 ng/mL, a threshold thought to be necessary to achieve cytogenetic response. Although survival and overall molecular response in TIDEL-II is excellent, this strategy was found to be of only marginal benefit in those who failed to achieve EMR. The subsequent study, Pinnacle, aims to study the combination of pegylated interferon and nilotinib in a similar context. Enrolment in this study is ongoing.

The same prognostic marker (EMR) may identify patients unlikely to achieve deep molecular responses (DMR), a milestone associated with excellent long term event free survival. It is also commonly stipulated as a pre-requisite to participation in treatment cessation studies. Consistent performance of *BCR-ABL1* qRT-PCR assays of a sufficient quality to determine this may be a challenge for some laboratories. To avoid false negatives which may lead to inappropriate reassurance and cessation attempts, we have developed an improved protocol that reliably achieves detection sensitivity required to classify patients in DMR. This assay may also be used as a basis for further enhancing *BCR-ABL1* qRT-PCR sensitivity, aimed at identifying patients with an ultra-low level of residual disease. Such patients are hypothesised to have negligible risk of molecular recurrence upon treatment cessation, versus patients who have residual disease just below the current limit of detection. Such ultra-sensitive *BCR-ABL1* qRT-PCR may, in this manner, contribute to further elucidation of the prognostic markers of successful treatment cessation. The thesis will conclude with prospects in prognostic markers in CML research and clinical management.

Declaration of originality

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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David T O Yeung
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- My supervisors A/Prof Branford, Profs Hughes and Scott; and Postdoctoral researcher Dr Wendy Parker - for their mentorship, and offering opportunities to join their research program
- My parents and my wife, who provided emotional support and encouragement throughout my studies.

List of commonly used abbreviations

ALLG	Australasian Leukaemia and Lymphoma Group
AP	Accelerated Phase
ASH	American Society of Hematology
BC	Blast Crisis, synonymous with Blastic Phase or BP
BP	Blastic Phase, synonymous with Blast Crisis or BC
CCR / CCyR	Complete Cytogenetic Response
cDNA	Complementary DNA
CML	Chronic Myeloid Leukaemia
CP	Chronic Phase
C_t	cycle threshold ()
DMR	Deep molecular response; *BCR-ABL1* ≤0.01% or ≤0.0032% in literature, depending on author and context
dNTP	Deoxynucleotide triphosphate – substrate for RT and PCR reactions
dPCR	digital PCR
EDTA	Ethylenediaminetetraacetic acid. Cation chelator, reagent and anticoagulant.
ELN	European Leukemia Net
EMR	Early Molecular Response, equivalent to *BCR-ABL1* ≤10% at 3 months
Fig	Figure
gDNA	genomic DNA
GEP	Gene Expression Profile
GSP	Gene Specific Primer (for reverse transcription)
GTC	Guanidinium thiocyanate solution (TRIzol reagent, ThermoFisher, Waltham,. MA, USAF)
HSANZ	Haematology Association of Australia and New Zealand
IFN-α	Interferon-alfa
IRIS	International Randomized Study of Interferon Vs STI571 (study)
IS	International Scale
KD	Kinase Domain (Mutations)
KIR	Killer Immunoglobulin-like Receptor
mg	milligram
mL	millilitre
mM	millimolar

MMR	Major Molecular Response, equivalent to *BCR-ABL1* ≤0.1% IS
MRD	Minimal Residual Disease
mRNA	messenger RNA
NCCN	National Comprehensive Cancer Network (US)
ncRNA	non-coding RNA
ng	nanogram
nM	nanomolar
NGS	Next Generation Sequencing
Peg IFN-α	Pegylated Interferon-α
Ph	Philadelphia (Chromosome)
PCR	Polymerase Chain Reaction
PK	Pharmacokinetics
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative, reverse transcription, polymerase chain reaction
RH	Random Hexamer
rRNA	Ribosomal RNA
RT	Reverse transcription
TFR	Treatment Free Remission
TKI	Tyrosine Kinase Inhibitor
TIDEL	Therapeutic Intensification in De Novo Leukaemia (clinical study)
Tris	Tris-(hydroxymethyl)-aminomethane. Commonly used in buffer preparations
tRNA	transfer RNA

Preface

The following published and unpublished manuscripts, based on research that I have performed solely or with collaborators, form the basis of the thesis. A description of my contribution to each manuscript is provided in context in the next section, and a statement of authorship, as stipulated by university requirements, precede each manuscript.

I have summarised these manuscripts in the following list, and provided an estimate of my contribution to each project.

Chapters in Textbooks

Yeung DT & Hughes TP. Chronic Myeloid Leukaemia. In: Hoffbrand AV, Higgs DR, Keeling D & Mehtaand AB, eds. Postgraduate Haematology. 7th edition. Oxford, John Wiley & Sons; 2016: Chapter 24. Estimated contribution: 95%

Yeung DT & Branford S. Optimal Monitoring of CML Treatment: Molecular and Mutation Analysis. In: Kizaki M (Ed). Molecular Pathogenesis and Treatment of Chronic Myelogenous Leukemia, Tokyo, Springer; 2016: Chapter 7. Estimated contribution: 90%

Published Manuscripts in Peer Reviewed Journals

Yeung DT & Mauro MJ. Prognostic significance of early molecular response in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. Hematology Am Soc Hematol Educ Program. 2014 Dec 5;2014(1):240-3. Estimated contribution: 95%

Yeung DT, Osborn MP, White DL, Branford S, Braley J, Herschtal A, Kornhauser M, Issa S, Hiwase DK, Hertzberg M, Schwarer AP, Filshie R, Arthur CK, Kwan YL, Trotman J, Forsyth CJ, Taper J, Ross DM, Beresford J, Tam C, Mills AK, Grigg AP, Hughes TP; for the Australasian Leukaemia and Lymphoma Group. TIDEL-II: first-line use of imatinib in CML with early switch to nilotinib for failure to achieve time-dependent molecular targets. Blood. 2015 Feb 5;125(6):915-23. Estimated contribution: 85%

Yeung DT & Branford S. Monitoring disease response in chronic-phase chronic myeloid leukemia: the age of molecular assays? Hematology Am Soc Hematol Educ Program. 2012;2012:111-4. Estimated contribution: 90%

Branford S, Yeung DT, Ross DM, Prime JA, Field CR, Altamura HK, Yeoman AL, Georgievski J, Jamison BA, Phillis S, Sullivan B, Briggs NE, Hertzberg M, Seymour JF, Reynolds J, Hughes TP. Early molecular response and female sex strongly predict stable undetectable BCR-ABL1, the criteria for imatinib discontinuation in patients with CML. Blood. 2013 May 9;121(19):3818-24. Estimated contribution: 30%

Branford S, Yeung DT, Parker WT, Roberts ND, Purins L, Braley JA, Altamura HK, Yeoman AL, Georgievski J, Jamison BA, Phillis S, Donaldson Z, Leong M, Fletcher L, Seymour JF, Grigg AP, Ross DM, Hughes TP. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014 Jul 24;124(4):511-8. Estimated contribution: 40%

Branford S, Yeung DT, Prime JA, Choi SY, Bang JH, Park JE, Kim DW, Ross DM, Hughes TP. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. Blood. 2012 May 3;119(18):4264-71. Estimated contribution: 35%

Yeung DT, Tang C, Vidovic L, White DL, Branford S, Hughes TP, Yong AS. KIR2DL5B Genotype Predicts Treatment Response in CML Patients Treated with Outcome Directed Sequential Imatinib / Nilotinib Strategy. Blood. 2015;126(25):2720-2723. Estimated contribution: 85%

Manuscripts invited for re-submission after revision

Parker WT, Yeung DT, Yeoman A, Altamura H, Jamieson B, Field C, Hodgson JG, Lustgarten S, Rivera VM, Hughes TP & Branford S. The impact of multiple low-level BCR-ABL1 mutations on response to ponatinib. Invited manuscript from Section Editor, undergoing first revision. Estimated contribution: 40%

Manuscript planned for submission

Yeung DT, Parker WT, Phillis S, Georgievski J, Scott HS, Hughes TP & Branford S. Enhancing sensitivity of BCR-ABL1 detection using purified mRNA – letter to the editor. Manuscript prepared for submission to *Leukemia*. Estimated contribution: 90%

Peer reviewed, published manuscripts included in the appendix as reference

Parker WT, Yeoman AL, Jamison BA, Yeung DT, Scott HS, Hughes TP, Branford S. BCR-ABL1 kinase domain mutations may persist at very low levels for many years and lead to subsequent TKI resistance. Br J Cancer. 2013 Sep 17;109(6):1593-8. doi: 10.1038/bjc.2013.318. Epub 2013 Jun 25. Estimated contribution: 10%

Parker WT, Phillis SR, Yeung DT, Hughes TP, Scott HS, Branford S. Blood. 2014;124(1):153-5. Many BCR-ABL1 compound mutations reported in chronic myeloid leukemia patients may actually be artifacts due to PCR-mediated recombination. Estimated contribution: 10%