Institution: Imperial College London

Unit of Assessment: 01 Clinical Medicine

Title of case study: Invention and Development of a Globally Recognised Molecular Method of Monitoring Disease Response in Chronic Myeloid Leukaemia

1. Summary of the impact (indicative maximum 100 words)

The change in outcome for patients with chronic myeloid leukaemia (CML) is the outstanding cancer success story of the 21st century. All newly diagnosed patients now receive highly effective targeted life-long therapy with tyrosine kinase inhibitors and their response is monitored by a molecular test invented at Imperial College in the 1990s, to monitor patients after transplant. Improvements in methodology pioneered by Imperial staff, refined the test such that it is now a robust and accurate quantitative reflection of residual disease, and now in 2013 it is routinely used in both developed and developing countries to diagnose, determine management and predict outcome in CML.

2. Underpinning research (indicative maximum 500 words)

Key Imperial College London researchers:
Professor Nick Cross, Non-Clinical Senior Lecturer (1988-2001), now Professor of Human Genetics now at Wessex Regional Genetics Laboratory
Professor Jane Apperley, Professor of Haemato-oncology (1984-present)
Professor John Goldman, Senior Research Investigator (1970-present)
Professor Junia Melo, Professor of Molecular Haematology (1992-2007) now in Adelaide
Professor Francesco Dazzi, Professor of Stem Cell Biology (1996-present)
Dr David Marin, Reader in Onco-Haematology (2001-present)
Professor Letizia Foroni, Visiting Professor (NHS), Head of Molecular Haematology (2008-present)

Until 2001 the standard of care for patients with CML was allogeneic stem cell transplantation (allo-SCT) but the procedure itself carried a high level of mortality from the complication known as graft versus host disease (GvHD). This is due to the ability of the cells of the donor's immune system to recognise the patient as 'foreign' and establish a potentially fatal systemic rejection process. The incidence and severity of GvHD can be mitigated by removal of donor T-lymphocytes from the stem cells prior to their infusion into the patients but researchers at Imperial were the first to recognise that the removal of these allo-immune effector cells resulted in disease relapse. Work at Imperial and other centres recognised that infusion of small amounts of donor T-cells could restore durable remissions if given early in relapse. For this reason it became imperative to find a test that would identify early disease recurrence.

CML is characterised in all patients by the product of the Philadelphia (Ph') chromosome, a fusion oncogene known as BCR-ABL1, and this was exploited by our group at Imperial to identify recurrent leukaemia first by Southern blotting and then by polymerase chain reaction (PCR). In the early 1990s our group optimised a qualitative RNA-based PCR test (RT-PCR) that was more sensitive than Southern blotting and could detect the presence of small amounts of residual leukaemia (1). However because the test at that stage was qualitative rather than quantitative, we could not determine whether the residual leukaemia was slowly reducing in amount through killing by the donor T-cells, or slowly increasing and in need of further therapy. The breakthrough in 1993 came when we engineered a control gene which allowed quantification of the residual leukaemia (RT-qPCR) and more accurate assessment of the disease course (2). This technique was rapidly disseminated across transplant centres worldwide, often by their researchers learning the technique in our laboratories at Imperial, and became the gold standard for monitoring residual disease in CML after allo-SCT and facilitating effective salvage therapy (3).

In the last decade, two advances have led to RT-qPCR for BCR-ABL1 monitoring becoming the standard of care for all patients with CML, not just those undergoing allo-SCT. First, further

technological developments in RT-PCR analysis have resulted in the ready availability of commercial equipment for real-time quantitative PCR monitoring (RT-qPCR). Second, a new class of oral targeted agents, the tyrosine kinase inhibitors (TKI), specifically directed against the protein product of the BCR-ABL1 oncogene, have replaced allo-SCT as the treatment of choice in CML (4). The TKI are capable of inducing deep and durable remissions in CML, so deep that residual disease, and hence the need for continuing therapy, can only be determined by the RT-qPCR test originally developed, refined and optimised at Imperial (5). Our researchers, using this test to predict outcome, change therapy and identify early the 10-15% of patients for whom allo-SCT is still the optimal therapy, continue to publish widely and influence CML management worldwide (6).

3. References to the research (indicative maximum of six references)

(1) Cross, N.C., Hughes, T.P., Feng, L., O'Shea, P., Bungey, J., Marks, D.I., Ferrant, A., Martiat, P., Goldman, J.M. (1993). Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: correlations with acute graft-versus-host disease and relapse. *Br J Haematol*, 84 (1), 67-74. DOI. Times cited: 175 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 4.94

(2) Cross, N.C., Feng, L., Chase, A., Bungey, J., Hughes, T.P., Goldman, J.M. (1993). Competitive PCR to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. *Blood*, 82, 1929-1936. Times cited: 344 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 9.06

(3) Kaeda, J., O'Shea, D., Szydlo, R.M., Olavarria, E., Dazzi, F., Marin, D., Saunders, S., Khorashad, J.S., Cross, N.C., Goldman, J.M., Apperley, J.F. (2006). Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. *Blood*, 107 (10), 4171-4176. DOI. Times cited: 59 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 9.06

(4) Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., Talpaz, M., Druker, B., for the International STI571 CML Study Group (2002). Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*, 346 (9), 645-652. DOI. Times cited: 1200 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 51.65

(5) Hughes, T.P., Hochhaus, A., Branford, S., Müller, M.C., Kaeda, J.S., Foroni, L., Druker, B.J., Guilhot, F., Larson, R.A., O'Brien, S.G., Rudoltz, M.S., Mone, M., Wehrle, E., Modur, V., Goldman, J.M., Radich, J.P. (2010). Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). *Blood*, 116 (19), 3758-3765. DOI. Times cited: 116 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 9.06

(6) Marin, D., Ibrahim, A.R., Lucas, C., Gerrard, G., Wang, L., Szydlo, R.M., Clark, R.E., Apperley, J.F., Milojkovic, D., Bua, M., Pavlu, J., Paliompeis, C., Reid, A., Rezvani, K., Goldman, J.M., Foroni, L. (2012). Assessment of BCR-ABL1 Transcript Levels at 3 Months Is the Only Requirement for Predicting Outcome for Patients With Chronic Myeloid Leukemia Treated With Tyrosine Kinase Inhibitors. *J Clin Oncol*, 30, 232-238. DOI. Times cited: 60 (as at 7th November 2013 on ISI Web of Science). Journal Impact Factor: 18.03

4. Details of the impact (indicative maximum 750 words)

Impacts include: health and welfare, practitioners and services, public policy and services
Main beneficiaries include: patients, practitioners, European Leukaemia Network, NICE, WHO, CML Charities (Leukaemia and Lymphoma Research, Leuka, International CML Forum)

Qualitative PCR assays were first used to detect residual and/or relapsing disease after allo-SCT for CML. The presence of residual disease detectable only by molecular testing is often, but not always, the prelude to frank relapse with abnormal blood counts and inevitable progression to the terminal and fatal phase of the disease. Early relapse, i.e. before the blood counts become

abnormal, can be effectively treated with further infusions of donor T-lymphocytes (DLI), but this treatment carries the risk of potentially fatal GvHD. Because qualitative detection of residual disease did not inevitably lead to increasing tumour load, it became very important to develop a test that when measured serially, could identify increasing tumour cell numbers and direct therapy only to patients. When Professor Cross, at Imperial, developed a quantitative PCR test, DLI, given in escalating numbers every few months, it became the treatment of choice for patients with evidence by RT-qPCR of increasing tumour burden. Groups interested in CML sent their researchers to the laboratories of Professors Cross, Goldman and Melo at Imperial, to learn the techniques and return to their own centres to improve their local management. Many of these individuals are now amongst the most respected CML researchers in the world and include Timothy Hughes (Adelaide) Andreas Hochhaus, (Jena) Andreas Reiter (Mannheim), Paolo de Fabritis (Rome), Francois Mahon (Bordeaux), and Michael Deininger (Salt Lake City).

The impact of both qualitative and quantitative PCR assays for the detection of residual disease in CML has had far ranging effects on other haematological malignancies. Now any acute myeloid leukaemia characterised by the presence of a fusion oncogene is diagnosed and monitored by RT-qPCR assays. The best known example is acute promyelocytic leukaemia in which the continuing presence or not of the PML-RAR oncogene after the first course of chemotherapy, determines the intensity of subsequent therapy [1]. Similarly the nature and intensity of therapy of acute lymphoblastic leukaemia, the commonest leukaemia of children, is dependent on the detection or not by RT-PCR of immunoglobulin and/or T-cell receptor gene rearrangements after initial treatment [2]. More recently (2010) RT-qPCR monitoring of the JAK2 mutation has been used to detect residual disease and monitor outcome of both SCT and chemotherpy with JAK2 inhibitors [3].

When the TKI were first introduced into general clinical practice in 2001, patients were initially monitored by blood counts and cytogenetic examinations for the Ph' chromosome. It soon became clear that in the majority of patients the Ph chromosome could not be detected in the bone marrow within 12 months of start of treatment. Discontinuing the drug lead to rapid relapse so there was an obvious need for a more sensitive test to determine the level of residual disease and the potential for long-term disease control. Professor Cross's original engineered competitive RT-PCR test could detect one malignant cell in 100,000 normal cells, a three log improvement over cytogenetic analysis. The advent of commercial equipment for real-time quantitative measurement of residual BCR-ABL1 RNA transcripts meant that this could be measured in diagnostic laboratories without a special interest in CML. As a result the use of RT-qPCR monitoring has become the standard of care for CML patients treated with TKI [4].

A group of recognised experts (including Professors Apperley and Goldman representing Imperial) in the field of CML came together in 2006 through the EuropeanLeukemiaNet (ELN), an EU-funded Network of Excellence, to develop guidelines for the management of CML. These guidelines were updated in 2009 and recommend the use of RT-qPCR for all patients, defined the optimal responder as a patient who had achieved a three log reduction in tumour load, as measured by RT-qPCR at 18 months [4]. The ELN recommendations and definitions of treatment failure were accepted by NICE in 2012 and are used to justify the use of the second and third generation TKI [5]. The latest version (2013) now defines the optimal responder as a patient whose BCR-ABL1: ABL1 ratio as measured by RT-qPCR, has fallen below 10% three months after start of therapy and recommends a change in treatment to a more potent TKI if this milestone has not been reached [6]. This recommendation is based on the Imperial outcome data described above. Those patients whose results demonstrate a tumour burden stably reduced below 4.5 logs should be considered for cessation of therapy. RT-qPCR monitoring, first introduced into clinical practice by the Imperial team, is now the accepted method of monitoring worldwide.

The RT-qPCR test is extremely sensitive and is subject to inherent variation depending on the precise methodology employed. In order to harmonise the test across the world, so that patient outcome can be compared in different countries and in different centres, and to facilitate multi-national multi-centre clinical trials. Professor Goldman has headed an international project to harmonise the assay. Together with Professors Cross (now at Salisbury) and Hughes (Adelaide)

and the UK National Institute for Biological Standards, they have introduced the concept of quality assurance and international standardised results by using local correction factors, and are close to producing internationally available standard materials. Recently this effort was recognised by the World Health Organisation (WHO) as the first genetic reference panel in 2010 [7]. It is notable that the molecular pathology laboratory at Imperial remains the pre-eminent monitoring laboratory in Europe, providing the testing for pivotal phase III commercial TKI studies of imatinib, dasatinib and nilotinib and the UK NCRI CML SPIRIT1, 2 and 3 studies.

The Imperial CML team have always sought to involve patients in their management and to 'own' their results, as manifested by the creation of an annual UK CML day for patients and carers and support for one of their patients to establish the UK CML Support Group. In the past few months the UK CML support group have collaborated with other national groups on a campaign to heighten awareness of the RT-PCR results, known as 'What's my PCR' [8].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[1] Grimwade, D., Jovanovic, J.V., Hills, R.K., Nugent, E.A., Patel, Y., Flora, R., et al. (2009). Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol*, 27 (22), 3650-3658. DOI

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[3] Alchalby, H., Badbaran, A., Bock, O., Fehse, B., Bacher, U., Zander, A.R., Kröger, N. (2010). Screening and monitoring of MPL W515L mutation with real-time PCR in patients with myelofibrosis undergoing allogeneic-SCT. *Bone Marrow Transplant*, 45 (9), 1404-1407. DOI

[4] Baccarani, M., Cortes, J., Pane, F., Niederwieser, D., Saglio, G., Apperley, J.F., Cervantes, F., Deininger, M., Guilhot, F., Hochhaus, A., Horowitz, M., Hughes, T., Kantarjian, H., Larson, R., Radich, J., Simonsson, B., Silver, R.T., Goldman, J.M., Hehlmann, R. (2009). Chronic myeloid leukemia. An update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol*, 27,6041-6051 DOI

[5] NICE guidance (2012). TA241 Leukaemia (chronic myeloid) - dasatinib, nilotinib, imatinib (intolerant, resistant): guidance http://guidance.nice.org.uk/TA241. Archived on 7th November 2013.

[6] Baccarani, M., Deininger, M.W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J.F., et al. (2013). European LeukemiaNet recommendations for the management of chronic myeloid. *Blood*, 122 (6), 872-884. DOI

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[8] http://whatismypcr.org/ (archived on 7th November 2013)