Safety and efficacy of pulsed imatinib with or without G-CSF *versus* continuous imatinib in chronic phase chronic myeloid leukaemia patients at 5 years follow-up

Despite their efficacy in inducing deep and durable responses in chronic phase (CP) chronic myeloid leukaemia (CML) patients, BCR-ABL1 tyrosine kinase inhibitors (TKI) do not eradicate leukaemia stem cells (LSC), as proven by the persistence of *BCR-ABL1*+ CD34+, colony forming, long-term culture-initiating cells in the bone marrow of patients in sustained molecular response 4·5 (a 4·5-log reduction of *BCR-ABL1* transcript levels, MR4·5) following TKI therapy (Chomel *et al*, 2011).

CML LSC survival is independent of BCR-ABL1 kinase activity (Hamilton *et al*, 2012) and their quiescence is a putative TKI-resistance mechanism causing disease persistence. Moreover TKI exert anti-proliferative rather than pro-apoptotic effects against CML LSCs and might further contribute to disease persistence (Graham *et al*, 2002). Promoting LSC cell-cycle entry using granulocyte-colony stimulating factor (G-CSF) has been shown *in vitro* to restore their sensitivity to TKI and enhance their eradication (Jorgensen *et al*, 2006).

Based on this evidence, we performed a randomized phase II study (GIMI, EudraCT 2004-000179-33), which compared the safety and efficacy of continuous imatinib (cIM) *versus* pulsed imatinib (pIM) alone or with G-CSF (pIM+G) therapy administered in 4-week cycles for 48 weeks (12 cycles in total) in CP CML patients with at least a complete cytogenetic response (CCyR) on IM (Drummond *et al*, 2009) (see reference for study design, primary and secondary endpoints, patient demographics and disease characteristics). The experimental arms were expected to improve CML LSCs eradication by reducing TKI-induced quiescence (pIM) and/or by actively pushing CML LSCs into cell-cycle (pIM+G). At 2 years follow-up no statistically significant differences for the study endpoints were observed, possibly due to the limited numbers (15 patients per arm). However, 6/30 patients across the two experimental arms exhibited either loss of CCyR or major molecular response (MMR) as compared with only 1/15 in the cIM arm, with all but one patient in the experimental arms regaining MMR on restarting continuous IM or nilotinib therapy (Drummond *et al*, 2009). These results raised some concerns that the experimental schedules might have contributed to the loss of response.

Subsequently, a mathematical model of the safety and efficacy of the IM and G-CSF combination suggested that this approach might be detrimental in the short- to medium-term for patients with persistent disease treated with IM, by increasing the LSCs burden through enhanced proliferation, thus in turn increasing the risk of acquiring a resistance mutation and of disease progression. However, in the long-term (>2500 d, i.e. 6·8 years, from start of treatment), such an approach was predicted to prove beneficial as it would deplete the CML LSCs by increasing their susceptibility to TKI (Foo *et al*, 2009).

Here we report the 5-year follow-up data for the GIMI study. 41/45 patients were available for analysis; four patients had died (one only as a result of CML progression). The median follow-up was 5·67 years. Using an intention to treat analysis, both CCyR and MMR rates were similar among treatment arms with no differences in progression rates. 5/15 patients in the cIM arm compared to 3/15 patients in each experimental arm changed treatment to second generation TKI (Table I).

Table I. Patient responses at trial entry and 5 years follow-up and number of patients who have interrupted imatinib therapy in each arm.

	cIM	pIM	pIM+G
Best response at trial entry			
MMR (% of total)	7 (46·7)	8 (53·3)	10 (66·7)
CCyR (% of total)	8 (53·3)	7 (46·7)	5 (33·3)
Total (% of total)	15 (100)	15 (100)	15 (100)
Best response at follow-up evaluation			
MMR or better (% of total)	13 (86·7)	13 (86·7)	11 (73·3)
CCyR (% of total)	0 (0)	2 (13·3)	1 (6·7)
CHR (% of total)	1 (6·65)	0 (0)	0 (0)
Dead (% of total)*	1 (6·65)	0 (0)	3 (20)
Total (% of total)	15 (100)	15 (100)	15 (100)
Change to second generation TKI†			
Dasatinib (% of total)	3 (20)	0 (0)	2 (13·3)
Nilotinib (% of total)	2 (13·3)	3 (20)	1 (6·6)
Total (% of total)	5 (33·3)	3 (20)	3 (20)

cIM, continous imatinib; pIM, pulsed imatinib; pIM+G, pIM + granulocyte colony-stimulating factor; MMR, major molecular response; CCyR, complete cytogenetic response; CHR, complete haematological response; TKI, tyrosine kinase inhibitor.

Note that one patient in the cIM and one patient in the pIM+G arm are currently off TKI therapy but still in MMR or better.

*Causes of death were: sudden death (cIM), myocardial infarction (pIM+G), metastatic prostate cancer (pIM+G), progression of chronic myeloid leukaemia (pIM+G).

†Causes of interruption of allocated study treatment were: intolerance (n = 3 for cIM and n = 1 for pIM and pIM+G), disease progression (n = 1 for cIM), resistance (n = 1 for pIM arm), suboptimal response (n = 1 for pIM arm and n = 2 for pIM+G arm) and physician's decision (n = 1 for cIM).

© 2013 John Wiley & Sons Ltd

Of note, the reduction in *BCR-ABL1* transcript levels between trial entry (baseline) and 5 years was highly statistically significant in both the pIM (1·10 median log reduction, $P = 0{\cdot}013$) and the pIM+G arms (1·43 median log reduction, $P = 0{\cdot}002$), while not significant in the cIM arm (1·06 median log reduction, $P = 0{\cdot}060$) (Fig 1). However, a comparison of the change from baseline between treatment arms shows that, although this was lower in the cIM arm, it was not significantly different from the change in either the pIM ($P = 0{\cdot}678$) or pIM+G arm ($P = 0{\cdot}528$).

Although the small number of patients demands caution in interpreting these data, these findings are reassuring regarding the safety of the experimental therapeutic approaches. Moreover, the deeper and significant reduction in *BCR-ABL1* transcript levels in the experimental arms compared to the cIM arm at 5 years provocatively suggests that in the long-term, as suggested by the mathematical model, these treatment strategies might become beneficial to CP CML patients with persistent disease. However caution is required when making inferences based on the mathematical model of IM and G-CSF treatment, as it predicted the effects of an indefinite duration of this treatment strategy while in our study the combination treatment was only continued for 48 weeks. Nevertheless, considering these findings, we believe that a therapeutic strategy aiming to reverse LSC quiescence in CML combining IM and G-CSF could be safely pursued and only larger studies might be able to provide a definitive answer on its efficacy.

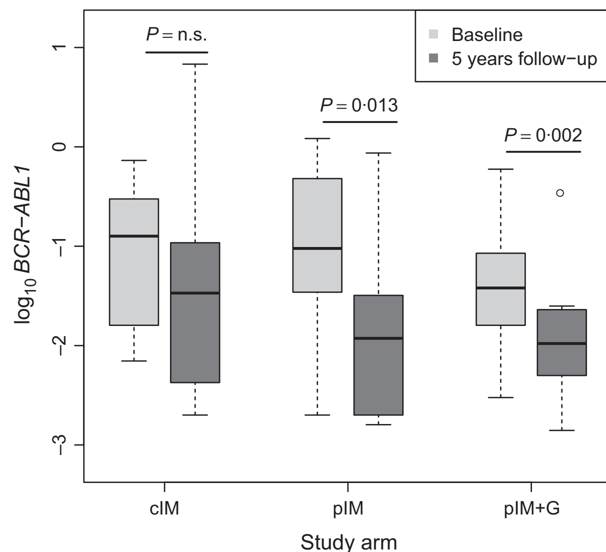

Fig 1. Comparison of baseline and 5 years follow-up *BCR-ABL1* transcript levels for each treatment arm. *BCR-ABL1* transcript levels were measured by quantitative real time-polymerase chain reaction from the peripheral blood of all patients at baseline and 5 years follow-up according to a standard protocol as previously described (Drummond *et al*, 2009). Data are presented as the median ± range of $\log_{10}$ *BCR-ABL1* transcript levels at baseline and 5 years follow-up within each treatment arm. The change in logged baseline and 5 years follow-up *BCR-ABL1* transcript levels within each treatment arm were compared using a Wilcoxon signed-rank test. Significance values are shown in the figure. cIM, continous imatinib; pIM, pulsed imatinib; pIM+G, pIM + granulocyte colony-stimulating factor.

Acknowledgements

This research was supported by the Leukaemia Research Fund CTAP (UK), Chugai Pharmaceutical Co. Ltd, Chugai Pharma, France and Novartis Pharmaceuticals (UK). We are grateful to Lindsay Mitchell and Karen Stewart for their help with recruitment and assessment of patients. We thank the 'Délégation à la recherche clinique des Hospices Civils de Lyon' for their support in setting up this trial in France, Elodie Gadolet and Madeleine Etienne, CRAs, for their help in collecting the data for the French patients. Drs Steve O'Brien, Heather Jorgensen and Alex McMahon assisted in reviewing the data. This study was supported by the Glasgow Experimental Cancer Medicine Centre, which is funded by Cancer Research UK and by the Chief Scientist's Office, Scotland. P.G. was funded by Medical Research Council UK clinical research training fellowship grant G1000288, N.H. was funded by the Leukaemia & Lymphoma Research UK clinical research training fellowship grant 05086 and T.L.H. was funded by Cancer Research UK Programme grant C11074/A11008.

Authorship contributions

PG analysed data and wrote the manuscript, JS TH and JP analysed data and reviewed the manuscript, NH, FEN, RC, JT, LM, and MD looked after patients, collected data and reviewed the manuscript, GW collected data, FM reviewed the manuscript and TLH designed trial, looked after patients, collected/analysed data and reviewed manuscript.

Conflict of interest disclosures

The authors declare no competing financial interests.

Paolo Gallipoli[1]
Jon Stobo[2]
Nicholas Heaney[3]
Franck E. Nicolini[4]
Richard Clark[5]
George Wilson[2]
Jane Tighe[6]
Lorna McLintock[7]
Timothy Hughes[8]
Franziska Michor[9]
James Paul[2]
Mark Drummond[3]
and Tessa L. Holyoake[1]

[1]*Paul O'Gorman Leukaemia Research Centre, University of Glasgow,* [2]*Cancer Research UK, Clinical Trials Unit, University of Glasgow,*

[3]Department of Haematology, Beatson West of Scotland Cancer Centre, Glasgow, UK, [4]Haematology Department, Centre Hospitalier Lyon Sud, Lyon, France, [5]Department of Haematology, Royal Liverpool University Hospital, Liverpool, [6]Department of Haematology, Aberdeen Royal Infirmary, Aberdeen, [7]Department of Haematology, Victoria Hospital, Kirkcaldy, UK, [8]Department of Haematology, SA Pathology, Adelaide, SA, Australia and [9]Department of Biostatistics, Dana-Farber Cancer Institute, Boston, MA, USA

E-mail: tessa.holyoake@glasgow.ac.uk

Keywords: therapy, *BCR-ABL1*, chronic myeloid leukaemia, trials, stem cells

First published online 19 August 2013
doi: 10.1111/bjh.12532

References

Chomel, J.C., Bonnet, M.L., Sorel, N., Bertrand, A., Meunier, M.C., Fichelson, S., Melkus, M., Bennaceur-Griscelli, A., Guilhot, F. & Turhan, A.G. (2011) Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood*, **118**, 3657–3660.

Drummond, M.W., Heaney, N., Kaeda, J., Nicolini, F.E., Clark, R.E., Wilson, G., Shepherd, P., Tighe, J., McLintock, L., Hughes, T. & Holyoake, T.L. (2009) A pilot study of continuous imatinib vs pulsed imatinib with or without G-CSF in CML patients who have achieved a complete cytogenetic response. *Leukemia*, **23**, 1199–1201.

Foo, J., Drummond, M.W., Clarkson, B., Holyoake, T. & Michor, F. (2009) Eradication of chronic myeloid leukemia stem cells: a novel mathematical model predicts no therapeutic benefit of adding G-CSF to imatinib. *Plos Computational Biology*, **5**, e1000503.

Graham, S.M., Jorgensen, H.G., Allan, E., Pearson, C., Alcorn, M.J., Richmond, L. & Holyoake, T.L. (2002) Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*, **99**, 319–325.

Hamilton, A., Helgason, G.V., Schemionek, M., Zhang, B., Myssina, S., Allan, E.K., Nicolini, F.E., Muller-Tidow, C., Bhatia, R., Brunton, V.G., Koschmieder, S. & Holyoake, T.L. (2012) Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood*, **119**, 1501–1510.

Jorgensen, H.G., Copland, M., Allan, E.K., Jiang, X., Eaves, A., Eaves, C. & Holyoake, T.L. (2006) Intermittent exposure of primitive quiescent chronic myeloid leukemia cells to granulocyte-colony stimulating factor in vitro promotes their elimination by imatinib mesylate. *Clinical Cancer Research*, **12**, 626–633.

A prenatal origin of childhood essential thrombocythaemia

In children, the myeloproliferative neoplasms (MPN) polycythaemia vera (PV) and essential thrombocythaemia (ET) are estimated to have a prevalence of approximately 40- to 90-fold lower than that seen in adults with sporadic, paediatric ET patients exhibiting a significantly lower prevalence of the *JAK2* V617F (Teofili *et al*, 2008). The incidence of the *JAK2* V617F is known to increase with age, in both ET and PV, through adolescence, into adulthood and old age (Cario *et al*, 2008; Randi *et al*, 2011), suggesting that an increasing degree of underlying genomic instability is partly responsible for the acquisition of this mutation. Furthermore, those children with ET harbouring this mutation also display a lower *JAK2* V617F allele burden (Teofili *et al*, 2009). Although several clinical and biological similarities exist between adult and paediatric ET, these aforementioned observations suggest differences in the aetiology of this disease in childhood and implicate some alternative pathogenetic mechanism(s).

Genetic analysis of antenatal blood spots (ABS; Guthrie cards) has demonstrated that clonotypic translocations, mutations and gene rearrangements associated with many subtypes of paediatric acute lymphoblastic leukaemia, and to a lesser extent, paediatric acute myeloid leukaemia, are present at birth and have therefore been generated *in utero*. These backtracking studies have provided significant insights into the pathogenesis, natural history and aetiology of these leukaemias (Wiemels *et al*, 2009). We have previously described the somatically acquired, *in utero* origin of the *JAK2* V617F mutation in an infant with PV, thus providing some insight into the origins of childhood MPN (Kelly *et al*, 2008). Whether all genotypes and phenotypes of paediatric MPN, especially those seen in younger patients, have a prenatal origin is unknown. To expand on this theme, we describe herein the prenatal origin of the *JAK2* V617F in a child with ET.

A four-and-a-half year-old female presented with persistent urinary tract infections associated with a thrombocytosis (platelet count 801–989 $\times$ 10^9/l). Antibiotic treatment ameliorated the infections yet the thrombocytosis remained. Significant platelet anisocytosis and giant platelets were observed on the peripheral blood film. A bone marrow aspirate revealed a normocellular, particular marrow with evidence of trilineage haematopoiesis. All cell lines were represented to maturity with abundant megakaryocytes, some with dysmorphic features, such as hyperchromatic and naked nuclei. Megakaryocyte clustering was evident and there was no obvious reticulin fibrosis. The *JAK2* V617F was detected in the peripheral blood by qualitative polymerase chain reaction (PCR) (Baxter *et al*, 2005) and determined as 31·2% of total *JAK2* alleles by quantitative PCR (qPCR; Larsen *et al*, 2007). Parental consent was sought and granted to access the

© 2013 John Wiley & Sons Ltd