Institution: University of Bristol

Unit of Assessment: 1 – Clinical Medicine

Title of case study: Minimal residual disease assessment in acute lymphoblastic leukaemia allows safe individualisation of chemotherapy and reduction of treatment toxicity.

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

Researchers at the University of Bristol have developed tests to track low-level leukaemia – 'minimal residual disease' (MRD) – in children with acute lymphoblastic leukaemia (ALL) down to levels thousands of times lower than detectable by light microscopy. These tests have become the gold standard for monitoring of leukaemic response in clinical trials. MRD testing has been shown in 2013 to allow safe de-intensification of treatment for one-fifth of patients treated nationally, with substantial savings in toxicity and treatment-related expense. The same techniques have also improved worldwide understanding of how disease clearance is related to success after haemopoietic stem cell transplantation.

2. Underpinning research (indicative maximum 500 words)

Acute lymphoblastic leukaemia (ALL) affects approximately 400 children per year in the UK and is the commonest childhood leukaemia. It develops when a lymphocyte precursor cell undergoes malignant change and copies itself uncontrollably to form the leukaemia clone. This usually occurs at a stage when the cell is going through the process of rearranging its immunoglobulin and/or T-cell receptor genes, and therefore these provide a genetic signature and means of accurate identification. From 1990-2007, research by clinicians and scientists at the University of Bristol demonstrated that these genetic changes allow extremely sensitive detection of low-level leukaemia cells (MRD). The key advances were achieved by identifying each child's leukaemic signature and then using polymerase chain reaction (PCR) amplification to effectively provide a molecular microscope up to 5000 times more powerful than light microscopy.

The researchers involved in these studies included Potter (1990-1993), Steward (1990-2001), Knechtli (1994-98), Goulden (1995-2006), Moppett (1999-2003) and Hancock (2002-2007). The research was funded by more than £5m of grants, principally from the Leukaemia Research Fund. It also attracted conference prizes (British Paediatric Association, British Society of Haematology) and led to the award of six PhDs.

Studies on the fundamentals of leukaemia biology and treatment response

Research on MRD at the University of Bristol elucidated many poorly-understood areas of leukaemia behaviour and management. The Bristol team were the first to prove that bone marrow disease was almost always present at submicroscopic levels in patients who had developed 'isolated relapse' (in either the central nervous system or testes), thereby validating the decision to use powerful systemic chemotherapy rather than localised treatment in such patients.[1] In 1998 they showed that poor early clearance of MRD identified children with a higher risk of subsequent relapse in MRC-funded trials.[2] Bristol researchers were also the first to analyse MRD levels before and after bone marrow transplantation in order to determine why some patients relapsed so quickly after transplantation; these studies have since been widely replicated and reported.[3,4]

Developing a safe system applicable to clinical trials

The first major contribution to recent trials was via a large study of relapsed patients that determined the stability of the genetic signatures with time and how to optimise the detection system for maximum reliability.[5] With Professor Jacques van Dongen and European colleagues, Bristol researchers then founded the EuroMRD group in 2001 to agree the genetic targets and technical aspects of MRD detection. These have since formed the basis for inter-patient comparison and treatment stratification in UK MRC-funded trials for first line treatment, relapsed

and infant leukaemia (ALL 2003, UKALL R3 and Interfant-06) and BFM group trials in mainland Europe. Previous University of Bristol MRD researchers now act as the Chief Investigator for UKALL 2011 (Goulden) and the clinical and molecular MRD co-ordinators respectively (Moppett, Hancock). MRD quantitation has been used successfully to determine the safety and efficacy of individualised reduction of intensification chemotherapy in patients treated on ALL 2003.[6]

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

[1] Goulden NJ, Langlands K, Steward CG et al (including Potter). PCR assessment of bone marrow status in 'isolated' extramedullary relapse of childhood B-precursor acute lymphoblastic leukaemia. Br J Haematol. 1994 Jun;87(2):282-5. PMID: 7947268

[2] Goulden NJ, Knechtli CJ, Garland RJ et al (including Langlands, Hancock, Potter, Steward). Minimal residual disease analysis for the prediction of relapse in children with standard-risk acute lymphoblastic leukaemia. Br J Haematol. 1998 Jan;100(1):235-44. PubMed PMID: 9450818.

[3] Knechtli CJ, Goulden NJ, Hancock JP et al (including Steward). Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood. 1998 Dec 1;92(11):4072-9. PubMed PMID: 9834212.

[4] Knechtli CJ, Goulden NJ, Hancock JP et al (including Potter, Steward). Minimal residual disease status as a predictor of relapse after allogeneic bone marrow transplantation for children with acute lymphoblastic leukaemia. Br J Haematol. 1998 Aug;102(3):860-71. PubMed PMID: 9722317.

[5] Steward CG, Goulden NJ, Katz F et al (including Langlands, Potter). A polymerase chain reaction study of the stability of Ig heavy-chain and T-cell receptor delta gene rearrangements between presentation and relapse of childhood B-lineage acute lymphoblastic leukemia. Blood. 1994 Mar 1;83(5):1355-62. PubMed PMID: 8118037.

[6] Vora A, Goulden N, Wade R (including Hancock). Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013 Mar;14(3):199-209. PubMed PMID: 23395119.

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

4.1 Establishment of methods/early studies

By the early 1990s it was apparent that the gene rearrangements present in the originating clones sometimes underwent further changes that might invalidate MRD detection. Steward et al [5] were the first to publish a series investigating this in detail, showing that the problem could be overcome by tracking multiple rearrangements detected at diagnosis; this approach still underpins MRD analysis to the present day. Collaboration was established with three other European groups in order to develop uniform systems; this led to the formation of the European Study Group on MRD detection in ALL (ESG-MRD-ALL, now EuroMRD; Hancock, Goulden and Moppett were founder members) and to the development of agreed testing systems in Europe. Euro-MRD now embraces 43 laboratories in Europe, Australia, the US, Japan and Israel.

4.2 Underpinning Modern Clinical Trials in Paediatric and Adult ALL

Studies of patients in the UK being treated on standard MRC leukaemia protocols showed that patients with isolated extramedullary relapses of ALL almost invariably had bone marrow disease [1] and that slow early clearance of disease was strongly correlated with subsequent relapse ([2] and output [a]). The research to demonstrate this had been part-funded by the Leukaemia

Research Fund (LRF, now Leukaemia & Lymphoma Research) and led to the LRF establishing ongoing support of MRD studies in all UK trials of ALL therapy. A network of national laboratories was established to perform this work on a regional basis, managed and funded via the central laboratory in Bristol. In 2007 routine MRD testing was transferred to the NHS Pathology Laboratories at Southmead Hospital, Bristol (where it is coordinated by Hancock and Moppett), thereby taking the technology to fully validated clinical testing.

MRD assessment is now widely regarded as the most sensitive and specific predictor of relapse risk in children with ALL during remission. All children and young adults treated in the UK since 2003 for either *de novo* or relapsed ALL have had their MRD measured and used in the entry criteria and/or randomisation procedures of the following trials: ALL 2003, UKALL R3 (relapsed and refractory ALL, open until 31/12/13, output [b]) and Interfant-06 (infant ALL, open until 30/6/14).

The MRC ALL 2003 trial, which ran from 2003 to 2011 and involved 3207 patients, reported in 2013 ([6] and output [c]); 521 children and young adult patients assessed as being at low risk on the basis of MRD assays were assigned to receive either one or two delayed intensification (DI) chemotherapy courses. There was no significant difference in outcome. The importance of this is highlighted by the fact that there were 74 episodes of grade 3-4 toxicity affecting 45 patients (17% of the whole cohort) in those who received two DI courses. The future use of a single intensification course for MRD low-risk patients will therefore avoid much unnecessary toxicity and hence patient suffering and cost (output [d]). The current ALL trial, UKALL 2011, is built on this finding and again utilises MRD as the critical determinant of stratification/randomisation (output [e]).

4.3 Rationalising use of haematopoietic stem cell transplantation (HSCT)

HSCT has conventionally been applied to those with high-risk features of their leukaemia at presentation or with relapsed disease. However, these procedures carry high transplant related mortality (20-30%) and an average cost of £100-150k per procedure. MRD researchers at Bristol were the first to highlight the strong correlation between persistent high level MRD going into transplant or re-emergence of MRD soon after transplantation with post-transplant relapse.[3,4] In all of the clinical trials mentioned above, MRD is used to determine which patients would go onto haematopoietic stem cell transplantation. Bader (Frankfurt) learned methods of MRD assessment in the Bristol laboratory and subsequently conducted a prospective trial using PCR MRD techniques via the European ALL-REZ BFM Study Group. In 2009 this study confirmed the strong correlation between pre-transplant MRD and outcome in mainland European patients treated on BFM protocols (output [f]). Bader and colleagues are now giving additional donor T-cells to patients with re-emergent MRD after transplantation and have reported successful reversion of incipient relapse (output [g]). Many groups around the world have since studied peri-transplant MRD and the Bristol research is widely cited (examples shown in outputs [a] and [h]).

4.4 Establishment of national leukaemia cell banking

Residual DNA samples from UKALL 2003 MRD studies were used to establish the LLR/CCLG Childhood Leukaemia Cell Bank, now being centralised at the UK Biobank in Manchester. Since inception over 18,090 samples from 3,175 leukaemic patients have been collected, comprising DNA and viable cells from patients on the ALL2003, ALLR3, ALL97 and ALL2011 interim protocols. This collection will play a pivotal role in future UK leukaemia research.

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

[a] The contribution of Bristol MRD clinicians/scientists in outlining the importance of MRD analysis at end of induction and in the pre- and post-transplant setting is explained by inclusion of the references 1, 26, 29 and 39 in the current essay on "Clinical use of MRD detection in ALL" in UpToDate, written by US clinicians, corroborating 4.2 and 4.4:
http://www.uptodate.com/contents/clinical-use-of-minimal-residual-disease-detection-in-acute-lymphoblastic-leukemia

[b] and [c] MRD assessment has been critical to the study design and randomisations in the most recent UK trials for *de novo* and relapsed childhood ALL, corroborating 4.2. The role of MRD in these protocols is outlined in two trial summaries from www.ClinicalTrials.gov:
ALLR3 (http://clinicaltrials.gov/ct2/show/NCT00967057)
MRC ALL2003 (http://clinicaltrials.gov/show/NCT00222612)

[d] MRD testing in the ALL2003 trial showed that a low risk group of patients could be identified and treated with just one course of intensification therapy, reducing costs and side effects. This corroborates 4.3 and is described in reference [6].

[e] The current ALL trial, UKALL 2011, utilises MRD as the critical determinant of randomisation, corroborating 4.3:
https://leukaemialymphomaresearch.org.uk/information/childhood-leukaemia/acute-lymphoblastic-leukaemia/treatment#UKALL%202011

[f] The Bristol demonstration of the importance of pre-transplant MRD has led to wider international study, corroborating 4.4, as shown by this reference from the European BFM Group:
Bader P, Kreyenberg H, Henze GH et al. Prognostic value of MRD quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol. 2009 Jan 20;27(3):377-84. PMID: 19064980.

[g] Post-transplant MRD analysis has allowed post-graft immune manipulation to reduce relapse rates, corroborating 4.4, as detailed here:
Pulsipher MA, Bader P, Klingebiel T, Cooper LJ. Allogeneic transplantation for pediatric acute lymphoblastic leukemia: the emerging role of peritransplantation minimal residual disease/chimerism monitoring and novel chemotherapeutic, molecular, and immune approaches aimed at preventing relapse. Biol Blood Marrow Transplant. 2009 Jan;15(1 Suppl):62-71. PMID: 19147081.

[h] The work on pre- and post-transplant MRD led to inclusion in a major textbook on transplantation, corroborating 4.4:
Clinical Bone Marrow and Blood Stem Cell Transplantation, 3rd Edition (ed. K Atkinson) p 1671 references Knechtli et al and an original figure is reproduced as figure 105.11. Can be supplied on request.