Institution: University of Bristol		
Unit of Assessment: 1) Clinical Medicine		
Title of case study: Childhood leukaemia survival rates increase thanks to new cell preservation methods and international cell bank		
Period when the underpinning research was undertaken: 2000 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Allison Blair	Principal Clinical Scientist	09/2010 – present
Nicholas Goulden	Consultant Senior Lecturer	01/1999 – 07/2006
Charlotte Cox	Honorary Research Assistant	09/2000 – 06/2015
Paraskevi Diamanti	Honorary Research Associate	01/2005 – present
Benjamin Ede	Research Associate	04/2019 – present
Period when the claimed impact occurred: 1st August 2013 - 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Research by the University of Bristol has led directly to systems for processing and storing leukaemia cells, which have led to lifesaving and life-changing treatments for over 430 children with acute lymphoblastic leukaemia (ALL) in the UK and USA. These treatments are the result of research enabled by CellBank, a UK repository of paediatric leukaemia cells which uses the processing systems developed by Bristol.

Vital insights from these CellBank-enabled projects have changed ALL treatment protocols in Europe, North America, Australia, New Zealand and Saudi Arabia. An intensified treatment approach has reduced relapse rates for children with a particular genetic abnormality from 75% to 20%. As a result, 5-year survival rates for this patient group have increased from 29% to 78%, saving the lives of at least 344 children in the UK and USA alone. Further, a lifesaving drug for an otherwise fatal form of ALL has been identified and is undergoing phase 1 clinical trials. In addition, new sample processing and distribution facilities for NHS diagnostic laboratories, has saved the NHS time and resources, improved patient experience and reduced costs for patients' families.

2. Underpinning research

Need for improved diagnostic testing and translational research in paediatric leukaemia

Approximately 1 in 20,000 children suffer from leukaemia. Improvements in therapy over the last 15 years have enabled remission rates of over 90%. However, 15-20% of children diagnosed with ALL suffer a relapse, due to a failure to entirely eradicate the leukaemia cells during treatment. Relapsed ALL accounts for 50 deaths in the UK per year. Unfortunately, it can be difficult to obtain accurate diagnoses of ALL, due to the poor quality of cell samples taken from children, particularly infants (babies up to 18 months old). Therefore, some indicators of high-risk disease may be missed by clinicians, and children may be treated on less intensive/inappropriate protocols.

Leukaemia cells are extracted from patients, primarily for diagnostic testing. Excess material can be frozen, in a process called cryopreservation, to enable long-term storage for future clinical investigation or research. In the past, paediatric leukaemia cell samples deteriorated very quickly (in less than 3 days), which had very real implications for clinicians and scientists; cells extracted late on a Friday, for example, would be no longer suitable for analysis by Monday. In addition, the recovery of viable cells from frozen samples was substandard, which severely restricted research and consequently, understanding of ALL biology was limited. Research at the University of Bristol, led by Dr Blair, developed methods to enhance survival of patient samples for up to 8 weeks and improve the recovery of viable leukaemia cells for analysis following cryopreservation.

Development of cell growth and cryopreservation techniques

Cell growth. Early studies funded by the Leukaemia Research Fund (now Blood Cancer UK) and the Department of Health (DoH, 2001-2004), described a tissue culture assay that maintained the extracted ALL cells and permitted their growth over an 8-week period. This was the first description of long-term culture of paediatric ALL cells and the first description of stem cell populations in this malignancy [1-3]. Importantly, the leukaemia cells remained in good condition, buying valuable time to complete investigations. This system led to an important increase in the number of cells that can be tested, from an average of 730 cells using standard techniques, to >4,200 using the culture assay.

Cryopreservation. The group also developed methods for freezing and subsequent thawing of paediatric ALL samples, so frozen material could be used for research and clinical evaluation (2002-2004). The standard operating procedures (SOPs) developed for cryopreservation of leukaemia samples allowed at least 90% of the cells to be usable upon thawing, which was hitherto impossible. Notably, this permitted researchers to investigate leukaemia cells in more depth. Subsequent studies, funded by DoH and charitable trusts (2004-2018), used cryopreserved samples to reveal that ALL was more complex than other leukaemias in that many cell types could act as stem cells to initiate the disease [4,5]. A critical finding was that some stem cells are resistant to current therapeutic agents and consequently may cause relapse [3,4,5,6]. This work also identified a cell surface marker that can be targeted by immunotherapeutic approaches. The cryopreservation methods transformed the opportunities for storing viable ALL samples and underpinned the development of CellBank – a UK repository of leukaemia cell samples.

3. References to the research

1. **Cox C,** Evely R, Oakhill A, Pamphilon D, **Goulden N, Blair A**. Characterisation of acute lymphoblastic leukaemia progenitor cells. *Blood.* 2004; 104:2919-2925. DOI:10.1182/blood-2004-03-0901
2. **Cox C,** Martin H, Kearns P, Virgo P, Evely R, **Blair A**. Characterisation of a progenitor cell population in childhood T-cell acute lymphoblastic leukaemia. *Blood.* 2007; 109:674-682. DOI:10.1182/blood-2006-06-030445
3. **Cox C, Diamanti P**, Evely R, Kearns P, **Blair A**. Expression of CD133 on leukemia-initiating cells in childhood ALL. *Blood.* 2009; 113: 3287-96. DOI:10.1182/blood-2008-04-154187
4. **Diamanti P, Cox C, Blair A**. Comparison of childhood leukemia initiating cell populations in NOD/SCID and NSG mice. *Leukemia.* 2012; 26:376-380. DOI:10.1038/leu.2011.212
5. **Diamanti P, Cox C,** Moppett J, **Blair A**. Parthenolide eliminates leukemia-initiating cell populations and improves survival in xenografts of childhood acute lymphoblastic leukemia. *Blood.* 2013; 121:1384-1393. DOI:10.1182/blood-2012-08-448852
6. **Ede B,** Asmaro R**,** Moppett J, **Diamanti P, Blair A**. Investigating chemoresistance to improve sensitivity of childhood T-cell acute lymphoblastic leukemia to parthenolide. *Haematologica.* 2018; 103: 493-1501. DOI:10.3324/haematol.2017.186700

4. Details of the impact

Good quality ALL cells are needed to accurately diagnose childhood leukaemia and identify new and effective forms of treatment. Historically, diagnosis and research were hindered by difficulties in preserving extracted ALL cells in good quality and over long periods. Blair's research has made long-term cell preservation possible. In turn, this has enabled new research capabilities and projects which have resulted in changes to treatment programmes in the UK, Europe, North America, Australia and New Zealand and the identification of a drug to potentially treat a fatal form of ALL. At the heart of these international developments is CellBank, a UK repository of leukaemia cell samples which Blair played a key role in establishing and developing.

4.1 CellBank

CellBank provides a high-quality service for collecting, processing and storing cell samples from every child diagnosed with leukaemia in the UK (average 430/year). This world-class resource, the only bank of childhood blood cancers in Europe [A], is used by both clinicians and academics

from across the globe and was established in 2004 by the charity Blood Cancer UK. Blair played a key role in CellBank's establishment by providing the scientific expertise needed to develop its SOPs for leukaemia sample processing and storage, based on her research [1, 2], which was *"instrumental to the quality and capacity of services provided by CellBank, including cell processing services for the NHS*" (CellBank Chair/Director of Paediatric Bone Marrow Transplant Programme, Manchester University NHS Foundation Trust) [B]. The SOPs continue to inform practice to the present day, and Blair plays an "*integral role in the ongoing development and implementation of those protocols in a quality driven manner*" (Business Development Director, UK Biocentre) [C].

CellBank-funded staff process and store every childhood blood cancer sample from all 22 UK treatment centres according to SOPs developed by the Bristol group, which remain the national standard [C]. Around 1,600 biological samples are received each year from around 430 children, at diagnosis and various stages during therapy. As at 2020, CellBank holds over 100,000 samples, comprising bone marrow and peripheral blood cells, DNA, plasma and cerebrospinal fluid, from over 8,200 children and young adults with leukaemia [A].

In 2017, CellBank was awarded the UK Biobank of the Year award by the UK Clinical Research Collaboration Tissue Directory and Coordination Centre (a forum for clinical research organisations) and was runner up in 2019. It was commended as a 'Significant and valuable resource that is firmly established' and noted for 'The very targeted use of the collection'. The panel was particularly impressed with the role of CellBank in coordinating samples to facilitate international research into rare diseases [D].

4.2 New capabilities and infrastructure through CellBank

CellBank has employed 20 staff and created new sample processing and supply services for the NHS and academia [C]. Since 2010, UK Biocentre have processed samples on behalf of CellBank for the NHS. UK Biocentre staff extract DNA from the samples and distribute it to NHS genetic laboratories for diagnostic testing and disease monitoring. Excess DNA is stored and can be sent to laboratories for repeat analyses. This facility: "*saves time and resources for these NHS laboratories, it prevents repeated sampling of patients and ensures accurate diagnoses and monitoring the response to treatment*" (CellBank Chair/Director of Paediatric Bone Marrow Transplant Programme, Manchester University NHS Foundation Trust) [B]. Currently (2020), the NHS requests 20 such repeat samples per year [C].

Further, CellBank has been supplying samples for approved academic research projects and collaborations with pharmaceutical companies, such as Bristol-Myers Squibb, since 2007. This has enabled a large body of academic work that has, in turn, generated vital insights in the search for new life-saving treatments and quality of life improvements for patients (see Section 4.3). In this REF period, 48 national and international projects (Australia, Europe, USA) have utilised samples from CellBank, meaning its clinical benefits are being shared across the world [A].

The success of CellBank, which "*can be put down to Allison's help in creating the protocols that have been used*" (Business Development Director UK Biocentre) [C], has been key to the wider success of UK Biocentre, the service arm of UK BioBank and the organisation that implements CellBank. UK Biocentre runs three other studies that use protocols based on those developed for CellBank [C]. CellBank has further leveraged funds for major projects that contribute to a better understanding of many diseases beyond ALL, and which pave the way for new treatments. The Business Development Director of UK BioCentre explains: "*hosting* [CellBank] *has allowed UK Biocentre to demonstrate … that it can provide a quality driven service and deliver results that contribute to successful publications.* Since CellBank, UK Biocentre has *"gone on to host >100 projects from other organisations … It is without doubt that the CellBank has provided a springboard to attracting £25 million pounds* [in 2014] *in funding from the National Institute for Health Research for the development of the National Biosample Centre* [which opened in 2015] *(run by UK Biocentre)"* and has led to *"successful collaborations with many projects such as the world renowned 100,000 genomes project.*" [C].

4.3 Impacts on patient health

CellBank-enabled research has produced findings that underpin new ALL treatments, as follows:

Intensified treatment for patients with iAMP21 genetic abnormality. CellBank-enabled research has shown the impacts of genetic abnormalities on the outcomes of children with ALL. A study, led by Professor Harrison from Newcastle University (Chair, UK Cancer Cytogenetics), reported a novel detection method for the high risk iAMP21 genetic abnormality [E]. The same group subsequently demonstrated that patients with this abnormality (~2% of ALL cases), had a poor outcome in the treatment protocol used at that time (UKALL2003) [E]. This finding led to a revision of that treatment protocol, so that patients with iAMP21 were identified prospectively and treated more intensively. All subsequent UK childhood ALL protocols (UKALL2011, introduced in 2012, and ALLTogether1, a European protocol which commenced in 2020) use this intensified approach [F]. These protocols were developed and used by clinicians in all treatment centres in the UK. Studies published from UK and international consortia in 2014 and 2015 have shown that this intensified treatment reduces relapse rates from >75% to <20% and markedly improved the 5-year survival rates from 29% to 78%, resulting in at least 344 lives saved since 2013 [G].

Several international groups, including the Children's Oncology Group, which compromises over 200 treatment centres in the USA, 15 in Canada, 8 in Australia, together with Auckland and Christchurch, New Zealand, and Riyadh, Saudi Arabia, have implemented this intensified treatment approach [f]. Under ALLtogether1, this approach for iAMP21 cases is now the standard in many countries across Europe including Belgium, Denmark, Estonia, Finland, France, Iceland, Lithuania, the Netherlands, Norway, Portugal and Sweden [F], with therefore considerable likelihood of saving more lives.

Venetoclax: new therapy for rare, fatal form of ALL. A potential therapy for young children with an extremely rare and uniformly fatal form of ALL has been identified using samples from CellBank. This form arises due to a specific genetic mutation and is fatal within 2 years of diagnosis. There are no known survivors, indicating that current forms of therapy are wholly ineffective for this patient group. CellBank supplied live cells from children with this mutation for an international study (Austria, China, Czech Republic, France, Germany, Italy, Switzerland and Turkey). The study found that these cases overexpressed the BCl-2 gene, which could be targeted by using a drug (Venetoclax) that inhibits the gene [H]. The results have led to an international (Australia, Canada, France, Germany, the Netherlands, Switzerland, UK and USA) phase 1 clinical trial for Venetoclax, conducted by international health services in collaboration with industry partners (AbbVie and Roche-Genentech), which opened in November 2017 [H].

Sprycel: new treatment for Philadelphia positive ALL. CellBank supplied samples from 7 patients to Bristol-Myers Squibb in 2013 for their Sprycel testing programme and international clinical trial. In 2019, Sprycel was approved for use in children with Philadelphia positive ALL (representing 3% of paediatric patients) in Europe, Canada and the USA [I]. It is the only second-generation tyrosine kinase inhibitor approved for paediatric patients one year of age and older with newly diagnosed Philadelphia positive ALL [I]. In combination with chemotherapy, Sprycel reduced relapse risk from 34% to 20% and increased event free survival at 4 years from 49% to 71% [I].

4.4 Economic impacts

While the absolute number of childhood ALL cases is small, the global burden in terms of years of life lost, is substantial. Childhood leukaemia accounts for 34% of all the years of life lost globally (11.23 million), according to the Global Burden of Disease Study 2017. Informal costs for families, associated with care at specialist treatment centres and productivity losses to society are very high. A relevant study in China [j] provides some indication of the scale of costs. This concludes that "*The financial burden faced by … families with a child with ALL*" are "*tremendous*", with average non-medical expenses (e.g. transport, accommodation) amounting to USD5220 per family, and indirect costs (lost productivity through e.g. unpaid leave, reduction in work hours) averaging USD1677. Prevention of relapse in 314 of 392 iAMP21 cases in the US markedly reduced treatment costs. In the UK, preventing relapse saves the NHS around GBP150,000 per patient [B]. The total saving for 38 iAMP 21 cases treated in the current REF period in the UK

alone [B], could amount to over GBP4,560,000, given that 80% cases do not relapse. The global economic burden will also be substantially reduced now that all children with iAMP 21 in the UK, USA and several European countries are treated with this intensified approach.

5. Sources to corroborate the impact

[A] **Childhood Leukaemia CellBank** (2020). i) Supporting statement – CellBank Administrator
ii) Email correspondence – CellBank Administrator
iii) List of approved CellBank projects

[B] **CellBank, Manchester University NHS Foundation Trust** (2020) Supporting statement - Chair CellBank and Director Paediatric Bone Marrow Transplant Programme

[C] **UK Biocentre** (2020). i) Supporting statement – Business Development Director
ii) Email correspondence – Business Development Director
iii) Email correspondence – Project Manager

[D] i) **Tissue Directory and Coordination Centre** (2017). Biobank of the Year Award
ii) **Tissue Directory and Coordination Centre** (2019). Honourable Mention Certificate

[E] i) **Robinson *et al*** (2007). Intrachromosomal amplification of chromosome 21 (iAMP21) may arise from a breakage-fusion-bridge cycle. *Genes Chromosomes Cancer.* 46, 318-326. DOI:10.1002/gcc.20412
ii) **Moorman *et al.*** (2013). Risk-Directed Treatment Intensification Significantly Reduces the Risk of Relapse Among Children and Adolescents with Acute Lymphoblastic Leukemia and Intrachromosomal Amplification of Chromosome 21: A Comparison of the MRC ALL97/99 and UKALL2003 Trials. *J Clin Oncol* 31:3389-3396. DOI:10.1200/JCO.2013.48.9377

[F] i) **UKALL2011** (2013). United Kingdom Trial for children and young adults with Acute lymphoblastic Leukaemia and Lymphoma 2011 protocol – *p.37.*
ii) ALLTogether1 protocol Version 1.3 June (2020) – *p.77.*
iii) **COG ALL trials**: Heerema *et al* (2013). Intrachromosomal amplification of chromosome 21 is associated with inferior outcomes in children with acute lymphoblastic leukemia treated in contemporary standard-risk children's oncology group studies. *J Clin Oncol,* 31(27): 3397-3402. DOI:10.1200/JCO.2013.49.1308 – *p.3401.*

[G] i) **Harrison *et al*.** (2014) An international study of intrachromosomal amplification of chromosome 21 (iAMP21): cytogenetic characterization and outcome. Leukemia. 28(5):1015-102. DOI:10.1038/leu.2013.317
ii) **Harrison CJ**. (2015) Blood Spotlight on iAMP21 acute lymphoblastic leukemia (ALL), a high-risk pediatric disease. Blood. 125: 1383–1386. DOI:10.1182/blood-2014-08-569228

[H] i) Fischer *et al.* (2015) Genomics and drug profiling of fatal TCF3-HLF positive acute lymphoblastic leukaemia identifies recurrent mutation patterns and therapeutic options. Nat. Genet. 47, 1020-1029. DOI:10.1038/ng.3362 *CellBank thanked in acknowledgements*
ii) ClinicalTrials.gov (2020). NCT03236857: **A Phase 1 Study** of the Safety and Pharmacokinetics of Venetoclax in Pediatric and Young Adult Patients With Elapsed or Refractory Malignancies

[I] i) ClinicalTrials.gov (2020). NCT01460160: **A Phase 2 Multi-Center, Historically Controlled Study** of Dasatinib Added to Standard Chemotherapy in Pediatric Patients With Newly Diagnosed Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia
ii) **Bristol Myers Squibb** (2019) Press release: Bristol-Myers Squibb's Sprycel® (dasatinib) Tablets Now Approved
iii) **European Medicines Agency** (2019). Sprycel
iv) **Shen *et al.*** (2020) Effect of Dasatinib vs Imatinib in the Treatment of Pediatric Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA Oncol, 6(3):358-366. DOI:10.1001/jamaoncol.2019.5868

[J] **Ren Y, Li X.** (2019) Direct and indirect costs of families with a child with acute lymphoblastic leukaemia in an academic hospital in China: a crosssectional survey. BMJ Open 9:e030511. DOI:10.1136/ bmjopen-2019-030511