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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31st December 2015
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  and for their fund raising efforts, for their encouragement and interest in our work, and putting
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  correlative studies
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- My parents and my wife, who provided emotional support and encouragement throughout my
  studies.
### List of commonly used abbreviations

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


Published Manuscripts in Peer Reviewed Journals


**Estimated contribution: 90%**


**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%