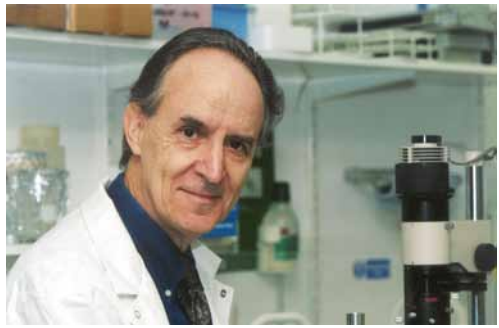
A SCIENTIST'S JOURNEY

Mel Greaves



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I have been very fortunate to have spent most of my professional life doing research on childhood leukaemia, although as a young biologist, I could so easily have taken a different path. We all make these choices by accident or design and in my case, there were three compelling reasons. First, as a biology undergraduate at University College London (UCL), I was inspired by the Nobel Prize winner, Sir Peter Medawar. He made me believe that fascinating and challenging science could be coupled to important medical problems. Second, I discovered, pretty much by accident, around 1973, that childhood leukaemia was a problem crying out for some biological investigation. Clinicians, in particular, Donald Pinkel, were beginning to make real inroads into the therapeutic control of childhood leukaemia. However, it was evident that there was a wall of ignorance about the basis of leukaemias' clinical variability, its biology and causation. So, at that time, an opportunity existed and, serendipitously, immunology, the subject I had specialised in, provided a passport into that territory. Thirdly, and probably most significantly, at the time I was first confronted, in a London children's hospital, with pale and bald 2 to 5 year olds, in a leukaemia treatment ward, my own son and daughter were of the same age. Children at

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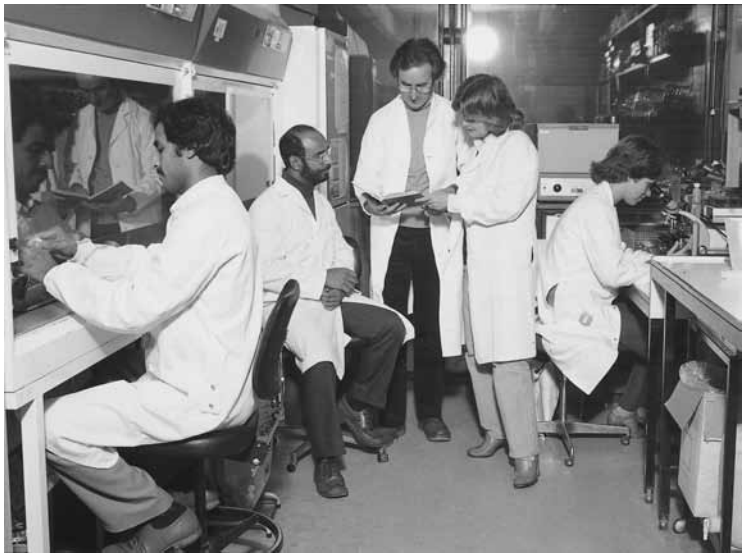

Fig. 11. Lab shot 1980. Author in centre.

that age are very special indeed and it was impossible not to imagine, if only fleetingly, your own family in the same position. Hooked!

A year or so later, my decision was strongly endorsed by meeting a girl cured of leukaemia. She was at a special school for educationally subnormal children where my wife was working. Her father turned out to be a fellow biologist at UCL. More to the point, her leukaemia had been cured but her life had been effectively sabotaged physically and mentally, probably due to the irradiation to the brain, part of the treatment given. This brought home, vividly, the message that potentially curative therapy was an incredibly blunt instrument with harsh potential for collateral damage. And that much remained to be done.

Questions, Questions and Yet More Questions

"*Progress in science consists not so much of finding the right answers… as of deciding what questions are sensible*". Joseph Needham, 1936

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Over the years, I have observed some superb scientists plying their trade. One thing they all seem to have had in common was the ability to synthesise the essence of a problem and come up with a laser-sharp but intrinsically simple question that goes straight to the heart of the matter. They ask the right question. And, invariably, the answer then appears to fall miraculously onto their plate. It may surprise lay readers to be told that this is not the way most scientists usually operate. This begs the question, of course, of what constitutes the right question. By definition, it has to address the central core of a problem and have the capacity, should it be answered, of resolving it. But in addition, it has to be a question that is ready or ripe for answering. As in music, sports, comedy and much else in life, timing is everything. A question that is ready for resolving is one where the essential framework of knowledge or understanding, though incomplete, is sufficiently mature that the experiment to test it can be intelligently designed. And, critically, there has to be the appropriate enabling technology. Creative ideas are the lifeblood of science but without technical innovation as the vehicle, nothing really moves ahead to convert speculation into fact. This has been especially true in the biology of leukaemia. So, in what follows as my personal narrative of a journey with leukaemia, the milestones are marked by questions as much as answers. These all relate ultimately to the same concerns that a parent of a patient would have: what exactly is leukaemia? What is wrong with the white blood cells? When did the white blood cells start to go wrong and what caused it? Or more pointedly: Why is it that my child has leukaemia?

It would be great if the answers to these important questions were to be simple:

— All leukaemias are one distinct disease entity with a single cellular defect — call it X.
— It is invariably caused by exposure to just one thing — call it Y.
— And it can be cured by a pill — call it Z.

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You can make this kind of simplistic argument for many infectious diseases, tuberculosis for example. But leukaemia, and cancer in general, is just not like that. The challenge for any biologist tackling a biomedical problem of this kind is to take on board the inherent complexity, indeed to exploit it, but then to seek to distil out the major principles or consistent features involved.

You can only begin to do this if you are either naïve or very optimistic that such challenges are resolvable. You can only make progress if you are dogged, persistent and resilient to setbacks. In for the long haul, one step at a time. My apologies if this sounds like the attributes of a round-the-world lone yachtsman. It is not actually like that. Progress made in science does depend upon the imagination and drive of individuals. However, its execution and success is a highly social activity dependent upon teamwork, international collaborations and the constant reiterative search for consensual solutions. It demands the slow and careful construction of a plausible narrative. Leukaemia, AIDS, global warming … these are all similar types of challenge.

What follows is a personal narrative. It will appear self-referential and disguise the fact that everything that is done and every advance that is made exploits, to some extent, knowledge and insight provided by other fellow travellers.

Who's Who?

As outlined in the introductory chapter, for a hundred years acute leukaemias have been divided into two distinct types defined by simple, anatomical features of the cells visible down a microscope of modest magnification. Lymphoid-looking or myeloid-looking large (blast) cells gives us acute lymphoblastic leukaemia (ALL) and acute myeloblastic leukaemia (AML). These correspond to the two major types of white cells in our blood. For a long time, these two types of leukaemias were each treated clinically as if they were homogeneous disease entities. For someone, coming from the research field

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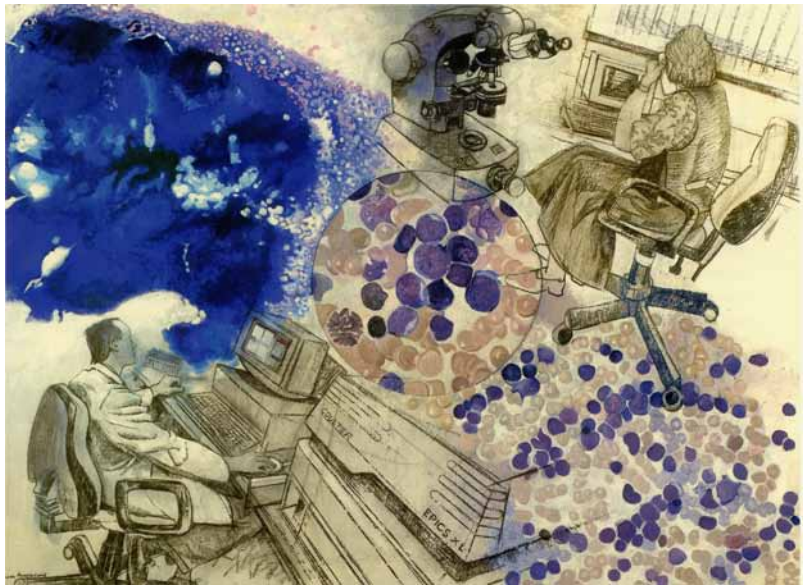

'*Typing the Leukaemic Cells*' by Susan Macfarlane.

"I am shown amazing colours and shapes under the microscope and must try to do them some justice." To confirm that a child has leukaemia a bone marrow examination is essential. A Consultant Haematologist studies small samples of bone marrow smeared onto glass slides and then stained to assess the degree of marrow infiltration by leukaemic cells. The appearance down the microscope of a bone marrow infiltrated by acute lymphoblastic leukaemia, with the leukaemic cells stained blue, is shown in the painting at three different magnifications; low power top left, middle power bottom right and high power in the centre. One of the cells at 8 o'clock in the latter group is dividing, known as mitosis. The other member of the laboratory staff in the painting uses a flow cytometer which sorts the leukaemic cells according to any surface 'markers' present. The leukaemic cells have previously been reacted with a large series of fluorescent 'marker' proteins, or antibodies, which bind to any specific 'markers' present on the surface of the leukaemic cells. It is the identification of the specific 'markers' on the leukaemic cells which allows the leukaemia to be accurately typed. Oil on Canvas. 100 × 135 cm. (Courtesy of Euan and Angus Mackay and Dr Geoffrey Farrer-Brown).

of lymphocyte biology, such as myself, it was, a 'no-brainer' to ask exactly what kind of cells they were and how they might relate to the normal developmental biology and cellular hierarchy of the blood cell system. In the early 1970s, we already had the immunological tools to ask that question, as did just one or two other teams, in Paris and at

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St. Jude in Memphis. It quickly became apparent that ALL, the major type of leukaemia in children, could be subdivided into two major lineages — T- and B-cell leukaemia which corresponded to immature cells or precursors in those two normal lymphocyte lineages. We were able to classify ALL then into T precursor (T) ALL and the more common (∼75%) variant, B precursor or common (c) ALL (see Fig. 3 in introductory chapter). We were also able to generate, for the first time, antibodies that specifically identified cALL. A third infrequent (5%) subtype of ALL was prevalent in infants and of indefinite lineage but was later found to be a very primitive B lineage progenitor (pro-B ALL). A final fourth subtype was very rare (∼2%) and had a mature B-cell phenotype.

The consequences of these relatively straightforward descriptive observations were considerable. Firstly, they led to the establishment of a national immuno-diagnostic service for childhood ALL in the United Kingdom. This referral centre used panels of discriminatory antibodies coupled with what was at the time a very novel automated single cell flow system using laser light to identify cells. These methods are used throughout the world today for the diagnosis and classification of leukaemia. Secondly, we were able to exploit the ongoing clinical trials for leukaemia in the 1970s to demonstrate that these subtypes had different prognostic outcomes. cALL was found to have a relatively good outcome, while pro-B infant ALL and B ALL had very poor outcomes. The treatment was subsequently modified or tailored to the various subtypes. Thirdly, these first tentative steps into the biology of childhood ALL suggested that very immature blood cells somehow become arrested in their normal development. Years later, we learned more about the biochemical mechanism that traps cells in this state of continuous proliferation. To me, at that time, this was redolent of the Queen's comment to Alice:

'Now, here, you see, it takes all the running you can do, to keep in the same place'
(The Red Queen to Alice in *Through the Looking Glass* by Lewis Carroll)

After classifying more than 1500 cases of childhood ALL in the UK, we found out the peak age of incidence for cALL was two to five

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years old. This peak (see Fig. 4 in introductory chapter) of leukaemia incidence in children became something of a personal obsession. Surely the peak was pertinent to the natural history of ALL in children and especially to its etiology? To explore this further, we undertook a rather ambitious plan in the early 1980s to similarly subclassify childhood ALL in many different centres throughout the world to see how universal these age matched subtypes were.

An international consortium was set up with colleagues in the Far East, Brazil, Chile, South Africa and other countries. Essentially, the results obtained in other countries was similar to that in the UK. However, the incidence of cALL in South African black children and Mapuche Indians of Chile was around 10-fold less. They had no marked 2–5 year peak age of incidence. This was a tantalising finding and was a fit with something else. This peak of incidence of childhood ALL was most apparent in the developed or affluent countries. Epidemiological registry data suggested that this peak of incidence of ALL emerged in the UK and USA between 1920 and 1940. However, it appeared later in Japan (1960s) and later still in

Fig. 12. The author (right) with Don Pinkel (left) and Professor David Galton (centre) at a conference in Wilsede, Germany (1982).

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China and in US black children (1970s). There were potentially trivial explanations for this: the disease might be underdiagnosed in certain social or geographic settings, and/or children might have died of pre-emptive infection before their leukaemia was recognised. However, the incidence of ALL but not AML appeared to be associated with affluence. With this in mind, I came up with a speculative idea of what the predominant cause of the major subtype of childhood ALL might be — and then spent much of the next 20 years trying to validate it. More on that later. But first, back to the beginning.

When Does it Start: Early Beginnings?

For common adult cancers, there are epidemiological clues to suggest that the malignant clone usually evolves over many years or decades, mostly in a clinically covert state in unsuspecting patients. Childhood cancers such as acute leukaemia clearly cannot take that long for the problem to surface. For a minority of cancers in children, bilateral tumours of the eye being the best example, their diagnosis early in life reflects the fact that they are kick-started by the inheritance of a mutant gene. This is very rare in leukaemia. So, for a child aged between two and five years old suffering from leukaemia, when is the cancer initiated? The production of white blood cells begins very early during the development of the unborn child — around six to seven weeks in the embryo. The white blood cells are formed first around the major aortic blood vessel, then, in the liver of the developing foetus. Eventually, blood production shifts to the bone marrow, continuing in this tissue after birth and indeed throughout life. So, in theory, leukaemia could begin anytime from the sixth week of gestation in pregnancy. A more tractable idea might be that it could start sometime before birth. I certainly was not the only one to consider this possibility. The problem, as always, was how to ask the question. Some twenty or more years ago, the Director of the Leukaemia Research Fund charity, Gordon Piller, said to me "Well, why don't you just screen hundreds of newborn babies for leukaemic cells?"

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Quite so, but at the time, it just wasn't technically feasible. But the question wouldn't go away and a solution was eventually found.

Twins Show the Way

A key step was the acquisition of a sensitive and specific test for numerically rare leukaemia cells. The chromosomal, molecular abnormalities in leukaemia cells provide just the right marker for such a test. Research in many different laboratories, beginning in the 1980s and still continuing today, has identified a plethora of chromosomal alterations and subtle DNA changes or mutations in leukaemia cells — more than 200 in all. The particular constellation of genetic errors in any patients' leukaemic cells has a major impact on the response to therapy. Indeed, they are probably the main determinant of the eventual outcome since they exert a profound influence on drug sensitivity. For this reason, genetic profiling is now becoming the diagnostic method of choice for sub-classification of leukaemia. Additionally, there is much excitement and capital investment in the idea that the mutant genes of cancer cells might be the Achilles heel providing ideal specific targets for therapy. This idea has led to some success in the treatment of chronic myeloid leukaemia in adults.

Fortunately, a few of these manifold genetic changes are predominant or common. The leukaemic cells from many children with the same subtype of leukaemia, say cALL, will have what appears to be the same chromosome change and underlying molecular lesion or mutation (and therefore similar clinical response). But at the very precise level of DNA sequence changes, each mutation of the leukaemic cells is completely unique. This enabled us to design molecular probes that would only bind to and thus identify the abnormal genes of leukaemic cells of individual patients, even when these are incredibly few in number. We should then be in business for very sensitive detection of leukaemic cells. But were we? How can we *back*track in time with a patient that is already diagnosed with leukaemia? Just weeks before being diagnosed with ALL the patient

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will have appeared to have been a healthy and perfectly normal child. How can you find out what happened five years before?

The answer came from an unexpected source. In the early 1980s, I was running the national diagnostic service for the biological classification of childhood leukaemia linked to the ongoing MRC clinical trials. Over a period of two weeks, we had two bone marrow samples arrive for screening from the same hospital Great Ormond Street Hospital in London (GOSH) and with the same surname. I thought this might be a repeat sample on the same patient so I telephoned a clinical colleague only to be told that, no, they were from identical twins, both of whom had ALL. I had never heard of such a double diagnosis before and was intrigued enough to look into past clinical reports of twins with leukaemia. I discovered that the first such twin pair was recorded in Germany in 1880. Since that time, there had been around 50 or so such cases reported, all of the same sex and likely to have been identical twins. During the 1960s and 1970s, there was a flurry of debate on this topic and various possible explanations profered. The most likely explanation was that the twins, being genetically identical (they are derived from the splitting of a single fertilised egg), had coinherited the same leukaemia predisposition gene. But this seemed at odds with the fact that a high rate of disease concordance was not seen for other paediatric cancers and there was little or no evidence for a greatly increased risk of leukaemia in non-identical twins (who are derived from two fertilised eggs) or in siblings (who still share 50% of their genes) or families generally. One alternative and radical suggestion appealed as very plausible. This was based on the recognised fact that most (though not all) identical twins, whilst in the womb, share a single placenta with vascular connections between them. The consequence of this is that the twins literally share each other's blood and are blood cell chimaeras. It is this arrangement, something of an accident of nature, that gives rise to some of the serious medical complications of twinning. It was speculated that both the twins will have leukaemia due to spread from one twin to the other, via their shared blood supply. But how can you

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show that two twins share the *same* clone of leukaemia cells derived from just one of them? There was no satisfactory way of answering this question in the 1980s. However, if we had our hands on the precise chromosomal change that actually initiated the disease, then this might be resolvable. We began a very slow process of collecting samples on twin pairs from a network of colleagues from all over the world. This kind of project takes time. If the chance of any child having leukaemia is around one in two thousand, then the probability of having a pair of twins in a family, both with leukaemia, is something like one in two million.

I should interject something personal here. Lest I sound like a cold blooded scientist, let me recall how I felt about pairs of twins with leukaemia. In a nutshell, schizophrenic. On the one hand, I was excited that we might have a crucial lead in discovering how leukaemia develops. On the other hand, I felt very sad for the parents of children with leukaemia and even more so for parents of twins with leukaemia: a double burden. As we progressed in our research, we uncovered the world's only triplet pairs with leukaemia. How bad can things get? In one set of triplets from Manchester, the two identical twins who shared a single placenta both had ALL, the non-identical twin with her own placenta was healthy. In the other set of triplets from the Slovac Republic, all three girls shared the same placenta and, tragically, all three developed ALL.

We first studied three very informative twin pairs of infants with the pro-B variant of ALL These came from Chile (see Fig. 13(A)), Guatemala (via St Jude Children's Hospital, Memphis) and Scotland. By that time, we already knew that a particular DNA change or gene alteration was common in infant ALL. The gene involved had the code name *MLL* and we had already shown that in a set of unrelated infant patients with this leukaemia that the precise position in the DNA sequence of the gene where it was damaged was unique to each patient. Therefore, specific probes could be developed.

The excitement for me here came not so much from the answer, which I sensed was obvious, but from the realisation that the question

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that we always wanted to ask could be addressed. Seeing for the first time that the leukaemic cells from each pair of twins shared exactly the same unique mutation was one of those rare eureka moments. The critical point here was that such a unique mutation was not coinherited by the twins, and could only have happened once only in a single cell. The only credible explanation was that in these pairs of twins, leukaemia was initiated by a gene mutation in one cell in one foetus with the resultant proliferating clone of cells spreading, via their shared blood, to the co-twin in the womb. Over the next ten years, we confirmed this shared single cell or clonal, pre-natal origin of childhood leukaemia in many other twin pairs with different chromosomal, DNA-based lesions; they all shared the same single initiating event or mishap in their blood cell DNA.

These twin experiments were not without clinical consequence. The type of leukaemia prevalent in infants is highly malignant and the prognosis is very poor. What happened in two of our pairs of twins (including the pair featured Fig. 13A) was this: one twin of each pair was unwell and clinically diagnosed with leukaemia. They were treated accordingly but unfortunately, they did not survive. At the same time, the co-twin was clinically well and the blood picture was normal. However, we decided to look at the bone marrow of the healthy co-twin. What we saw there were unmistakable signs of leukaemia. The co-twin had the same leukaemia, in molecular signature, as the sibling but at a less advanced stage. These children were then given chemotherapy and are alive and well today. We presume that it is because we were able to detect the disease and start chemotherapy early enough.

Now, leukaemia in twins is no different biologically or clinically from leukaemia in non-twinned children. There just happens to be two developing babies nourished by the same placenta. It seemed reasonable to assume that if leukaemia starts before birth in the twin context, the same must hold for other non-twinned children with leukaemia.

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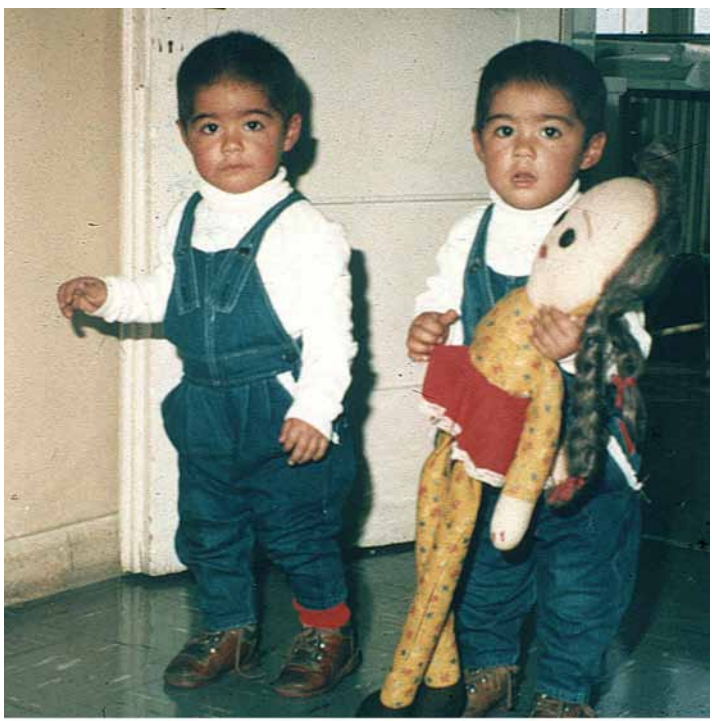
But there was a catch, a serious caveat to consider. For twin children in the peak of incidence of ALL at two to five years of age, the concordance rate is not 100% but 10%. This translates to a risk of around 1 in 10 for a twin whose identical twin sibling already has leukaemia. This is still a 100-fold increase in risk compared to another, non-twinned sibling. It could be argued therefore that concordant pairs of twins are very special and that for the 90% that are *dis*cordant for leukaemia, i.e. only one of the pair has leukaemia, and similarly for the majority of non-twinned children, leukaemia was *not* initiated before birth.

Fortunately, a solution to this conundrum emerged.



(A)

Fig. 13A. Twin patients. Both these infants, from Chile, had acute leukaemia and were the first twin pair in which we demonstrated a common pre-natal origin of the disease, in 1993. (with permission of parents)

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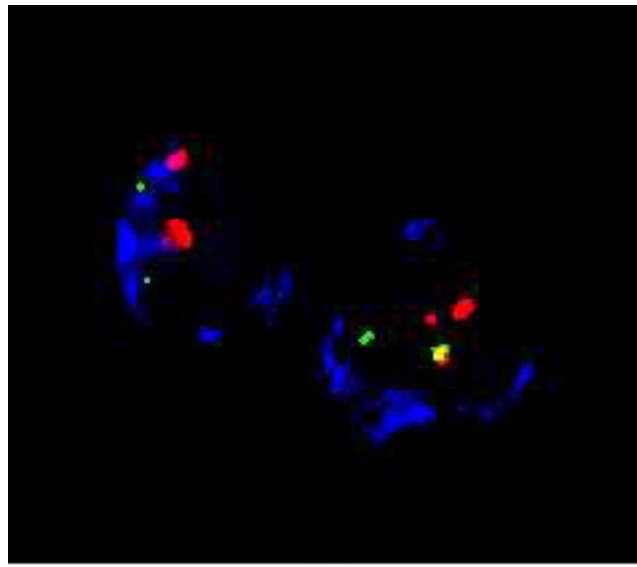
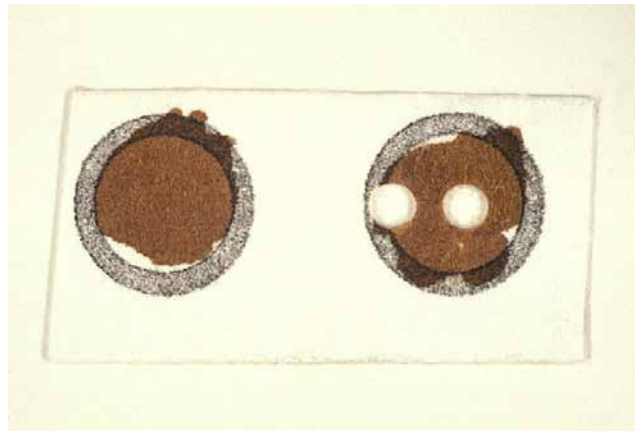


(B)

Fig. 13B. Visualising abnormal genes by I-FISH. I-FISH: *F*luorescence *I*n *S*itu *H*ybridisation to identify specific genes combined with (I) immune antibodies (blue) to recognise cell type. Two cells from a normal baby's cord blood. Cell on the left is normal immature B cell: 2 red genes, 2 green genes. Cell on the right has red/green fusion (=yellow), the marker of chromosome exchange of DNA (=translocation) and leukaemia initiation.



(C)

Fig. 13C. Neonatal blood spots (Guthrie cards). Cards with single blood drops taken in the first week of life: a vital, archived source of blood cell DNA.

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Forensic Blood Spots

Readers will know that from a single spot of blood found at the scene of a crime or on the clothes of a suspect, forensic scientists can identify the source of that blood using very sensitive DNA 'fingerprinting' methods. It turns out that we were able to do something very similar to this for leukaemia. Over a beer at a conference in Germany sometime around 1995, I was telling colleagues that what I desperately needed was an accessible source of blood from newborns. One obvious source was the cord or even placenta itself that was usually discarded (my daughter's placenta was used to fertilise the garden roses). But, ideally, what was needed was a source of routinely stored blood, including that from individuals who eventually develop leukaemia. A clinical colleague pointed out the existence of so-called Guthrie cards (see Fig. 13(C)). These are literally cards onto which a single drop of blood is collected, usually by a district nurse, by heel prick from the baby's foot in the first week of life. The dried neonatal blood spot is archived, often for many years, and is routinely used to screen for an inborn metabolic defect called PKU. This was just what we needed! Don Pinkel told me that when Dr Guthrie was developing the idea of the blood spot cards 50 years ago, his paediatric department chief told him that he should stop his research as it had no possible relevance to leukaemia. A nice twist in the story as it has transpired.

In the 1980s, it was discovered that the DNA from the white blood cells in the dried blood spot remains relatively intact. It was thus possible to extract the DNA and test it for inherited mutations involved in, for example, sickle cell anaemia or cystic fibrosis. In such situations, every white cell in the spot (some 30,000 in all) will have carried the mutation making detection relatively easy. This was especially so when the polymerase chain reaction (PCR) method was invented by the Nobel Prize-winning and the somewhat maverick scientist, Kary Mullis. This technique can generate millions of copies of pieces of DNA or genes present at extremely low levels.

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But the argument we had to make was more demanding. It went like this:

IF	we are right about the origins of leukaemia transmission from twin to twin *in utero*, then leukaemic cells had to be present in their blood at some time (and not just in their liver or bone marrow where they are probably spawned);
and IF	leukaemia in twins is biologically exactly the same as leukaemia in most non-twinned children;
THEN	the leukaemic mutation might be detectable in the archived blood spot of a patient with leukaemia;
PROVIDED	that the mutation used as a marker for the leukaemic cells really was the first or initiating genetic change, rather than a later (post-natal) alteration;
and IF	the number of leukaemia cells in the blood at birth, and several years before the disease was diagnosed, was, at the very least, present at more than 1 cell per single blood drop — or 1 in 30,000 white cells;
and IF	the PCR test is working optimally to detect very few mutant cells (not a given).

This looks like a rather too long list of ifs and buts. But this is how science operates. The art form is using some judgement to decide if what's being proposed as a question is too much of a long shot or is worth a serious punt. It was, in this case, the latter. It turned out that in most cases of leukaemia in children aged anything between a few months old and 12 years, we could indeed identify the unique leukaemia mutation in their blood spots, thereby proving unambiguously that leukaemia usually starts before birth. Whether it always does so is another matter. It may not, we cannot tell. And, just to make it very clear: the leukaemia mutations in the blood spots, as in the twin cells, are early acquisitions in the developing baby's blood cells. They are not inherited from parents.

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Cords Galore

The very satisfying blood spot result carried another important implication. This was that in the context of the nine out of ten twin pairs that were *dis*cordant for leukaemia, it was likely that the twin who remained healthy harboured leukaemic cells generated and shared before birth, but that some trigger and associated secondary, but still essential, DNA mutations were absent. This accorded with a two-hit model that I had conjured up as a speculative scenario for leukaemia development many years before. This postulated that a minimum of two independent DNA or gene mishaps/mutations in a blood cell, one before birth, one after, had to occur for acute leukaemia to emerge clinically. We have, in fact, only recently been able to show that leukaemic cells with just the one or first genetic "hit" are indeed present and persistent in the blood of a healthy child whose co-twin has ALL (see Epilogue at end of chapter). The two-hit model was important to endorse not least because it would identify two distinct time windows, one before and one after birth when critical events and, possibly, critical exposures might be causing leukaemia. But then the twins' observations prompted an additional thought. If in nine times out of ten, hit number two didn't happen to the second twin already primed for leukaemia by hit one, how often does leukaemia arise before birth but never mature to a diagnosis? Compared to, that is, with the risk of 1 in 2000 for the disease itself. Leukaemia is a rare disease but is its initiation in the womb rare? This is not an esoteric or academic point. Epidemiologists trying to get at the cause of a disease such as childhood leukaemia regard it as a relatively rare occurrence.

In order to answer the question, we then did at long last what Gordon Piller had, with some prescience, suggested we do about 20 years previously. We screened several hundred cord bloods taken at birth of normal healthy babies for the presence of leukaemic cells carrying specific leukaemia-causing chromosome changes — the same genetic changes shared by twins and present in neonatal blood spots of patients with leukaemia. This experiment was a real headache logistically, ethically and technically. So, that was another slog, this

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time for around four years in all. But eventually, we got there in 2002. We could, for the first time, visualise the covert mutation harbouring leukaemic cells (Fig. 13(B)). And the striking numerical result was this: for every single child with leukaemia there appears to be around 100 children born with a leukaemic clone of cells on board but which never sees the light of day, clinically speaking. This finding should change the way we think about leukaemia; at least it did for me. As many as 1 in 20 children may lead perfectly normal, healthy lives, unaware of the presence since birth of what we now call the *pre*-leukaemic population. Cells latent with malignant potential but lacking the full credentials. Just one percent of children harbouring such clones go on to develop ALL.

'*Cytogenetics: A Lesson on Typing the Chromosomes*' by Susan Macfarlane.
"*A Consultant Scientist is showing a trainee the work done in the Cytogenetics laboratory*." Depicted are the microscope, computer and a small square representation of a photo of chromosomes printed against a black background that shows up the luminous colours of the fluorescent DNA probes which identify specific gene defects. Study of chromosome pattern is very helpful in identifying different types of leukaemic cells and predicting the likely response to treatment. Oil on Canvas. 24 × 45.5 cm. (Courtesy of Euan and Angus Mackay and Dr Geoffrey Farrer-Brown).

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Making Babies and Playing Roulette?

Whatever causes the first hit in the womb has to be common; indeed, there is a technical argument, not detailed here, that says the causative exposure has to be ubiquitous or present in all of us as developing babies. My own view reflects a decided preference (or bias perhaps) that I have towards the evolutionary explanations for our vulnerability to medical mishaps. Contrary to what some like to believe, we have not been intelligently engineered by millions of years of evolution to near perfection. The slow evolutionary process, driven predominantly by natural selection of random genetic variations, cobbles the best available solutions together. The inevitable consequence is that we have manifold imperfections, compromises and trade-offs on board. It's nothing short of amazing to me that a normal baby develops from a fertilised egg. But the error rate is high; many early embryos die spontaneously, around 1 percent of us are born with abnormalities and all of us are carrying mutations of one sort or another. This really shouldn't be surprising given the magnitude of the task of making something as complex as a human baby. The architectural challenge of assembling a fully functional baby's body involves massive cell migration and cell death, three-dimensional tissue assembly and billions of rounds of proliferation that require faithful copying, in each cell, of the entire genetic code in the face of constant assault by DNA-damaging products of our internal, metabolic processes. My view is that many childhood leukaemias, and probably most paediatric cancers, may be initiated as a consequence of this natural, error-prone process. That's not to say that the process is not susceptible to outside influence. The fact that babies born with greater than anticipated average weight have significantly increased risk of leukaemia may indicate that maternal diet can influence the number of cells at risk from those otherwise spontaneous events in the developing baby. It may seem counter-intuitive that such a critical process as making a healthy baby should frequently generate mutant premalignant clones. Surely, you might say, evolution itself should have weeded out this sloppy control? It probably

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would have done so if "one hit" had been enough to produce a lethal cancer. But that's the point: one hit is necessary to get the pathological process moving but it is insufficient and in itself harmless. Clearly what really matters, the real bottleneck, is whatever triggers the conversion of the silent pre-leukaemic clone to full blown leukaemia, usually at around two to five years of age at the peak. So what might that be?

What's the Trigger?

The quest for an understanding of the cause of childhood leukaemia is a very long-running saga — 100 years or more. To be frank, we still do not have a definite answer. The explanations paraded are legion. Here is a selection of postulated causal exposures from the published epidemiological literature (Table 1).

Beggar's belief, doesn't it? There are several problems here. One is, with the best will in the world, epidemiologists haven't been able to define the question well enough and have found it difficult to design large, statistically robust and well controlled studies. For a

Table 1. Postulated causal exposures.

- Car exhaust fumes
- Pesticides
- Ionising radiation
- Non-ionising electromagnetic fields
- Electric fields
- Vitamin K injection at birth
- Hot dogs or hamburgers (depending on whether the patient was in California or Colorado)
- Domestic animals
- Organic dust from cotton, wool or synthetic fibres
- Natural light deprivation through melatonin disruption
- Artificial, fluorescent light exposure in hospital neonatal care units
- Parental cigarette smoking
- Maternal medicinal drug taking (during pregnancy)
- Maternal alcohol consumption (during pregnancy)
- Chemical contamination in drinking water
- Infections

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long time, they assumed that childhood leukaemia was one disease with one cause awaiting to be discovered. This has zero chance of being correct. It is not like lung cancer and cigarette smoking; and even lung cancer cause is not quite as simple as it seems.

What the biology suggests is this: that there are several very different kinds of leukaemia and the related cancer, lymphoma. We know that leukaemia can sometimes be caused by high dose irradiation (the Hiroshima bomb experience), by some chemicals (benzene), and by particular viruses (one called EBV for a type of childhood lymphoma, another called HTLV-1 in adult lymphoma/leukaemia in West Africa and Japan). In domesticated animals (cats, cattle and chickens), common leukaemias and lymphomas are mostly caused by specific viruses. Acute leukaemias in children and adults can also arise, most ironically, as a consequence of the DNA-damaging properties of cancer treatment drugs: so cured of one cancer but leukaemia arriving as "collateral damage". In these examples, leukaemias are usually of particular subtypes and are clearly linked to specific and unusual insults or exposures.

So the lesson from this is that there is no universal or single cause of childhood leukaemia. But maybe major and minor or rare causes allied to different types of blood cell cancers. The other message from biology is that leukaemia, as I've tried to describe, develops in discrete stages. This immediately posits the distinction between what might cause initiation (in the womb) and what triggers the disease later in life, more proximate to a diagnosis. I've already explained why I believe that the trigger after birth is the key practical question. But what might that trigger be?

In 1988, I published a highly speculative article outlining what I thought might be a major causal mechanism for childhood ALL. The motivation was finding an explanation for the striking peak incidence of common (B-cell precursor) ALL at two to five years of age that appeared to track with affluence in society. It went like this:

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— Childhood ALL develops in two discrete stages or mutational hits, one before, one after birth. The first "hit" results in a persistent but clinically silent premalignant clone of leukaemic cells.
— The prenatal events, chromosome changes, were effectively accidental or spontaneous, i.e. no external causes.
— The post-natal "trigger" was predominantly an abnormal immunological response to one or more common infection(s) that could be viral, bacterial or either. The striking peak incidence of ALL between two and five years of age is then a reflection of the timing of the trigger itself, which effectively precipitates the disease.

As I've outlined above, our subsequent research showed that the minimal two-hit model was essentially correct. But what about an infectious trigger?

The idea that childhood leukaemia might be caused by infection itself was certainly not new. Back in the early part of the 20th century, clinicians considered this a possibility and observed that the incidence peak at two to five years of age coincided with the timing of common infections such as measles. They were, however, put off the track of an infectious origin by the lack of evidence and, in part, because it was apparent that leukaemia didn't transmit from person to person. Some, however, were probably on the right track, or rather the track I believe is leading to an answer:

> *"We incline on our evidence to the belief that the solution of the problem of leukaemia lies rather in some peculiar reaction to infection than in the existence of some specific infective agent".*
> (J Poynton, H Thursfield and D Paterson, Great Ormond Street Hospital for Sick Children, London, **1922**)

Parenthetically, when I was cogitating these ideas in the mid 1980s, I had an informal meeting with the then Secretary of State for Health, the very youthful Dr David Owen who was himself trained as a doctor. His son had been diagnosed with ALL and he told me he had a strong suspicion that it was triggered by a measles attack. Very

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interesting, I thought. There has, on the other hand, never been any credible evidence to support a *direct* role of infection, i.e. a virus that infects blood cells and converts them into leukaemic derivatives. As scientists discovered in the 1970s that leukaemias in cattle, cats and chickens was caused by particular viruses, the instinctive reaction was: if it's true for domesticated animals, then why not domesticated humans? With colleagues in Glasgow, we've spent ten years using every available molecular probing test to identify an offending, specific virus in childhood leukaemia cells. It does not appear to exist and almost certainly does not exist.

When, in 1988, we suggested an infectious trigger might be the cause of leukaemia, an insightful epidemiologist, Leo Kinlen, came up independently with a similar explanation. In his case, he was concerned to explain the cluster of cases of childhood leukaemia around the nuclear reprocessing plant at Sellafield, in the area of Cumbria, UK. Folklore and conventional wisdom insisted that this cluster must be caused either by exposure of young children to radiation leaks (which certainly occurred) or that children were inheriting leukaemia-predisposing mutations from their dads who worked at the reprocessing plant and themselves had been exposed to radiation. Kinlen suggested a radically different alternative. The village of Seascale next to the Sellafield plant was an unusual social artefact and rapid mixing of families brought together from all over the UK for occupational reasons may have resulted in the contagious spread of a leukaemia-causing virus. Over the following 15 years or so, Kinlen and colleagues investigated more than a dozen other situations of "sudden population mixing", including the construction of rural new towns and army camps. In each situation, they documented a transient increase in childhood leukaemia shortly after the "mixing" occurred. Kinlen favours a specific virus, as found in animal leukaemias, but this, as I've indicated, is unlikely to be the case.

Marked clusters of childhood leukaemia are not common but they have been described several times. One of the first was in the district of Niles, a suburb of Chicago in 1957 to 1960 and the latest is the

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most marked of all: 14 cases over four years where only one would normally be anticipated. And where is this? Around a "top gun" naval air base in the small town of Fallon, Nevada. The answer is obvious particularly if you've seen the Hollywood movies "*A Civil Action*" and "*Erin Brockovich*"? Surely it must be the leaking, carcinogenic jet fuel? Scientists are trying to study the cluster but the place is crowded out with litigation lawyers and filmmakers. The best bet for Fallon is that it is some unusual immune response to infection related to population mixing. But proving the real cause of a cluster such as this is extremely difficult.

Paradox, or Grandma's Wisdom?

If infection is in some way responsible for triggering ALL, then why on earth should it be linked to affluence; surely the opposite should apply — more proverty equals more infection?

It comes back to the unanticipated consequences of evolution to which we are inevitably subjected. The immune system of humans is extraordinarily dynamic. Rather like the brain, it is not born hard-wired but learns, and its component parts become architecturally networked, by experience in early life; in the case of the immune system by natural infection. Paradoxically, it actually needs infection to set it up for proper function and if deprived of these "priming" exposures, it malfunctions. The cellular basis of this exposure-dependent design is relatively well defined in mice, less so in humans. The corollary is this: the way the system has evolved or adapted is to *anticipate* infection very early in life, in the womb and shortly after birth. Moreover, evolution has continually refined the system by Darwinian natural selection. Survivors of past infectious plagues were almost certainly the accidental genetic beneficiaries of more potent anti-infectious responses. So imagine now a Victorian slum-dwelling infant is transported by a tardis to the 21st century Western society city suburb. Where are the necessary infections? Largely eradicated of course. And with some considerable benefit since infant

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and childhood mortality from infectious causes has declined dramatically. But maybe at a cost. A poorly primed immune system can react inappropriately later, say when children mix with their peers at school and inevitably share common infections. Triggering leukaemia via an unregulated immune response in susceptible individuals could, I suspect, be one such consequence. And it may be not the only one. Shortly after this "delayed infection" idea was proposed for leukaemia, essentially the same idea, the *hygiene hypothesis*, was suggested for the epidemic of allergies and type I diabetes, multiple sclerosis and certain other autoimmune diseases of young people, all of which appear to be afflictions of affluent and modern societies. The highest risk for all these problems is in the same region — Scandinavia. This really is an idea that I find irresistible, that we have a slew of modern illnesses that are the paradoxical consequence of progress. And that this is due to a mismatch between our past evolutionary programming (of the immune system) and our current lifestyles. Interesting maybe, and plausible, but is it right?

Scientific explanations often start out as no more than hunches, then there will be a search for supportive evidence. Definite proof can be hard to find and so it has proven to be though we are certainly much closer than we were 20 years ago. If the idea is that it's not a specific virus that causes leukaemia but actually an aberrant, dysregulated immune system somehow stressing the bone marrow and triggering preexistent leukaemia cells spawned before birth, how can this be either endorsed or disproved? It isn't at all easy to pose this question decisively, especially as we have had no idea what sorts of infections might be important. One could guess, from our evolutionary selection viewpoint, mycobacteria (related to those causing tuberculosis) or even common bacterial or viral respiratory infections might be involved, but we really don't know. The idea itself has attracted considerable international and media attention, in part, I suspect because of the dearth of plausible alternative explanations of the causes of childhood leukaemia. But also maybe because it smells of common sense, or grandma's wisdom: "a little bit of dirt does you good"?

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One test we thought might prove informative was formulated as part of a very large and expensive (£15 million plus) nationwide study of children with leukaemia initiated around 1990 in the UK and which epidemiologists, clinicians and biologists, for the first time, got their heads together on this question. A parallel study, replicating the UK effort, was set up some years later in California. Over 1500 children with ALL were studied along with AML, other cancers and, for comparison, 4000 or so normal healthy children. We had a broad brief to look at all possible causes of leukaemia that had been of public concern, including infection, ionising radiation, non-ionising radiation from electromagnetic fields (EMF) (power lines, in house wiring, etc.), chemicals and genetic, inherited factors. Ten years later, the first results emerged. Ionising radiation and EMF appeared to have a minor, if any, role contrary to what some scientists and environmentalists believed, and still believe. This remains a highly contentious and unresolved issue. And one can understand why some parents, including Delena Fernandes and her husband, as she explains (Chapter 6), still focus on what must seem to them an obvious and local hazardous exposure. We cannot say that no cases of childhood leukaemia are ever caused by such radiation exposures, only that the evidence suggests that most are not. The most striking and positive data to emerge from both the UK and Californian studies were that infants who attended playgroups or crèches in the first year of life and had multiple contacts with other children had a reduced risk of childhood ALL. Other studies had similarly shown they had a reduced risk of allergies and type I diabetes. Playgroups are well recognised as hotbeds of infection spread and so the paradox was that somehow infection early in life appears to protect against ALL. But this is exactly what the hypothesis predicted. The results look convincing.

Home and dry? Not quite. Epidemiology is a very complex art form and there are always caveats and contradictory data. The detailed arguments do not matter here. It is suffice to say that more direct evidence is required. So, the hunt goes on. As it happens, we have

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recently uncovered, in the laboratory, a specific biochemical mechanism in which a hormonal product of a very active immune system can selectively stimulate the outgrowth of preleukaemic clones of cells. This certainly endorses the credibility of the aberrant immune response explanation. Now other doors have been opened up by technological innovation, with the human genome project in particular. It is now possible, in a way that was inconceivable 20 years ago, to scrutinise the entire human DNA code for variations in genes between individuals that might increase or decrease susceptibility to disease. This is already paying rich dividends in breast and colon cancers and certain autoimmune diseases. Needless to say, the prospect of being able to identify who is most at risk of what disease is far-reaching. For childhood leukaemia, the definitive, or what I hope will be the definitive, study is being planned just now as I write. The anticipation here is that the DNA variations that confer susceptibility will include genes that encode components of the immune system, or if not, then other functions that signify relevant exposures. For example, if variations in genes that encode enzymes that detoxify certain classes of toxic environmental chemicals turn out to be important, then this would automatically finger the corresponding chemicals as prime suspects. We will just have to wait and see.

I remain, as always, naïvely optimistic that there will be a clear outcome. But if it's not what I suspect, then here's the unwritten rule of scientific life: you have to be prepared to jettison your pet ideas and move on. If I'm right, on the other hand, then the prospect would be for some kind of prophylactic vaccine given to all infants that would prevent a substantial fraction, though not all, of childhood leukaemia. Now, that's a hope worth holding on to.

Journey's End?

Scientific research, and the knowledge base it provides constantly evolves. By the time this book is published, there will be some new insights into the biology of childhood leukaemia. Just in the past few

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years, we have learnt that leukaemic cell clones (and those of cancer in general) are organised in a kind of cellular hierarchy with a very small fraction (< 1%) of primitive founder or stem cells sustaining the disease. This is a critical new insight since these cells, rather than the bulk disease, are the focus of evolutionary changes in the clone over time and must be the essential target for therapeutic elimination. We now see a different and more focussed target (see Epilogue below for a twin 'twist in the tale'). The chase never ends but after thirty or so years of travel in the landscape of childhood leukaemia, it's timely to reflect on where I, and fellow white-coated voyagers, are on the map? Well, we are one heck of a distance from the starting point for sure. But, to be realistic, the ultimate objective is still, tantalisingly, some way off. And, indeed, will remain so as long as some children like Georgie, whose story is told by her mum, Nicola, in Chapter 5 still succumb to their leukaemia. Or while others suffer long term side effects of the treatment and the causes are not ambiguously nailed as avoidable or preventable exposures. But the grounds for optimism are very tangible. We started off this journey in a fog, but, equipped with the extraordinarily illuminating tools of molecular biology and genetics, we can now see what paths to take. There will be false turns, setbacks and surprises, as always in science, but the prospects for more specific targeted and minimally toxic therapy are not just pipe dreams. No magic bullet, no quick fix but an eventual resolution nonetheless. And I have no doubt also that, before long, causation will no longer be a mystery to befuddle scientists, physicians, parents and patients, alike.

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Epilogue: A Tale of Two Twins

Fig. 14. Twins, Olivia and Isabella Murphy. (with permission of parents)

The Murphy family live just outside London. In 2003, Sarah gave birth to identical twin girls, Olivia and Isabella. As infants and toddlers, their development and general health were perfectly normal. But in 2005, aged 2½, Olivia was diagnosed with ALL. This was, to say the very least, bad news, but the mitigating fact was that Olivia's ALL was of the very good prognosis subtype — B cell precursor (common) ALL with the *TEL-AML1* fusion gene. She underwent the now standard two year regime of combination chemotherapy, and at the time of writing (April 2008), she is off treatment, in complete remission, and has an excellent chance of complete recovery. But alas, things are not that simple. Firstly, during her therapy, Olivia developed an attack of shingles (chicken pox) and as a consequence, she lost the sight in one eye. This was almost certainly a consequence of the immunosuppressive impact of the drugs given to combat the leukaemia — a tragic but not uncommon

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consequence of "collateral" damage of non-specific or toxic, drugs. This understandably caused the parents considerable distress. The parents were interviewed as part of a series of TV films on childhood leukaemia made by the company Mentorn and shown on BBC1 in July 2007. In the film, Dad, Jason, recalled how Olivia's loss of sight had shocked and upset him almost as much as the leukaemia itself. He and his wife were terrified that she might be left blind if the other eye also succumbed, which, fortunately, it has not.

Jason and Sarah also vividly recalled another aspect of their emotional engagement with leukaemia that is unique to their situation as the parents of twins and where one twin has leukaemia and the other does not. The twins are genetically identical and before Olivia developed ALL, they looked indistinguishable to anyone but the parents. As the parents watched Olivia transit through all the traumas and difficulties of treatment, they inevitably looked at Olivia's appearance in comparison with how she should and would, without ALL and chemotherapy, have looked like — just like her twin sister. Aside from the temporary loss of her hair, Olivia's growth slowed down such that she was smaller in size than Isabella and, as the parents reflected, she could be taken for her younger sister. Now, off treatment, Olivia's hair has grown back. Curiously, it is a somewhat different colour and more curly than before. The girls do look equally healthy and lovely now.

But then the parents have had another challenge to face. As Isabella is Olivia's identical twin sister, what about her risk of developing ALL? This very worrying question will inevitably occur to any parents in this situation and they will certainly ask their doctor about it. In the relatively recent past, some parents have been misinformed by doctors who obviously had the children's best interest at heart but were uncertain about the underlying biology. I know of contrasting situations where the

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parents have in one case been told words to the effect that "it's inevitable the second twin will get leukaemia", to another where the paediatrician said the chance was "less than one in a million". Both are way off the mark. The first thing that parents should be told is that there is a significantly increased risk but this has little or nothing to do with coinheritance of some "susceptibility" gene by the twins and that, by the same token, any other sibling would have no or very little risk inherited.

When Olivia was diagnosed and with the help of her physician Dr Philip Ancliff at Great Ormond Street Hospital in London, we were able to discuss the actual risk involved and what might be done about it. We know from our longstanding studies that the risk of a "double diagnosis" comes from the sharing of a single placenta in the womb and the consequent sharing of blood. Olivia and Isabella did have a single placenta (some 40 percent of identical twins do not). Based on the available data on concordance of disease in twins with ALL of the common subtype, we calculated that at the time Olivia was diagnosed, the risk to her healthy twin sister Isabella was approximately 10 percent or one in ten. This is of course significantly increased (by 200 times) from the standard risk of any child of one in 2000 but there was still a 90 percent or so probability that Isabella would *not* develop ALL; good or bad odds depending on how you look at it (and whether you're a parent or an observer!). As was detailed earlier in this chapter, ALL begins with one mutant cell arising in the developing baby in the womb but only emerges as a clinical disease if a second, independent mutation arises in the same cell clone within the following few years. The chances are stacked against the second "hit" occurring at all such that in 90 percent of identical twin pairs, when one does have the second "hit" and hence ALL, the other will not. Sarah and Jason were remarkably astute in readily comprehending this complexity, to the point that Sarah

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made a very perceptive observation. This was that although Olivia but not Isabella had ALL, the first hit generating the leukaemic clone could have happened in Isabella. This is absolutely correct as a possibility but there is no way of knowing.

The advice given to the parents in this case was that it would be prudent to serially monitor Isabella's blood for signs of ALL. If the results were consistently negative, then this would be reassuring but if the one in ten chance came up, then at least the disease would be caught very early and the prospects for cure would be very high. This wasn't an easy call for Sarah and Jason. Isabella would have to have blood taken regularly, not a happy prospect for any child or her parents and, additionally, they would, in effect, be in constant anticipation of a possible positive result. Nevertheless, they opted for this course. So, for the past three years, every four to six weeks Isabella has provided a blood sample that has been sent across London from Great Ormond Street Hospital to my laboratory. What we have found has taught us a great deal about the biology of ALL so that the family have made a real contribution to our understanding and deserve our gratitude. What we had long anticipated from our previous twin studies, coupled with the neonatal blood spots, was that Isabella would have, in her blood, what we call the "pre-leukaemic" cell clone. These are cells that are shared by the twins and have "hit 1" mutation, which in Olivia's case, was the common fusion gene called *TEL-AML1*. Two further predictions were as follows. First, that these cells, in Isabella, should only be present at the low level we observed before in newborn cord blood — which would be around one per every 1000 normal lymphocytes. This would be millions-fold less than present in Olivia's blood at diagnosis. And, second, that these rare cells, though harbouring malignant potential, would lack the additional mutational gene changes present at diagnosis in Olivia's ALL cells. This is exactly what was found in the serial

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blood samples of Isabella's blood. From the purely academic perspective, this has given us a unique insight. It also provided us with a source of pre-malignant ALL cells that we are using for further research so that we could determine their inherent risk of evolving to full blown ALL. From the family's perspective, we could reassure them that the presence of such cells, given the twin context, was as anticipated (but previously unproven) and that these pre-leukaemic cells have remained silent, docile, and at the same very low level. So what now? Our twin data set suggest that the 10 percent risk declines with age and will be very low by the time Isabella is a teenager. Most twins develop ALL within one or two years of each other, and in the many twin pairs we have studied, only one has developed ALL much later (age 14, nine years after her sister).

The Murphy twins offered us another unique opportunity. They provided a chance to interrogate different stages of the leukaemic process; to, in effect, watch a process unfolding that is normally invisible. In collaboration with a colleague, Professor Tariq Enver and his team in Oxford, we were able to identify, for the first time, in Isabella, the rare stem cells that drive and sustain the pre-leukaemic phase and which, in Olivia's case, evolved to stem cells generating florid or clinical leukaemia. This stem cell evolutionary process we could show was associated with the acquisition, in Olivia's stem cells (but not in Isabella's), of additional genetic mutations. Stem cells in leukaemia, and other cancers, are the most critical cellular component of the disease. These relatively rare cells continuously spawn large numbers of clonal progeny cells (which cause the pathology), they are the focus for further malignant evolution by mutation and, most importantly, the cells that the therapy has to silence or eliminate. Having a handle on these cells in childhood leukaemia is a very significant advance.

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We published these exciting studies in the journal *Science* at the beginning of 2008. These attracted extensive media coverage in the UK and abroad. Why was this so? We would like to think that the science was innovative and certainly there is a great deal of public interest in stem cells. But, undoubtedly, what really "sold" the story was the role played by two rather special little girls.

Acknowledgements

The author wishes to express his gratitude for those funding bodies and organisations that have generously supported his research over many years. These include The Imperial Cancer Research Fund (now part of CR-UK), The Institute of Cancer Research (London), The Kay Kendall Leukaemia Fund (UK) and the Leukaemia Research Fund (UK). Many thanks also to the many colleagues who have been fellow travellers and wonderful companions on this journey.

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WHITE BLOOD
Personal Journeys with Childhood Leukaemia
edited by Mel Greaves (The Institute of Cancer Research, UK)
ISBN: 978-981-279-039-2 - 2008 World Scientific Pub Co