

TIMELINE

Leukaemia ‘firsts’ in cancer research and treatment

Mel Greaves

Abstract | Our understanding of cancer biology has been radically transformed over recent years with a more realistic grasp of its multilayered cellular and genetic complexity. These advances are being translated into more selective and effective treatment of cancers and, although there are still considerable challenges, particularly with drug resistance and metastatic disease, many patients with otherwise lethal malignancies now enjoy protracted remissions or cure. One largely unheralded theme of this story is the extent to which new biological insights and novel clinical applications have their origins with leukaemia and related blood cell cancers, including lymphoma. In this Timeline article, I review the remarkable and ground-breaking role that studies in leukaemia have had at the forefront of this progress.

Physicians, pathologists and surgeons have pondered the nature of cancer for centuries¹, but real insights into its biological character and successful treatment, beyond early surgical intervention, are the product of scientific advances in the second half of the twentieth century. This began with relatively simple cell biology and animal modelling, but, enabled by technological innovation, has progressed to intimate genomic portraits of the disease and more rational therapeutics. Some of the many steps and the pioneering protagonists crucially involved in this historical progression tend to become anonymous or ignored in the glare of contemporary progress. An historical perspective matters in so far as it can attribute credit in a more equitable fashion and may provide a philosophical and practical framework within which to understand the conditions under which science, and its medical applications, evolve. With the explosion of research in cancer genomics, insights into cancer as a dynamic ecosystem and the optimism for more efficacious, rationally designed therapies, now is a good time to consider how we came to be where we are now. In this Timeline article, I make the case that research into the leukaemias and related blood cell cancers has been pivotal

in providing many of the crucial biological insights and new treatment modalities that shape the current research agenda in oncology (FIG. 1).

Cancer biology

Genetics. We have known since the prescient observations of Theodor Boveri, more than 100 years ago, that cancer cells acquire chromosomal abnormalities, which hinted that the underlying driver of tumour growth is genetic change². During the 1950s and 1960s, chromosomal abnormalities were described as scrambled karyotypes in the metaphase spreads of leukaemic and other cancer cells, but their causal significance remained obscure³. Unquestionably, a major breakthrough was the first identification, through simple microscopy, of a specific chromosomal abnormality consistently associated with one type of cancer — the Philadelphia (Ph) chromosome in chronic myeloid leukaemia (CML). Named after the city in which it was discovered by David Hungerford and Peter Nowell⁴, the Ph chromosome seemed at the time to be a small or partially deleted version of chromosome 21. Improvements in chromosome staining, or banding methods, and the astute observations of Janet Rowley⁵,

led to identification of the reciprocal chromosome translocation involving chromosomes 22 and 9. This was a game changer. Later, the underlying chimeric fusion gene *BCR-ABL1* (REF. 6), and its function in activating the ABL1 kinase⁷, were uncovered; this sequence of basic biological discoveries culminated in successful therapeutic targeting of CML and Ph-positive acute lymphoblastic leukaemia (ALL) via small-molecule ABL kinase inhibitors⁴ (see below).

Although recombinant fusion genes were later described in sarcomas and for many types of cancer⁸, the first to be identified by DNA sequencing were from blood cell cancers: t(8;14) in Burkitt's lymphoma^{9,10} and t(14;18) in follicular lymphoma^{11,12}, both of which involved deregulation of oncogene expression by its juxtaposition to the constitutively active immunoglobulin heavy chain (*IGH*) locus in germinal centre B cells¹³. The discovery that the gene on chromosome 18 deregulated by this juxtaposition to the *IGH* locus (*BCL2*) controlled cell death pathways changed the perspective of cancer as solely a disease of compulsive proliferation and offered up new therapeutic strategies¹⁴.

The prominence of leukaemias and lymphomas in the identification of fusion genes via chromosome translocation owes much to the relative simplicity of their altered genomes and karyotypes, compared with the complexity seen in common epithelial carcinomas. In the more modern genomics period, the first reports of genome-wide expression¹⁵, copy number profiling¹⁶ and sequencing¹⁷ in cancer were all with acute leukaemias, albeit followed in short order by similar studies in common epithelial carcinomas.

The genomics of all major cancer types are now in full investigation. The challenges are to build into the picture the intracлонаl complexity and dynamic shifts in cell population genetics over time in individual patients. The development of tools for serial monitoring of cancer cell populations and their genetics will be crucial for this.

Clonal evolution. Observations by cytogeneticists in the 1960s and 1970s of chromosomal diversity and changes over time, particularly, but not exclusively,

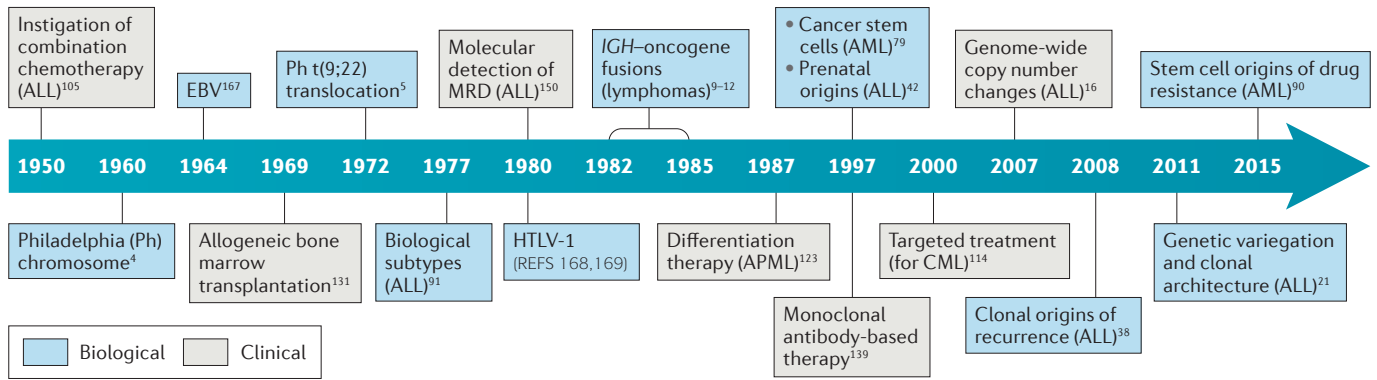


Figure 1 | Milestones in leukaemia research that have paved the way for scientific and clinical cancer discoveries. Discoveries in leukaemia and lymphoma important for the scientific advances in cancer research are shown with a blue background (biological) whereas the roles of leukaemia and lymphoma in leading clinical developments in cancer are shown with a beige background. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; APML, acute promyelocytic leukaemia; CML, chronic myeloid leukaemia; EBV, Epstein–Barr virus; HTLV-1, human T-lymphotropic virus 1; IGH, immunoglobulin heavy chain locus; MRD, minimal residual disease; Ph, Philadelphia chromosome.

in leukaemia, prompted Nowell to synthesize the emerging idea that cancers, although originating from a single transformed cell¹⁸, spawn progeny that genetically diversify and undergo sequential rounds of selection or gain competitive advantage¹⁹. His clonal evolution model (FIG. 2a), has been revisited with twenty-first-century cancer genomics and elaborated into a more dynamic picture of branching clonal evolution in the context of tissue ecosystem (or microenvironmental) and therapeutic selective pressures²⁰. Blood-borne leukaemias lend themselves to single-cell analysis, and genetic profiles of cells in paediatric ALL²¹ first revealed that even a ‘simple’ or diploid cancer can have clonally variegated genetics and a relatively complex branching or nonlinear phylogeny. Similar patterns were observed in acute myeloid leukaemia (AML)^{22,23}, chronic lymphocytic leukaemia (CLL)²⁴ and myeloma²⁵ (FIG. 2b). Clonal complexity was found to be even more pronounced in common solid tumours, including renal carcinoma²⁶, breast cancer²⁷, glioblastoma²⁸, prostate cancer²⁹ and colorectal cancer³⁰. In these tumours, unlike in leukaemia, subclones are frequently topographically segregated in the tissue of origin^{28,31,32}. This reflects space–time evolution and poses a major dilemma for biopsies, which may thus provide a biased sample of the tumour³¹. The development of high-throughput, multiplexed single-cell genetic analysis in leukaemias³³ and single-cell whole-genome sequencing in other cancers^{34,35} has substantially improved the resolution of clonal complexity in cancer.

Nowell’s model implied what evolutionary biologists call selective sweep, with subclonal or single-clone dominance. Acute leukaemias

seldom look like this at diagnosis, nor do many major cancers^{30,36} implying that most subclones have no clear fitness advantages over others. However, in accordance with Nowell’s model (FIG. 2a), further progression of disease may include stringent subclonal selection — or a sweep. Relapses in acute leukaemia^{37,38} or lymphoma³⁹ can be backtracked to subclones in the primary diagnostic sample that are very minor in size. Nowell postulated (FIG. 2a) that disease recurrence or metastatic lesions in solid tumours should be monoclonal in origin and this appears to be mostly correct, though some cancers seed multiple independent metastatic lesions from the primary tumour and multiple independent drug-resistant subclones^{29,40}.

Clonal trajectories or evolutionary histories of cells in cancer can be reconstructed via the phylogenetic trees inferred from the genomics of single, snapshot diagnostic samples^{30,36}. The timing, sequence and clonal architecture of the earliest events may, however, be lost or buried in subsequent clonal expansion. The inherent blood-borne character and relatively brief natural histories of leukaemia have, however, provided a time window on early or founder events that are more difficult to access in solid tumours. Many, if not most, paediatric cancers are likely to originate during embryonic and fetal development⁴¹, but childhood ALL is the only paediatric cancer in which it has been possible to backtrack clonal evolution to ancestral or founder cells that mutate *in utero*⁴². This was achieved through the study of monozygotic twins⁴³ and by PCR-based scrutiny of archived neonatal blood spots of patients⁴⁴ or stored cord blood⁴⁵. Monozygotic twins with

concordant or discordant acute leukaemia have provided unique insights into the natural history of cancer and identification of probable founder or initiating mutations⁴³. This is possible because identical twins who share a single, or monochorionic, placenta (~60% of monozygotic twins) are natural blood cell chimaeras consequent on intraplacental anastomoses⁴⁶. In the leukaemia context, early or preleukaemic mutational events arising in one fetus of the twin pair are shared, but later, postnatal, events are independent. This natural experiment of nature has helped to define the *in utero* versus postnatal sequence of genetic events in childhood ALL^{47–49} and we assume that similar principles apply to other types of paediatric solid tumour.

Ancestral or precursor clones and their genetic lesions have also been identified for AML. These are discernible as clinically silent but numerically expanded clones in the blood of many ageing but otherwise healthy adults^{50,51}. The silent precursor lesions that spawn overt AML continue to exist in the clinical, acute, phase from which they can be isolated via their stem cell-like immunophenotypes^{52,53} and, crucially, they have been shown to selectively survive chemotherapy and possibly provide a reservoir for later relapses^{52–54}, as also documented in ALL⁵⁵. Similar identification of the early stages of epithelial and other common solid tumours is substantially more difficult, exceptions being the identification of clinically silent precursor lesions through autopsy or screening⁵⁶. Recently, however, precursor lesions for squamous cell carcinoma have been identified and genetically characterized by the careful genomic scrutiny of normal skin samples of ageing individuals⁵⁷.

Overall, this Darwinian, evolutionary perspective has changed the way we view the progression of cancer, has highlighted the paradoxical, selective role that therapy can play in the emergence of resistance^{58–60} and has major implications for cancer therapy. It rationalizes our limited success in controlling or eradicating advanced cancers, endorses the concept of early diagnosis and intervention, and provides new therapeutic avenues to explore that exploit cancer's inherently Darwinian character^{58,59,61,62}.

Cellular origins. The precise cellular origin of a cancer constrains the resultant genotypic changes and influences the phenotypic features and clinical responses of the disease in individual patients^{63,64}. It has long been assumed that major subtypes of cancer, for example, breast, skin, prostate, or blood, reflect their origins from the equivalent, normal tissue. But beyond that, we have known very little of the specific cell types in which cancers originate. Historically, pathologists tended to regard the poorly differentiated state of many cancers as indicative of a dedifferentiation process, implying that cancer cells lose or scramble their mature phenotypic features. This interpretation was turned on its head, initially by Barry Pierce and colleagues⁶⁵, who, using teratocarcinomas as a model, posited the idea that cancer could be regarded as a caricature of normal developmental processes in which some of the cancer cell population (ostensibly the stem cells) failed to differentiate. Early evidence for the stem cell origins of cancer derived from the studies of Philip Fialkow¹⁸ using X-linked glucose-6-phosphate dehydrogenase (*G6PD*) as a marker of monoclonality in female patients. This indicated that the cancer clone in CML, despite its relatively mature granulocytic phenotype, originated from lympho-myeloid stem cells⁶⁶. It is now widely regarded that many, if not most, malignant tumours originate from stem or progenitor cells, the development of which is skewed by favouring self-renewal over differentiation. This could, and maybe should, be regarded as a hallmark feature of cancer⁶⁷ but usually is not. The primary evidence for a differentiation block in cancer was derived from studies on blood cell cancers with mouse leukaemic cell modelling⁶⁸, leukaemias elicited with viral oncogenes in chickens⁶⁹ and inferences from the phenotypic features of human acute leukaemias^{52,70} and germinal centre-derived lymphomas⁷¹. In recent years, comprehensive gene expression profiling and mouse modelling have led to the identification of

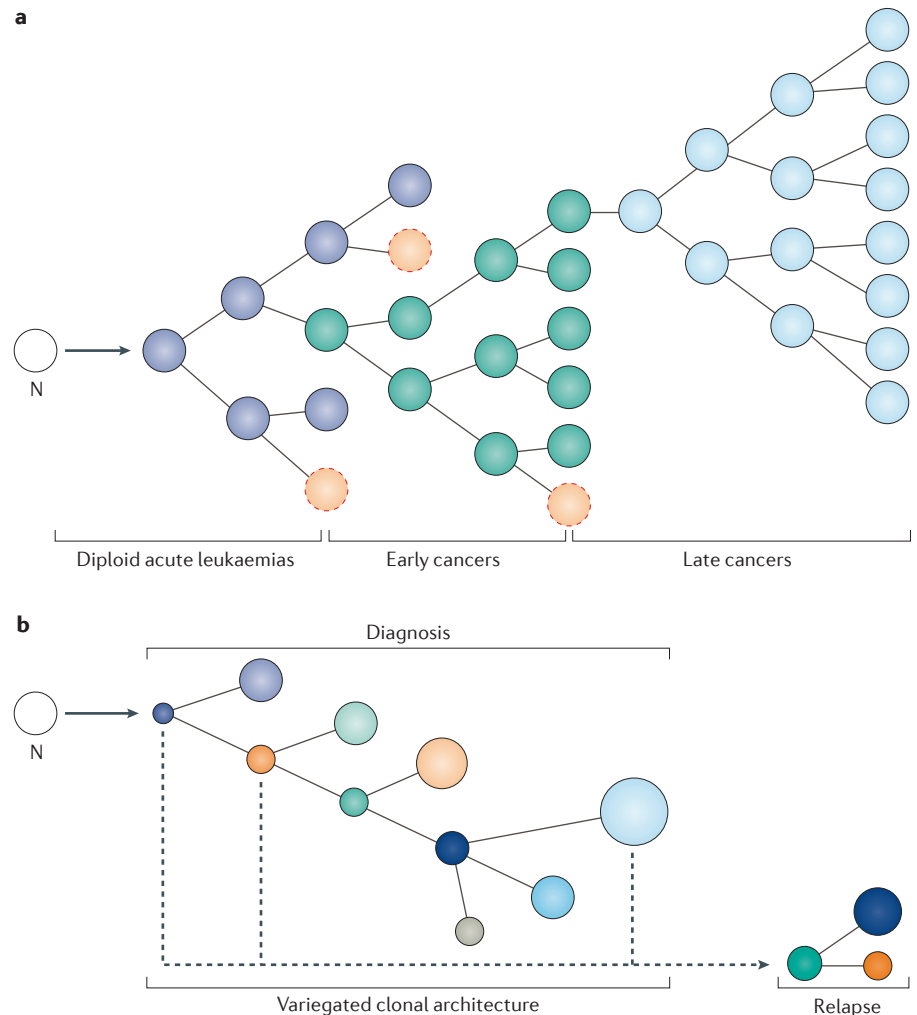


Figure 2 | Clonal evolution models. a | A simplified version of the model provided by Peter Nowell in his seminal 1976 paper¹⁹. Different colours represent acquired mutations except those that are orange with a dashed outline, which are dead. N = normal cells. The model suggests linear, clonal succession. **b** | Clonal structure revealed in acute lymphoblastic leukaemia²¹ (and other leukaemias). This model embodies nonlinear or branching clonal architecture, complex dynamics and the potential for relapse to emerge from early or late, minor or major subclones. Circles of different colours and sizes represent genetically distinct subclones of varying cell population size. Dotted lines indicate that relapse can originate from subclones throughout the evolutionary tree. Note also that relapse clones continue to diversify.

plausible candidate stem or progenitor cells for several non-blood cancers — central nervous system tumours being a particularly good example^{72–74}.

These insights go beyond a purely descriptive value. A focus on the cells of origin helps to rationalize the plethora of phenotypic subtypes of cancer and provides a developmental context for the different subtypes of cancer that we see in the very young compared with adults^{75–77}.

Stem cells as cellular 'drivers' of cancer.

The important idea that most, if not all, cancers, irrespective of their precise cell of origin, might have a stem component that

sustains disease is longstanding, with early supportive experimental evidence from teratocarcinomas⁶⁵ (see Timeline of research in REF 78). Formal demonstration that a subpopulation of self-renewing cells can propagate cancer required the development of an appropriate *in vivo* biological model, and this was first achieved by John Dick and colleagues, using AML, in the late 1990s⁷⁹. Xenotransplantation of human cancer cells into immunodeficient mice, piggy-backed onto a model developed for the study of normal haematopoiesis, has become the standard assay for cancer stem cells⁶⁰. The cancer stem cell hypothesis has proved to be somewhat contentious, principally

because the phenotypes and frequencies of these cells are so variable, between cancers and over time with progression of disease in individual patients^{80,81}. Measured stem cell output also varies according to the particular murine model used^{23,82}. However, this does not seriously challenge the view that cells with self-renewing capacity are crucial cellular drivers of disease and key targets for therapeutic control. Evidence endorsing this view comes from the finding, mostly but not exclusively in leukaemia, that quantitative stem cell burden is prognostic^{83,84} and that self-renewal itself^{59,60} or the stem cell niche⁸⁵ provide tractable therapeutic targets. Cancer stem cells were found to be genetically and functionally diverse in individual patients with ALL^{21,86}. This observation, also seen in AML²³ and now in some solid tumours^{35,87,88}, led to the concept that self-renewing or stem cells are the crucial units, or substrate, for evolutionary selection in cancer, responsible for generating and sustaining subclonal architecture, metastases and drug-resistant recurrence^{59,60,89}. Recent data, again in acute leukaemia, validate the stem cell origins of drug resistance⁹⁰.

The cancer stem cell concept, led by studies in leukaemia, has changed the way we view the cellular epigenetic plasticity within a genetically homogeneous clone. It is a fundamental feature of most, if not all, cancers and sits at the centre of our attempts to develop more effective therapies.

Differential diagnosis and prognosis

Maximizing the efficacy of available therapies and trialling new therapies requires optimal classification of patients into biologically and clinically or prognostically distinct subgroups. In this regard, childhood ALL stands out as pivotal in the advances made. In the 1970s and 1980s, morphologically uniform ALLs were systematically dissected into biological subgroups using cell or lineage selective antibodies and the new technology of flow cytometry⁹¹. This was facilitated by earlier technology for analysing normal lymphoid cells and the ease of access of non-adherent, single leukaemic cells in blood or marrow aspirates. The subgroups identified were classified in comparison with normal lymphoid cell development as of B or T lineage, with the former being divided into a common B cell precursor subtype (CD10⁺) and rarer subtypes that corresponded to very early or pro-B ALL and more mature (cell surface immunoglobulin-positive (IG⁺)) B cells. The latter were then reclassified as B lymphoblastic or Burkitt-like lymphoma, which led to a

radical and successful change in their therapy. The execution of these studies in the context of clinical trials facilitated the demonstration of prognostic relevance^{92–94}, the refinement and standardization of assays (with monoclonal antibody panels) and subsequent modification of treatment schedules for prognostic subgroups. Although the classification schemes that emerged — for ALL and then for B and T cell lymphomas⁹⁵, were, in retrospect, rather simplistic, this was, arguably, the beginning of biologically driven personalized medicine for cancer.

Subtype definition by cell type and/or immunophenotype is still currently used, but more definitive prognostic subgrouping has been forged, again first in the acute leukaemias, by genetic subtyping. This began with chromosome karyotype-level subclassification, but later incorporated probing for specific and recurrent genetic lesions by fluorescence *in situ* hybridization (FISH) or PCR^{96,97}. This has led, via genomics, to better classification of high-risk cases in leukaemia⁹⁸. Many other cancers are now systematically classified into discrete biological and clinical subtypes based on genetic features, striking examples being breast cancer⁹⁹, colorectal cancer¹⁰⁰ and paediatric medulloblastomas¹⁰¹.

Arguably, we still lack accurate prognostication for common cancers with very variable time frames of progression or eventual outcome, prostate cancers being a clear example. One way to remedy this situation would be to use crucial evolutionary parameters of the disease. For example, the probability of a cancer progressing or developing drug resistance might be expected to be dependent on the pool size of potential stem cells and their genetic diversity. The quantitative burden of stem cells does seem to be associated with clinical outcome in several studies (see above) as do some measures of genetic diversity of the whole tumour or paired biopsy samples^{102,103}. Mathematical modelling of drug resistance in the context of clonal evolution would be of value, and CML has provided an ideal test bed for this¹⁰⁴.

Cancer treatment

Many of the outstanding successes of cancer treatment have come from innovative studies and trials in leukaemia. These include combination chemotherapy, stem cell transplantation, ‘differentiation’ therapy, monoclonal antibody therapy and targeted treatment.

Chemotherapy. In the mid-twentieth century, 100% of children with acute leukaemia died and medical intervention was considered

by many to be inappropriate, pointless and unethical¹⁰⁵. A small number of pioneers (FIG. 3) changed this dismal landscape and over several decades the elaboration of drug combinations and schedules (involving antifolates, corticosteroids and cytotoxic drugs) in the setting of large-scale national and international clinical trials led to a high cure rate¹⁰⁵. Overall, this now stands at ~90% for ALL¹⁰⁶ and close to 100% for one major subtype, ALL with the ETS variant 6–runt-related transcription factor 1 (*ETV6–RUNX1*) fusion¹⁰⁷. Childhood leukaemia, along with choriocarcinoma¹⁰⁸ is the first otherwise lethal cancer to be systematically cured by chemotherapy. It is, in this age of hyperbole, a genuine medical triumph.

Success with acute leukaemias, and particularly paediatric ALL, has been extended to Hodgkin’s lymphoma, some non-Hodgkin’s lymphomas, a few paediatric cancers (Wilms’ tumour) and particularly to testicular cancer¹⁰⁹ in young adult males, but to date, has had more limited success in common epithelial cancers of adults. The reasons for this are not entirely clear. Part of the explanation may lie in the fact that malignant lymphoid cells may still retain the apoptosis propensity of the normal cells in which they arise⁷⁵. Additionally, and perhaps crucially, the curable lymphoid cancers, though inherently malignant, have minimal genetic diversity, very little evidence of genetic instability or strong selection for mutations in *TP53* (which encodes p53)¹¹⁰. This may greatly reduce the probability of drug resistance. The reasons for this are uncertain but one possibility is that a lack of causal genotoxic exposure or hypoxic stress in the developing tumour means there is no selective pressure for the expression of these adverse genotypes. Additionally, leukaemias, which usually have a relatively short latency (~2–5 years), readily disseminate and therefore prompt diagnostic symptoms early in their evolutionary trajectory. Testicular germ cell tumours^{111,112} share these clinically advantageous features with lymphoid cancers, but common epithelial cancers do not. Under these circumstances, drug resistance would be expected to emerge much more readily in the latter. The genetic complexity of common adult cancers is the major barrier to successful treatment. Improvements in outcome will require combinatorial therapy and appropriate scheduling to thwart the evolutionary resilience of the disease^{59,113}.

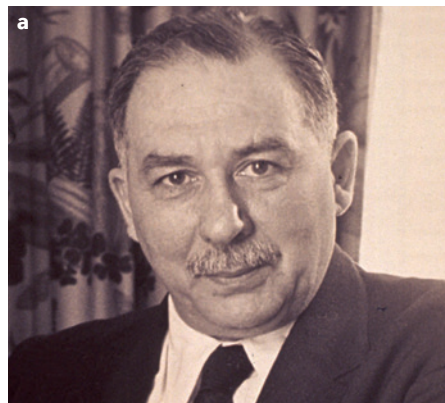
Targeted therapies. Leukaemia has provided the paradigm for targeted treatment in cancer, directed by the underlying

genetic abnormality. Until recently CML was an intractable disease with inevitable progression to a more malignant acute phase, and effective control was only ever achieved via allogeneic bone marrow transplantation. CML is now systematically and successfully controlled by treatment with small-molecule tyrosine kinase inhibitors (TKIs) of ABL, such as imatinib, providing a wonderful example of basic biological insights being translated into very tangible patient benefit¹¹⁴. A uniform or consistent genetic abnormality that encodes an enzyme doubtless made it an attractive candidate for this first exploration of targeted treatment.

TKI treatment does not entirely eliminate all the cancer clones; dormant but reactivatable stem cells persist¹¹⁵. Drug resistance as a result of stem cell dormancy, or quiescence, is a major problem in cancer therapy generally¹¹⁶, and research on CML and other leukaemias is at the forefront of novel strategies to target these robust cells^{117,118}. Relapse in CML can also occur via the emergence of subclones with resistance mutations in the ABL kinase domain. Drug resistance in cancer is predicted, by analogy with antibiotic resistance in bacteria and the Luria–Delbrück principle, to arise by Darwinian selection of mutant subclones that pre-date exposure to drugs⁵⁹. This fundamental principle in cancer was first validated for ABL TKI resistance mutations in CML and ALL^{119,120}.

There was much anticipation that the success of targeted treatment in CML would herald a new era of cancer treatment for the more common adult cancers. This might still happen and remains a major focus of therapeutic research. But, in our enthusiasm, we may have forgotten that CML is different from epithelial carcinomas in terms of genetic complexity. Imatinib and related TKIs work effectively in CML because they target the founder or truncal mutation in every cell and can be administered at a relatively early stage of disease evolution when genetic diversity and the probability of on-board resistance mutations are not too severe. One object lesson from CML that applies to the targeted treatment of common adult cancers might then be to treat as early as possible and to target truncal mutations⁶².

Differentiation therapy. The notion that acute leukaemias originate in stem or precursor cells with imposed decoupling of proliferation and differentiation has been strongly endorsed by the finding that many of the recurrent and early, or founder, mutations driving AML are in genes encoding



Sidney Farber



Donald Pinkel

Figure 3 | Development of combination chemotherapy for leukaemia. Two of the pioneers in chemotherapy treatment for leukaemia, Sidney Farber (part **a**) and Donald Pinkel (part **b**). Other key players in the 1950s and 1960s included Emil (Tom) Frei, Emil Freireich, Gertrude Elion, James Holland and Joseph Burchenal (see REFS 105, 195 for more detail on this historical narrative). Part **a**: image courtesy of the National Library of Medicine; part **b**: reproduced with permission from REF. 105, Pinkel, D. in *White Blood: Personal Journeys with Childhood Leukaemia* (ed. Greaves, M.) Copyright © 2008, World Scientific.

epigenetic regulators of this process¹²¹. This encourages the idea that this altered state might be amenable to modification or reversal for therapeutic benefit. The concept that cancer cell clones can undergo differentiation can be traced back to the innovative studies of Pierce on teratocarcinomas⁶⁵ and, crucially, to studies on murine leukaemia cell lines by Leo Sachs^{68,122}. Translating this into the cancer clinic has been a long haul, but the stand-out success has been with acute promyelocytic leukaemia (APML)¹²³ (FIG. 4). In all cases of APML, differentiation is blocked by fusion genes incorporating the retinoic acid receptors; this led to the idea of over-riding this block with all-*trans* retinoic acid (ATRA), which very effectively restores differentiation competencies. Here, Zhu Chen, Zhen-Yi Wang and their colleagues in China played a major role along with Laurent Degos and researchers in France¹²³. Chinese researchers also discovered that arsenic, perhaps the oldest drug known to man, could achieve something similar¹²⁴. ATRA is now used in combination with arsenic trioxide and the long-term outlook for patients with APML has changed dramatically¹²³.

The success of ATRA and arsenic in controlling or curing APML is probably due to more than simply the reversal of differentiation arrest. Degradation of the ‘blocking’ fusion gene *PML-RARA* (encodes promyelocytic leukaemia–retinoic acid receptor α), and inhibition of stem cell self-renewal with a p53-dependent process, seems to be crucial¹²⁵. There is evidence that the epigenetic block to differentiation is

reversible in other types of leukaemia¹²⁶ and some solid tumours^{127,128}. As we learn more about epigenetic regulation of cancer cell phenotypes¹²⁹, the opportunities for imposing differentiation may become more tractable in many cancer types, as exemplified again by studies in AML¹³⁰.

Stem cell transplantation. Regenerative medicine (stem cell transplant) offers the prospect of effective treatment of a panoply of challenging medical conditions, including blindness and spinal cord injury. This very innovative tactic originated with bone marrow transplantation for leukaemia. Bone marrow or blood stem cell transplantation is essentially a rescue tactic that enables the replenishment of normal haematopoiesis following administration of potentially lethal levels of chemotherapy and radiation. It can also provide the added benefit of an anti-leukaemic, or graft-versus-leukaemia, immunological effect. Human leukocyte antigen (HLA)-matched sibling donor transplants were trialled for late-stage acute leukaemia by E. Donnall Thomas and his Seattle team in the 1970s (for which he was awarded the Nobel Prize in 1990) and its refinement has led to sustained remission and cures^{131,132} (see Timeline of haematopoietic stem cell transplantation in REF. 133). These pioneering clinical studies were underpinned by earlier experiments that demonstrated successful haematopoietic reconstitution of irradiated mice and outbred dogs (reviewed in REF. 131). Haematopoietic stem cell transplantation has expanded

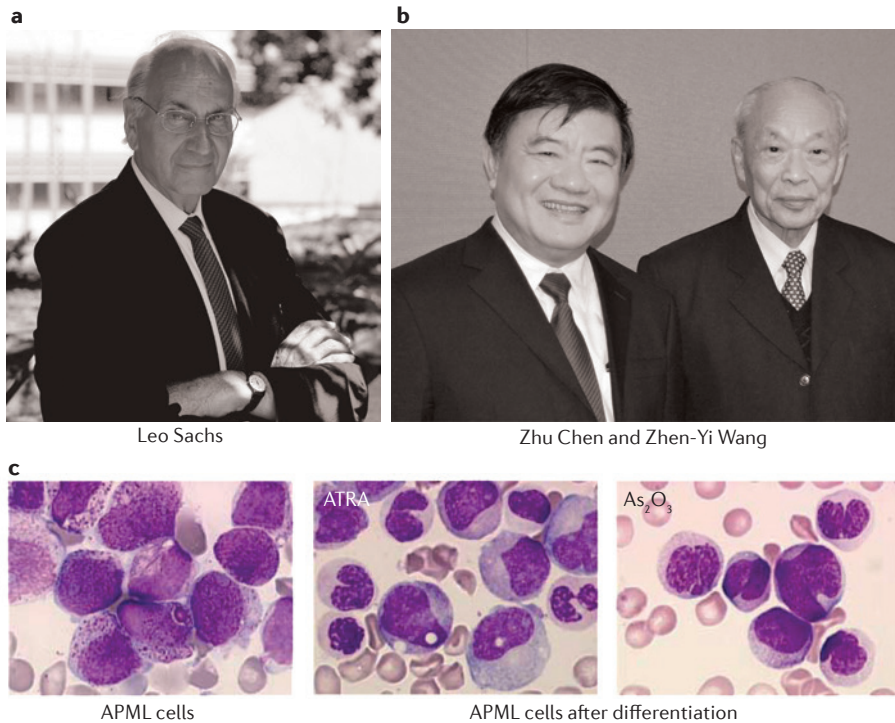


Figure 4 | **Reversing differentiation arrest in leukaemias.** **a** | Leo Sachs used murine leukaemic cell lines to demonstrate reversible proliferation/differentiation uncoupling in cancer⁶⁸. **b** | Zhu Chen, Zhen-Yi Wang and their colleagues in China developed all-trans retinoic acid (ATRA) as an effective therapeutic agent for acute promyelocytic leukaemia (APML)¹²³. **c** | Differentiation induction in APML by ATRA. Left panel: untreated blast-like leukaemic cells; middle panel: differentiated, granulocytic cells after treatment with ATRA; right panel: differentiated cells after treatment with arsenic trioxide (As₂O₃). Part **a**: image courtesy of the Weizmann Institute of Science, Israel; part **b**: image courtesy of the US National Foundation for Cancer Research; part **c**: reproduced from REF. 124, Nature Publishing Group.

to involve the use of peripheralized (or mobilized) blood stem cells or cord blood from newborns. Coupled with the establishment of donor registries, better preconditioning regimens, histocompatibility matching and control of graft-versus-host adverse effects, transplantation is now the treatment of choice for some patients with blood cell cancers for whom more conventional chemotherapy is not curative. Early (1980s) attempts to rescue patients with advanced solid cancers using autologous bone marrow transplantation were largely unsuccessful, either because the disease was resistant to high-dose chemotherapy regimens or because, as we now recognize¹³⁴, blood and tissues can be seeded with covert micrometastases relatively early in disease progression. Since the 1990s, allogeneic bone marrow transplantation has been successfully used in cases of advanced paediatric cancer, including neuroblastoma, Wilms' tumour and sarcomas¹³⁵. Allogeneic marrow transplants have been attempted in most common adult cancers, usually at a very advanced stage, with some success in germ cell tumours^{136,137}. And, as demonstrated

with leukaemia, a graft-versus-tumour effect can be beneficial¹³⁸ although concomitant graft-versus-host activity remains a significant problem.

Monoclonal antibody therapy. Bachireddy and colleagues recently reviewed in this journal¹³⁹ the part played by blood cell cancers in the initial, proof-of-concept therapeutic approach of immunotherapy with antibodies, beginning with anti-idiotypic antibodies in lymphoma and with the first clinically approved monoclonal antibody (rituximab for B cell malignancies) in 1997. These early endeavours have been followed up with immuno-engineering of T cells — or chimeric antigen receptors (CARs) — applied successfully to refractory B lineage leukaemias¹⁴⁰ and with radionucleotide-tagged monoclonal antibodies in leukaemia and some other cancers¹⁴¹.

After several decades of trial and tribulation, immunotherapy is now very much in vogue again in cancer therapy, propelled by a better understanding of tumour antigens^{142,143} and imaginative engineering of monoclonal antibodies or

T cells¹⁴⁴ to eliminate cancer cells. More rationally designed immunotherapies have had some striking successes, notably via immune checkpoint (for example, cytotoxic T lymphocyte-associated antigen 4 (CTLA4) or programmed cell death protein 1 (PD1; also known as PDCD1)) inhibition in melanoma and lung cancer^{145–148}.

Residual disease monitoring. Cancer surgery, radiation and/or chemotherapy may achieve substantial debulking of tumours, resulting in clinical remission, only for the disease to recur from within the original clone. The problem is post-therapy survival of subclinical disease or residual cancer cells. Clearly, it would be very beneficial for patient management to be able to detect residual cancer cells and serially monitor their re-expansion at an early presymptom stage. In principle, this requires specific and sensitive blood-based markers. The clear and encouraging precedent for this is with ALL.

The discovery that ALL had clone-specific rearrangements at *IGH* and T cell receptor (TCR) loci in accordance with the early lymphoid lineage origins of this type of cancer provided unique clonal markers for PCR-based monitoring of minimal residual disease (MRD) in blood^{149,150}. Persistent or re-emergent MRD is predictive of recurrence and provides a guide to patient treatment and management in ALL¹⁵¹. Sensitive (down to 10⁻⁵ or 10⁻⁶ leukaemia cells and mixed with normal cells) MRD screening has also been explored in AML by detection of mutational changes or unique immunophenotypes¹⁵². Molecular detection of MRD is more demanding for non-blood-borne cancers but may now be possible via sensitive and quantitative assays of cell-free tumour DNA in plasma^{153,154}. Given the variegated genetics of cancer subclones, it would make sense to monitor residual cancer cells via detection of the founder genetic lesion, if this is known, or early mutations that are in the trunk of the clonal evolutionary tree; these are likely to be stable markers with pan-clonal expression. The experience with ALL suggests that monitoring cancer in this way, via serial blood samples, should be clinically informative, or prognostic, and lead to improved management of patients and better outcomes. Early data, on breast cancer, are very promising in this regard¹⁵⁵.

Infectious causality

Some 15–20% of cancers worldwide have a specific association with microbial infection¹⁵⁶. The rate is even higher in less-developed societies at close to 40%¹⁵⁶.

A clear implication is the potential for prophylactic prevention. Harald zur Hausen was awarded the Nobel Prize in 2010 for his pioneering work on human papillomavirus (HPV) in cancer¹⁵⁷. HPV vaccines are now in use for preventing cervical cancer and there are good prospects for other viral cancers including hepatitis virus vaccines for liver cancer and Epstein–Barr virus (EBV) vaccines for lymphomas and nasopharyngeal carcinoma¹⁵⁸.

Research on animal and human leukaemias and lymphomas played a crucial role in the recognition of viral causes of cancer (see Timeline on viruses and cancer in REF. 159). More than 100 years ago, Vilhelm Ellermann and Olaf Bang¹⁶⁰ showed that avian lymphoid leukosis had an infectious aetiology, although the first cancer-associated virus was identified in a chicken sarcoma by Peyton Rous in 1911 (REF. 161). This observation lay dormant for several decades and the key observations on viruses and cancer came from Ludwig Gross's identification of murine leukaemia viruses¹⁶² and observations from others of naturally occurring leukaemia in cats^{163,164} and lymphoma in cattle¹⁶⁵, which were shown to be caused by feline leukaemia virus (FeLV) and bovine leukaemia virus (BLV), respectively. These animal-based studies greatly encouraged the search for viral and infectious causes of human cancers, and the development of a vaccine for FeLV¹⁶⁶ strongly endorsed the preventive or prophylactic potential.

The first viruses associated with human cancer were also discovered through the study of leukaemias and lymphomas — the DNA herpesvirus EBV in Burkitt's lymphoma in the 1960s¹⁶⁷ (see BOX 1) and the retrovirus human T-lymphotropic virus 1 (HTLV-1) in adult T cell leukaemia/lymphoma in the 1980s^{168,169}. Human lymphomas provide another striking precedent: the involvement of bacterial species in the causal pathway. The paradigm is *Helicobacter pylori* in gastric lymphoma¹⁷⁰. One striking and important consequence of these microbial links is the beneficial impact of antibiotics early in the disease¹⁷⁰. Bacteria indirectly affect lymphomas through chronic T cell stimulation. It is likely that *H. pylori* and other commensal bacterial species are significant contributors to disease progression in other more common cancers, particularly perhaps in gastrointestinal tumours¹⁷¹. Here, interplay of diet, natural gut microbiota and chronic inflammation has an impact on the

progression of disease^{172–174} and opens the door to anti-inflammatory drugs such as aspirin for reducing risk¹⁷⁵ or slowing down clonal evolution¹⁷⁶.

How come?

Leukaemia and lymphomas constitute no more than 10% of all cancers worldwide¹⁷⁷. So how is it that researchers and clinicians in this area seem to have punched well above their weight? As a young scientist, I was reliably informed that the smartest students opt for immunology. So it could be that leukaemia has attracted the brightest minds. Alas, the reasons for the success of leukaemia research are more prosaic.

Peter Medawar described scientific research as “The art of the soluble” (REF. 178), and as practitioners, scientists are, mostly, pragmatists. Leukaemia research has been more tractable because of the inherent, blood-borne nature of those cancers and the accessibility of the relevant tissues — blood

itself, and bone marrow. And what comes out of the blood, or marrow, are single cells that are relatively easy to purify and manipulate. The other related reason is that the biology of normal blood cell development and function has been substantially ahead of that of any other tissue, at least until recently, when, for example, the intestinal crypt biology was beautifully elucidated¹⁷⁹. This haematopoietic and immunological research included assays for stem and progenitor cells pioneered by Jim Till and Ernest McCulloch in Toronto¹⁸⁰, the identification of key growth factors, particularly by Donald Metcalf and his team in Melbourne¹⁸¹, and a dissection of immune cell diversity and its networked functions. Allied to this was the development of animal models, including transplantation, for both normal haematopoiesis research and leukaemia modelling. These models had a very significant impact on the development of clinical bone marrow transplantation and

Box 1 | Burkitt's lymphoma: from safari to paradigm

Burkitt's lymphoma provides an important precedent or paradigm for the recognition of multifactorial causation in cancer. In the case of Burkitt's lymphoma, this involves person-to-person transmission of Epstein–Barr virus (EBV), immunosuppression of T cells by mosquito-transmitted malaria and activation of *MYC* by chromosome translocation to the immunoglobulin heavy chain (*Igh*) locus¹⁹¹. The discoverer of this eponymous tumour was a surgeon, Denis Burkitt (see the figure part **a**), who, having identified this jaw-associated tumour in young patients in East Africa, embarked on what he described as a long (10,000 mile) safari to document its variable distribution in Africa¹⁹². This led to the identification of a link between the lymphoma and the prevalence of rainfall, which then implicated malaria. This association was later explained via the immunosuppressive impact of malaria infection providing an immunologically permissive environment for EBV to transform B cells. Serendipitously, Burkitt visited London early on in this adventure and gave a lecture that Tony Epstein attended. That led in a short time to identification of EBV by Epstein and his colleagues Yvonne Barr and Bert Achong, who cultured cells provided by Burkitt and examined them by electron microscopy (see the figure part **b**)¹⁶⁷. The development of Burkitt's lymphoma in the context of immunosuppressive malaria is paralleled by the greatly increased risk of EBV-associated lymphomas in iatrogenically immunosuppressed patients and similarly in patients with HIV/AIDS, who have high rates of EBV-positive lymphomas as well as human papillomavirus (HPV)-linked cancers and Kaposi's sarcomas associated with human herpesvirus 8 (HHV-8, also known as Kaposi's sarcoma-associated herpesvirus (KSHV))¹⁹³. EBV also has a causal role in nasopharyngeal carcinoma in China and South East Asia¹⁹⁴.

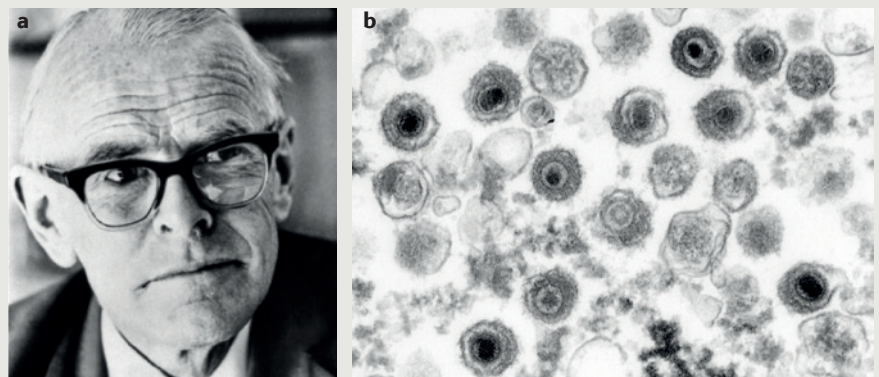


Figure part **a**, image courtesy of the National Library of Medicine/Science Photo Library; part **b**, image courtesy of the DKFZ German Cancer Center.

the early preclinical testing of anti-leukaemic drugs and, more generally, they helped to forge academic haematology as a biomedical discipline that links basic research to the clinic. Modelling of leukaemogenesis with murine and avian viruses was also pivotal, not least in highlighting the functional impact of oncogenes^{69,182}.

Much of the success of leukaemia research is therefore due to the good fortune of tissue accessibility and especially to the underpinning of a solid foundation of basic biology for the corresponding normal tissue. This can be contrasted with the brain and brain cancer where we are still largely in the dark.

Key technological innovations have also played a part. Monoclonal antibodies have provided a crucial tool for research and treatment. They are themselves a product of blood cell immunological research and perhaps not surprisingly, they were first used to analyse and purify human cell populations in normal and leukaemic blood. This was underpinned by the technological innovation of Len Herzenberg and colleagues¹⁸³, working with Becton Dickinson, of the flow-based sorter using rapid, single-cell interrogation and physical sorting by lasers. This exploited a method for single-droplet deflection in an electrical field designed originally for the ink jet printer.

The fact that acute leukaemia, although relatively rare as a cancer, is the most common paediatric cancer in developed societies has had a big influence. The emotional impact of a lethal cancer of childhood is substantial. It energized the establishment and successful execution of standardized, randomized clinical trials of combination chemotherapy on a national and international scale, leading over several decades to high cure rates. However, the place of children in the story has had another impact — on funding, without which the research success would not have occurred. Leukaemia has, for a long time, been funded particularly well and perhaps disproportionately as a single type of cancer. In the United Kingdom for example, a leukaemia-focused charity, the Leukaemia Research Fund (subsequently known as Leukaemia & Lymphoma Research and now as Bloodwise) was instigated more than 50 years ago by the parents of a child with acute leukaemia in Middlesbrough, United Kingdom¹⁸⁴. This evolved into a national charity that has provided some £500 million of funding for research into, and treatment of, blood cell cancers.

These crucial variables are not independent. Success breeds success. Better research begets more funding; more funding attracts more and better researchers. Advances in clinical trials encourage more endeavour and persistence in the challenge to outwit the disease.

There are also elements of serendipity in the success story of leukaemia research. The early identification of chromosomal translocations and fusion genes was contingent upon the relative simplicity of the cancer genome and chromosome karyotypes in leukaemia — compared with the marked complexity in carcinomas. The remarkable cure rate now standard in childhood ALL did not come quickly or easily and owes much not only to the persistence of its protagonists but also to the intrinsic biological character of this group of cancers.

In this Timeline article, I have tried to distil out a personal perspective of the key contributions that have their origins with leukaemias and lymphomas (FIG. 1). Inevitably, there are many other discoveries and important areas in cancer research, diagnosis and treatment in which the blood cell cancers have been less influential or largely irrelevant. These include early studies on chemical carcinogenesis¹⁸⁵, the first identification of consistent, single-nucleotide mutations (in RAS) in cancer by gene transfection studies with DNA from bladder cancer^{186,187}; research on tumour-associated angiogenesis and anti-angiogenesis pioneered by Judah Folkman¹⁸⁸; the first, comprehensive whole-genome screens¹⁸⁹; the first genome-wide association studies (GWAS) on inherited cancer susceptibility¹⁹⁰ as well as all studies related to metastases, whole-body imaging of tumours and surgical intervention.

A collective of thousands of scientists, clinicians, patients and their families, and fundraisers worldwide have made remarkable contributions to medical science and cancer via a focus on leukaemia and related blood cancers. It may be a less romantic narrative than that of one lone scientist stumbling one night on an antibiotic that cures fatal infections, but it is the way that applied science works, at its best. And it is an appropriate narrative that matches the complexity of cancer.

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- Greaves, M. *Cancer: The Evolutionary Legacy* (Oxford Univ. Press, 2000).
- Wright, N. A. Boveri at 100: cancer evolution, from preneoplasia to malignancy. *J. Pathol.* **234**, 146–151 (2014).
- Heim, S. & Mitelman, F. *Cancer Cytogenetics* (Alan R. Liss, 1987).
- Nowell, P. & Hungerford, D. A minute chromosome in human granulocytic leukemia. *Science* **132**, 1497 (1960).
- Rowley, J. D. Chromosome translocations: dangerous liaisons revisited. *Nat. Rev. Cancer* **1**, 245–250 (2001).
- Heisterkamp, N. *et al.* Localization of the *c-abl* oncogene adjacent to a translocation breakpoint in chronic myelocytic leukaemia. *Nature* **306**, 239–242 (1983).
- Konopka, J. B., Watanabe, S. M. & Witte, O. N. An alteration of the human *c-abl* protein in K562 leukemia cells unmasks associated tyrosine kinase activity. *Cell* **37**, 1035–1042 (1984).
- Mertens, F., Johansson, B., Fioretos, T. & Mitelman, F. The emerging complexity of gene fusions in cancer. *Nat. Rev. Cancer* **15**, 371–381 (2015).
- Dalla-Favera, R. *et al.* Human *c-myc* onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7824–7827 (1982).
- Taub, R. *et al.* Translocation of the *c-myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7837–7841 (1982).
- Cleary, M. L. & Sklar, J. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. *Proc. Natl Acad. Sci. USA* **82**, 7439–7443 (1985).
- Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. & Croce, C. M. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. *Science* **229**, 1390–1393 (1985).
- Küppers, R. & Dalla-Favera, R. Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene* **20**, 5580–5594 (2001).
- Cory, S. & Adams, J. M. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat. Rev. Cancer* **2**, 647–656 (2002).
- Golub, T. R. *et al.* Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* **286**, 531–537 (1999).
- Mullighan, C. G. *et al.* Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **446**, 758–764 (2007).
- Ley, T. J. *et al.* DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* **456**, 66–72 (2008).
- Fialkow, P. J. The origin and development of human tumors studied with cell markers. *N. Engl. J. Med.* **291**, 26–35 (1974).
- Nowell, P. C. The clonal evolution of tumor cell populations. *Science* **194**, 23–28 (1976).
- Greaves, M. & Maley, C. C. Clonal evolution in cancer. *Nature* **481**, 306–313 (2012).
- Anderson, K. *et al.* Genetic variegation of clonal architecture and propagating cells in leukaemia. *Nature* **469**, 356–361 (2011).
- Welch, J. S. *et al.* The origin and evolution of mutations in acute myeloid leukemia. *Cell* **150**, 264–278 (2012).
- Klco, T. M. *et al.* Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell* **25**, 379–392 (2014).
- Schuh, A. *et al.* Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns. *Blood* **120**, 4191–4196 (2012).
- Egan, J. B. *et al.* Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. *Blood* **120**, 1060–1066 (2012).
- Gerlinger, M. *et al.* Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nat. Genet.* **46**, 225–233 (2014).
- Yates, L. R. *et al.* Subclonal diversification of primary breast cancer revealed by multiregion sequencing. *Nat. Med.* **21**, 751–759 (2015).
- Sottoriva, A. *et al.* Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc. Natl Acad. Sci. USA* **110**, 4009–4014 (2013).

29. Gundem, G. *et al.* The evolutionary history of lethal metastatic prostate cancer. *Nature* **520**, 353–357 (2015).
30. Sottoriva, A. *et al.* A Big Bang model of human colorectal tumor growth. *Nat. Genet.* **47**, 209–216 (2015).
31. Gerlinger, M. *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
32. Boutros, P. C. *et al.* Spatial genomic heterogeneity within localized, multifocal prostate cancer. *Nat. Genet.* **47**, 736–745 (2015).
33. Potter, N. E. *et al.* Single-cell mutational profiling and clonal phylogeny in cancer. *Genome Res.* **23**, 2115–2125 (2013).
34. Navin, N. E. Cancer genomics: one cell at a time. *Genome Biol.* **15**, 452 (2014).
35. Eirew, P. *et al.* Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. *Nature* **518**, 422–426 (2015).
36. Yates, L. R. & Campbell, P. J. Evolution of the cancer genome. *Nat. Rev. Genet.* **13**, 795–806 (2012).
37. Ding, L. *et al.* Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature* **481**, 506–510 (2012).
38. Mullighan, C. G. *et al.* Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* **322**, 1377–1380 (2008).
39. Jiang, Y. *et al.* Deep sequencing reveals clonal evolution patterns and mutation events associated with relapse in B-cell lymphomas. *Genome Biol.* **15**, 432 (2014).
40. Juric, D. *et al.* Convergent loss of PTEN leads to clinical resistance to a PI(3)K α inhibitor. *Nature* **518**, 240–244 (2015).
41. Marshall, G. M. *et al.* The prenatal origins of cancer. *Nat. Rev. Cancer* **14**, 277–289 (2014).
42. Greaves, M. F. & Wiemels, J. Origins of chromosome translocations in childhood leukaemia. *Nat. Rev. Cancer* **3**, 639–649 (2003).
43. Greaves, M. F., Maia, A. T., Wiemels, J. L. & Ford, A. M. Leukemia in twins: lessons in natural history. *Blood* **102**, 2321–2333 (2003).
44. Wiemels, J. L. *et al.* Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet* **354**, 1499–1503 (1999).
45. Mori, H. *et al.* Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc. Natl Acad. Sci. USA* **99**, 8242–8247 (2002).
46. Strong, S. J. & Corney, G. *The Placenta in Twin Pregnancy* (Pergamon Press, 1967).
47. Ma, Y. *et al.* Developmental timing of mutations revealed by whole-genome sequencing of twins with acute lymphoblastic leukemia. *Proc. Natl Acad. Sci. USA* **110**, 7429–7433 (2013).
48. Cazzaniga, G. *et al.* Developmental origins and impact of *BCR-ABL1* fusion and *IKZF1* deletions in monozygotic twins with Ph⁺ acute lymphoblastic leukemia. *Blood* **118**, 5559–5565 (2011).
49. Bateman, C. M. *et al.* Acquisition of genome-wide copy number alterations in monozygotic twins with acute lymphoblastic leukemia. *Blood* **115**, 3553–3558 (2010).
50. Jaiswal, S. *et al.* Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* **371**, 2488–2498 (2014).
51. Genovese, G. *et al.* Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* **371**, 2477–2487 (2014).
52. Shlush, L. I. *et al.* Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* **506**, 328–333 (2014).
53. Corces-Zimmerman, M. R. & Majeti, R. Pre-leukemic evolution of hematopoietic stem cells: the importance of early mutations in leukemogenesis. *Leukemia* **28**, 2276–2282 (2014).
54. Zhang, X. *et al.* Genomic analysis of the clonal origin and evolution of acute promyelocytic leukemia in a unique patient with a very late (17 years) relapse. *Leukemia* **28**, 1751–1754 (2014).
55. Ford, A. M. *et al.* Protracted dormancy of pre-leukaemic stem cells. *Leukemia* **29**, 2202–2207 (2015).
56. Greaves, M. Does everyone develop covert cancer? *Nat. Rev. Cancer* **14**, 209–210 (2014).
57. Martincorena, I. *et al.* Tumor evolution: high burden and pervasive positive selection of somatic mutations in normal human skin. *Science* **348**, 880–886 (2015).
58. Aparicio, S. & Caldas, C. The implications of clonal genome evolution for cancer medicine. *N. Engl. J. Med.* **368**, 842–851 (2013).
59. Greaves, M. Evolutionary determinants of cancer. *Cancer Discov.* **5**, 806–820 (2015).
60. Kreso, A. & Dick, J. E. Evolution of the cancer stem cell model. *Cell Stem Cell* **14**, 275–291 (2014).
61. Yap, T. A., Gerlinger, M., Futreal, P. A., Pusztai, L. & Swanton, C. Intratumor heterogeneity: seeing the wood for the trees. *Sci. Transl. Med.* **4**, 127ps10 (2012).
62. McGranahan, N. & Swanton, C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* **27**, 15–26 (2015).
63. Polak, P. *et al.* Cell-of-origin chromatin organization shapes the mutational landscape of cancer. *Nature* **518**, 360–364 (2015).
64. Visvader, J. E. Cells of origin in cancer. *Nature* **469**, 314–322 (2011).
65. Pierce, G. B., Shikes, R. & Fink, L. M. *Cancer: A Problem of Developmental Biology* (Prentice Hall Inc., 1978).
66. Fialkow, P. J., Denman, A. M., Jacobson, R. J. & Lowenthal, M. N. Chronic myelocytic leukemia: origin of some lymphocytes from leukemic stem cells. *J. Clin. Invest.* **62**, 815–823 (1978).
67. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
68. Sachs, L. Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukaemia. *Nature* **274**, 535–539 (1978).
69. Beug, H., Hayman, M. J. & Graf, T. Leukaemia as a disease of differentiation: retroviruses causing acute leukaemias in chickens. *Cancer Surveys* **1**, 205–230 (1982).
70. Greaves, M. F. Differentiation-linked leukaemogenesis in lymphocytes. *Science* **234**, 697–704 (1986).
71. Küppers, R. Mechanisms of B-cell lymphoma pathogenesis. *Nat. Rev. Cancer* **5**, 251–262 (2005).
72. Zhu, Y. & Parada, L. F. The molecular and genetic basis of neurological tumours. *Nat. Rev. Cancer* **2**, 616–626 (2002).
73. Gibson, P. *et al.* Subtypes of medulloblastoma have distinct developmental origins. *Nature* **468**, 1095–1099 (2010).
74. Alcantara Llaguno, S. R. *et al.* Adult lineage-restricted CNS progenitors specify distinct glioblastoma subtypes. *Cancer Cell* **28**, 429–440 (2015).
75. Greaves, M. in *Nathan and Oski's Hematology and Oncology of Infancy and Childhood* (eds Orkin, S. H. *et al.*) 1229–1238 (Elsevier Saunders, 2015).
76. Paugh, B. S. *et al.* Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J. Clin. Oncol.* **28**, 3061–3068 (2010).
77. Filbin, M. G. & Stiles, C. D. Of brains and blood: developmental origins of glioma diversity? *Cancer Cell* **28**, 403–404 (2015).
78. Huntly, B. J. & Gilliland, D. G. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat. Rev. Cancer* **5**, 311–321 (2005).
79. Dick, J. E. Stem cell concepts renew cancer research. *Blood* **112**, 4793–4807 (2008).
80. Rosen, J. M. & Jordan, C. T. The increasing complexity of the cancer stem cell paradigm. *Science* **324**, 1670–1673 (2009).
81. Magee, J. A., Piskounova, E. & Morrison, S. J. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* **21**, 285–296 (2012).
82. Quintana, E. *et al.* Efficient tumour formation by single human melanoma cells. *Nature* **456**, 593–598 (2008).
83. Gentles, A. J., Plevritis, S. K., Majeti, R. & Alizadeh, A. A. Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. *JAMA* **304**, 2706–2715 (2010).
84. Eppert, K. *et al.* Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat. Med.* **17**, 1086–1093 (2011).
85. Hartwell, K. A. *et al.* Niche-based screening identifies small-molecule inhibitors of leukemia stem cells. *Nat. Chem. Biol.* **9**, 840–848 (2013).
86. Notta, F. *et al.* Evolution of human *BCR-ABL1* lymphoblastic leukaemia-initiating cells. *Nature* **469**, 362–367 (2011).
87. Piccirillo, S. G. M. *et al.* Genetic and functional diversity of propagating cells in glioblastoma. *Stem Cell Rep.* **4**, 7–15 (2015).
88. Meyer, M. *et al.* Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity. *Proc. Natl Acad. Sci. USA* **112**, 851–856 (2015).
89. Greaves, M. Cancer stem cells as 'units of selection'. *Evol. Appl.* **6**, 102–108 (2013).
90. Fong, C. Y. *et al.* BET inhibitor resistance emerges from leukaemia stem cells. *Nature* **525**, 538–542 (2015).
91. Greaves, M. F. Analysis of the clinical and biological significance of lymphoid phenotypes in acute leukemia. *Cancer Res.* **41**, 4752–4766 (1981).
92. Chessells, J. M., Hardisty, R. M., Rapson, N. T. & Greaves, M. F. Acute lymphoblastic leukaemia in children: classification and prognosis. *Lancet* **ii**, 1307–1309 (1977).
93. Sallan, S. E. *et al.* Cell surface antigens: prognostic implications in childhood acute lymphoblastic leukemia. *Blood* **55**, 395–402 (1980).
94. Greaves, M. F., Janosy, G., Peto, J. & Kay, H. Immunologically defined subclasses of acute lymphoblastic leukaemia in children: their relationship to presentation features and prognosis. *Br. J. Haematol.* **48**, 179–197 (1981).
95. Swerdlow, S. H. *et al.* (eds) *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (IARC, 2008).
96. Pui, C.-H., Relling, M. V. & Downing, J. R. Acute lymphoblastic leukemia. *N. Engl. J. Med.* **350**, 1535–1548 (2004).
97. Armstrong, S. A. & Look, A. T. Molecular genetics of acute lymphoblastic leukemia. *J. Clin. Oncol.* **23**, 6306–6315 (2005).
98. Hunger, S. P. & Mullighan, C. G. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. *Blood* **125**, 3977–3987 (2015).
99. Curtis, C. *et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* **486**, 346–352 (2012).
100. Sadanandam, A. *et al.* A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat. Med.* **19**, 619–625 (2013).
101. Taylor, M. D. *et al.* Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol.* **123**, 465–472 (2012).
102. Maley, C. C. *et al.* Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nat. Genet.* **38**, 468–473 (2006).
103. Mroz, E. A. *et al.* High intratumor genetic heterogeneity is related to worse outcome in patients with head and neck squamous cell carcinoma. *Cancer* **119**, 3034–3042 (2013).
104. Michor, F. *et al.* Dynamics of chronic myeloid leukaemia. *Nature* **435**, 1267–1270 (2005).
105. Pinkel, D. in *White Blood: Personal Journeys with Childhood Leukaemia* (ed. Greaves, M.) 13–46 (World Scientific, 2008).
106. Inaba, H., Greaves, M. & Mullighan, C. G. Acute lymphoblastic leukaemia. *Lancet* **381**, 1943–1955 (2013).
107. Bhojwani, D. *et al.* ETV6-RUNX1-positive childhood acute lymphoblastic leukemia: improved outcome with contemporary therapy. *Leukemia* **26**, 265–270 (2012).
108. Newlands, E. S. *et al.* Developments in chemotherapy for medium- and high-risk patients with gestational trophoblastic tumours (1979–1984). *Br. J. Obstet. Gynaecol.* **93**, 63–69 (1986).
109. Horwich, A., Nicol, D. & Huddart, R. Testicular germ cell tumours. *BMJ* **347**, f5526 (2013).
110. Papaemmanuil, E. *et al.* RAG-mediated recombination is the predominant driver of oncogenic rearrangement in *ETV6-RUNX1* acute lymphoblastic leukemia. *Nat. Genet.* **46**, 116–125 (2014).
111. Litchfield, K. *et al.* Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. *Nat. Commun.* **6**, 5973 (2015).
112. Gutekunst, M. *et al.* p53 hypersensitivity is the predominant mechanism of the unique responsiveness of testicular germ cell tumor (TGCT) cells to cisplatin. *PLoS ONE* **6**, e19198 (2011).
113. Al-Lazikani, B., Banerji, U. & Workman, P. Combinatorial drug therapy for cancer in the post-genomic era. *Nat. Biotechnol.* **30**, 679–692 (2012).
114. Druker, B. J. Perspectives on the development of imatinib and the future of cancer research. *Nat. Med.* **15**, 1149–1152 (2009).
115. Corbin, A. S. *et al.* Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of *BCR-ABL* activity. *J. Clin. Invest.* **121**, 396–409 (2011).
116. Sosa, M. S., Bragado, P. & Aguirre-Ghiso, J. A. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat. Rev. Cancer* **14**, 611–622 (2014).
117. Zhang, B. *et al.* Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer Cell* **17**, 427–442 (2010).

118. Essers, M. A. & Trumpp, A. Targeting leukemic stem cells by breaking their dormancy. *Mol. Oncol.* **4**, 443–450 (2010).
119. Roche-Lestienne, C. *et al.* Several types of mutations of the *Abl* gene can be found in chronic myeloid leukemia patients resistant to ST1571, and they can pre-exist to the onset of treatment. *Blood* **100**, 1014–1018 (2002).
120. Pfeifer, H. *et al.* Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with *de novo* Philadelphia-positive acute lymphoblastic leukemia (Ph⁺ ALL). *Blood* **110**, 727–734 (2007).
121. Shih, A. H., Abdel-Wahab, O., Patel, J. P. & Levine, R. L. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat. Rev. Cancer* **12**, 599–612 (2012).
122. Lotem, J. & Sachs, L. Epigenetics wins over genetics: induction of differentiation in tumor cells. *Semin. Cancer Biol.* **12**, 339–346 (2002).
123. Wang, Z. Y. & Chen, Z. Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood* **111**, 2505–2515 (2008).
124. Zhu, J., Chen, Z., Lallemand-Breitenbach, V. & de Thé, H. How acute promyelocytic leukaemia revived arsenic. *Nat. Rev. Cancer* **2**, 705–713 (2002).
125. Ablain, J. *et al.* Activation of a promyelocytic leukemia-tumor protein 53 axis underlies acute promyelocytic leukemia cure. *Nat. Med.* **20**, 167–174 (2014).
126. Schenk, T. *et al.* Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-*trans*-retinoic acid differentiation pathway in acute myeloid leukemia. *Nat. Med.* **18**, 605–611 (2012).
127. Zhang, X., Cruz, F. D., Terry, M., Remotti, F. & Matushansky, I. Terminal differentiation and loss of tumorigenicity of human cancers via pluripotency-based reprogramming. *Oncogene* **32**, 2249–2260 (2013).
128. Cruz, F. D. & Matushansky, I. Solid tumor differentiation therapy – is it possible? *Oncotarget* **3**, 559–567 (2012).
129. Laugesen, A. & Helin, K. Chromatin repressive complexes in stem cells, development, and cancer. *Cell Stem Cell* **14**, 735–751 (2014).
130. Sykes, S. M. *et al.* AKT/FOXO signaling enforces reversible differentiation blockade in myeloid leukemias. *Cell* **146**, 697–708 (2011).
131. Thomas, E. D. Bone marrow transplantation: a review. *Semin. Hematol.* **36**, 95–105 (1999).
132. Nivison-Smith, I. *et al.* Relative survival of long-term hematopoietic cell transplant recipients approaches general population rates. *Biol. Blood Marrow Transplant.* **15**, 1323–1330 (2009).
133. Jenq, R. R. & van den Brink, M. R. Allogeneic haematopoietic stem cell transplantation: individualized stem cell and immune therapy of cancer. *Nat. Rev. Cancer* **10**, 213–221 (2010).
134. Klein, C. A. Parallel progression of primary tumours and metastases. *Nat. Rev. Cancer* **9**, 302–312 (2009).
135. Pietras, W. Advances and changes in the treatment of children with nephroblastoma. *Adv. Clin. Exp. Med.* **21**, 809–820 (2012).
136. Necchi, A. *et al.* High-dose chemotherapy for germ cell tumors: do we have a model? *Expert Opin. Biol. Ther.* **15**, 33–44 (2015).
137. Sureda, A. *et al.* Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. *Bone Marrow Transplant.* **50**, 1037–1056 (2015).
138. Demirel, T. *et al.* Transplantation of allogeneic hematopoietic stem cells: an emerging treatment modality for solid tumors. *Nat. Clin. Pract. Oncol.* **5**, 256–267 (2008).
139. Bachireddy, P., Burkhardt, U. E., Rajasagi, M. & Wu, C. J. Haematological malignancies: at the forefront of immunotherapeutic innovation. *Nat. Rev. Cancer* **15**, 201–215 (2015).
140. Maude, S. L. *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371**, 1507–1517 (2014).
141. Larson, S. M., Carrasquillo, J. A., Cheung, N. K. & Press, O. W. Radioimmunotherapy of human tumours. *Nat. Rev. Cancer* **15**, 347–360 (2015).
142. Coullie, P. G., Van den Eynde, B. J., van der Bruggen, P. & Boon, T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. *Nat. Rev. Cancer* **14**, 135–146 (2014).
143. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
144. Weiner, G. J. Building better monoclonal antibody-based therapeutics. *Nat. Rev. Cancer* **15**, 361–370 (2015).
145. Adams, G. P. & Weiner, L. M. Monoclonal antibody therapy of cancer. *Nat. Biotechnol.* **23**, 1147–1157 (2005).
146. Miller, J. F. & Sadelain, M. The journey from discoveries in fundamental immunology to cancer immunotherapy. *Cancer Cell* **27**, 439–449 (2015).
147. Reiss, K. A., Forde, P. M. & Brahmer, J. R. Harnessing the power of the immune system via blockade of PD-1 and PD-L1: a promising new anticancer strategy. *Immunotherapy* **6**, 459–475 (2014).
148. Rizvi, N. A. *et al.* Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128 (2015).
149. Szczepanski, T., Orfao, A., van der Velden, V. H., San Miguel, J. F. & van Dongen, J. J. Minimal residual disease in leukaemia patients. *Lancet Oncol.* **2**, 409–417 (2001).
150. van Dongen, J. J. M. *et al.* Prognostic value of minimal residual disease in childhood acute lymphoblastic leukemia. *Lancet* **352**, 1731–1738 (1998).
151. van Dongen, J. J., van der Velden, V. H., Brüggemann, M. & Orfao, A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood* **125**, 3996–4009 (2015).
152. Hourigan, C. S. & Karp, J. E. Minimal residual disease in acute myeloid leukaemia. *Nat. Rev. Clin. Oncol.* **10**, 460–471 (2013).
153. Bettgeowda, C. *et al.* Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci. Transl. Med.* **6**, 224ra24 (2014).
154. Dawson, S. J. *et al.* Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N. Engl. J. Med.* **368**, 1199–1209 (2013).
155. Garcia-Murillas, I. *et al.* Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci. Transl. Med.* **7**, 302ra133 (2015).
156. Franceschi, S. & Herrero, R. in *IARC World Cancer Report 2014* (eds Stewart, B. W. & Wild, C. P.) 105–114 (IARC, 2014).
157. Zur Hausen, H. *Infections Causing Human Cancer* (Wiley-VCH, 2006).
158. Cohen, J. I., Fauci, A. S., Varmus, H. & Nabel, G. J. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci. Transl. Med.* **3**, 107fs7 (2011).
159. Moore, P. S. & Chang, Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer* **10**, 878–889 (2010).
160. Ellermann, V. & Bang, O. Experimentelle Leukämie bei Hühnern. *Zentralbl. Bakteriol.* **46**, 595 (1908).
161. Rous, P. A sarcoma of the fowl transmissible by agent separable from tumor cells. *J. Exp. Med.* **13**, 397–411 (1911).
162. Gross, L. *Oncogenic Viruses* (Pergamon Press, 1983).
163. Jarrett, W. F., Crawford, E. M., Martin, W. B. & Davie, F. A. Virus-like particle associated with leukemia (lymphosarcoma). *Nature* **202**, 567–569 (1964).
164. Hardy, W. D. *et al.* Horizontal transmission of feline leukaemia virus. *Nature* **244**, 266–269 (1973).
165. Burny, A. *et al.* Bovine leukemia virus involvement in enzootic bovine leukosis. *Adv. Cancer Res.* **28**, 251–311 (1978).
166. Jarrett, W. *et al.* Vaccination against feline leukaemia virus using a cell membrane antigen system. *Int. J. Cancer* **16**, 134–141 (1975).
167. Epstein, M. A., Achong, B. G. & Barr, Y. M. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* **i**, 702–703 (1964).
168. Gallo, R. C., Essex, M. E. & Gross, L. (eds) *Human T-cell Leukemia/Lymphoma Virus: The Family of Human T-Lymphotropic Retroviruses: Their Role in Malignancies and Association with AIDS* (Cold Spring Harbor Laboratory Press, 1984).
169. Matsuoka, M. & Jeang, K. T. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat. Rev. Cancer* **7**, 270–280 (2007).
170. Isaacson, P. G. & Du, M.-Q. MALT lymphoma: from morphology to molecules. *Nat. Rev. Cancer* **4**, 644–653 (2004).
171. Peek, R. M. Jr & Blaser, M. J. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat. Rev. Cancer* **2**, 28–37 (2002).
172. Schulz, M. D. *et al.* High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature* **514**, 508–512 (2014).
173. Arthur, J. C. *et al.* Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**, 120–123 (2012).
174. Elinav, E. *et al.* Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat. Rev. Cancer* **13**, 759–771 (2013).
175. Cuzick, J. *et al.* Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. *Lancet Oncol.* **10**, 501–507 (2009).
176. Kostadinov, R. L. *et al.* NSAIDs modulate clonal evolution in Barrett's esophagus. *PLoS Genet.* **9**, e1003553 (2013).
177. Stewart, B. W. & Wild, C. P. (eds) *World Cancer Report 2014* (IARC, 2014).
178. Medawar, P. B. *The Art of the Soluble* (Methuen & Co. Ltd, 1967).
179. Sato, T. & Clevers, H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* **340**, 1190–1194 (2013).
180. Wang, J. C. Y. & Dick, J. E. Cancer stem cells: lessons from leukemia. *Trends Cell Biol.* **15**, 494–501 (2005).
181. Metcalf, D. Hematopoietic cytokines. *Blood* **111**, 485–491 (2008).
182. Wyke, J. & Weiss, R. (eds) *Cancer Surveys: Viruses in Human and Animal Cancers* (Oxford Univ. Press, 1984).
183. Herzenberg, L. A. *et al.* The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. *Clin. Chem.* **48**, 1819–1827 (2002).
184. Piller, G. *Rays of Hope – The Story of the Leukaemia Research Fund* (Leukaemia Research Fund, 1994).
185. Luch, A. Nature and nurture – lessons from chemical carcinogenesis. *Nat. Rev. Cancer* **5**, 113–125 (2005).
186. Tabin, C. J. *et al.* Mechanism of activation of a human oncogene. *Nature* **300**, 143–149 (1982).
187. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* **300**, 149–152 (1982).
188. Zetter, B. R. The scientific contributions of M. Judah Folkman to cancer research. *Nat. Rev. Cancer* **8**, 647–654 (2008).
189. Campbell, P. J. *et al.* Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat. Genet.* **40**, 722–729 (2008).
190. Fletcher, O. & Houlston, R. S. Architecture of inherited susceptibility to common cancer. *Nat. Rev. Cancer* **10**, 353–361 (2010).
191. Lenoir, G. M., O'Conor, G. T. & Olweny, C. L. M. (eds) *Burkitt's Lymphoma: A Human Cancer Model* (WHO/IARC, 1985).
192. Burkitt, D. P. in *Burkitt's Lymphoma: A Human Cancer Model* (eds Lenoir, G. M., O'Conor, G. T. & Olweny, C. L. M.) 11–15 (IARC, 1985).
193. Boshoff, C. & Weiss, R. AIDS-related malignancies. *Nat. Rev. Cancer* **2**, 373–382 (2002).
194. Young, L. S. & Rickinson, A. B. Epstein-Barr virus: 40 years on. *Nat. Rev. Cancer* **4**, 757–768 (2004).
195. Laszlo, J. *The Cure of Childhood Leukaemia* (Rutgers Univ. Press, 1995).

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Competing interests statement

The author declares no competing interests.