Cancer stem cells and the cellular hierarchy in haematological malignancies

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Abstract

Malignancies in the haematopoietic system seem to depend on a small subset of so-called cancer stem cells (CSC) for their continued growth and progression – this was first described as the "sleeper-feeder theory" for leukaemia. The leukaemia stem cell was the first of such subsets to be described although the origins of these cells have been difficult to dissect. Consequently, their biology is not fully elucidated, which also holds true for the normal-tissue counterparts.

The stem cell concept describes stem cells to be of low frequency, self renewing and with multilineage potential based on phenomenology – a definition which may not hold strictly true for CSCs when studied in animals and humans *in vivo* and *in vitro*. Several studies have analysed the cellular hierarchy of the haematopoietic system by cell sorting of few and even single cells, tracking acquired genetic changes and performing transplantation model studies to document subsets within the differentiating hierarchy as potential CSC compartments.

In leukaemia the CSC has been described in the bone marrow compartment of haematopoietic stem cells (HSC); however, in other bone marrow disorders like multiple myeloma it is likely that the cell of origin is a more differentiated cell, like post-germinal memory B cells or plasmablasts. Studies performed so far have even indicated that the genetic events may occur in different B cell subsets in accordance with the stepwise oncogenesis of the disease.

Although our understanding of the nature and biology of these initiating cells remains unknown, the obvious existence of such cells has implications for understanding initial malignant transformation and disease metastasis or progression and, most important, the selection of individualised therapeutic strategies targeting the subsets harbouring the CSC function.

In the present review on stem cells in haematological malignancies we have focused on two topics, *first*, describing the stem cell concept in health and disease, and its "phenomenology", and *second*, describing the CSC compartments in leukaemia and multiple myeloma.

Introduction

One of the major puzzles in stem cell research in general is the construction and characterisation of the cellular hierarchy from the compartment of stem cells to mature end stage blood cells of any kind. Despite years of research, the exact construction and control of the process that leads from a quiescent self-renewing cell into a dynamic actively dividing and differentiating committed progenitor and end stage compartment is still largely unexplained. However, the hierarchy model claims that all mature blood cells derive from a bone compartment of marrow stem cells with self-renewing abilities. Differentiation into lineage-specific progenitors and mature blood cells is sequential and hierarchical, driven by cytokines and growth factors, interacting with cellular receptors and marrow stroma.

Recent research, however, has suggested that the stem cell compartment is not strictly hierarchical in structure, indicating that stem cells and their self-renewing capacity can change back and forth from engraftable stem cells to progenitor cells with cell cycle transit. In other words, the definition of stem and progenitor cells is difficult as pointed out by Potten and Loeffler, who realised that a stem cell's characteristics relate to its future potential and can only be studied effectively by allowing the cell to express that potential [1]. Therefore, to characterise a cell as a stem cell, allowing it to differentiate, the original cell is lost; at the same time one may only see a limited range of responses. The authors continued by drawing an analogy to the Heisenberg Uncertainty Principle. Originally formulated in quantum physics theory, it

says that "the very act of measuring the properties of a certain body inevitably alters the characteristics of that body, thus giving rise to uncertainty in the evaluation of its properties" [2]. Stem cell assays all observe the response after a perturbation to the system. So far, no scientist has identified the haematopoietic stem cell and it is still an open question as to how it should be defined? The answer is awaited and in the meantime we have to accept well studied and described protocols as a part of our bias.

A hierarchic model has been described for malignancies exemplified by acute and chronic myeloid leukaemia (AML, CML) and acute lymphoblastic leukaemia (ALL) and multiple myeloma (MM), suggesting that the malignant clone includes immature progenitors or stem cells present in the proliferating marrow compartment. These proliferating cells may originate from a non-dividing compartment of quiescent or dormant so-called cancer stem cells, which may be therapy resistant and responsible for disease recurrence following therapy. According to the "multistep-oncogenesis" theory, the myeloid and lymphoid malignancies develop as the consequence of a series of genetic alterations in such putative stem cells. It is unknown at which level(s) of haematopoietic differentiation the initial and/or cancer transforming genetic events occur, but the malignant process leads to a maturation stop and aggregation of immature, dedifferentiated cells of one or more lineages, easy to identify, characterise and quantitated by e.g. multiparametric flow cytometry and gene expression.

The practical usefulness and conviction of the hierarchical model rely on the emerging knowledge of stem cell biology which provides new insight into the pathogenesis of haematological malignancies. Despite these advances, most of the current prognostic and staging systems utilise relatively simple datasets. A major criticism of these systems is, however, that they rely on surrogate markers for the behaviour of the tumour. They are not capable of defining distinct biological groups and cannot, therefore, be used to define clinically meaningful subgroups, nor can they be used to predict response to the range of new specific treatments.

Progress in this area most likely relies on recent technological advances, which have enabled researchers to study single cell gene expression and also to address changes in cellular programming in a global fashion by analysing gene expression with microarrays. Lagging a little behind this approach is the technology for examining the protein compartment of the cell, but this is now rapidly developing and requires full evaluation in a clinical setting.

In the present condensed review we have focused on the stem cell concept in health and disease, and its "phenomenology" including the cancer stem cell (CSC) compartments in leukaemia and multiple myeloma as identified by novel technologies used in the field.

The stem cell concept in health and cancer

There is emerging evidence that understanding stem cell biology could provide new insights into cancer biology and reveal some of the mechanisms involved in cancer growth – including characterisation of CSCs. It is likely that normal adult stem cells are the targets of initial transforming mutations or translocations, and that cancer cell proliferation is the result of activated malignant transformed stem cells. Thus, putative cancer stem cells may share similarities with normal stem cells, in particular mechanisms that regulate self-renewal, which should be considered the most important variable described as "stemness" [3,4].

The normal stem cell concept

A normal stem cell is a unique low frequency cell type that is able to renew itself and also has the potency to produce progenitors of one or more specialised cell types. During the formation of an individual from the fertilised oocyte, cellular differentiation into specialised cell types, tissues and organs follows a strict pattern. The classical view is that during such processes the cells gradually lose the self renewal capacity and their plasticity and ability to develop into different directions.

Furthermore, stem cells may be categorised into two major classes of stem cells: pluripotent stem cells derived from early embryos, able to replenish all cell types in the animal, and multipotent stem cells located in various organs of the body, dedicated to the replenishment of specific tissues such as blood. Examples of such stem cells are embryonic stem and embryonic germ cells. Embryonic stem cells, which are derived from the inner cell mass of the blastocyst, can be cultured *in vitro* almost infinitely. Unlike embryonic stem cells, multipotent stem cells, which may be isolated from various tissues in foetal and adult animals, are lineage specific and include haematopoietic stem cells (HSCs), neuronal stem cells, and hepatic stem cells. In this review we consider HSCs and CSCs as a prototype of this category.

It is now half a century since bone-marrow reconstitution experiments, following lethal irradiation in mice, first indicated the existence of the HSC [5–

7]. Although this cell population is still not fully characterised, its discovery awakened the field of stem cell biology at the forefront of biological research. At around the same time that the existence of the HSC was postulated, observations were reported of the heterogeneous potential of tumour cells to self-renew both *in vitro* and *in vivo*. For example, in 1973, Ernest McCulloch and colleagues observed that only 1 in 100 to 1 in 10,000 murine myeloma cells had the ability to form colonies *in vitro*. In 1963, Robert Bruce and colleagues showed that only 1–4% of transplanted murine lymphoma cells formed colonies in spleens of recipient animals [8,9]. This low clonogenic potential was also observed in human AML blasts that formed colonies at low frequency in methylcellulose [10,11].

A normal stem cell is defined to repopulate the mature cells of the organ system that it serves. Although homeostatic pressures can dictate that a stem cell undergoes symmetric division to produce two daughter cells that are either both stem cells or both progenitor cells, stem cells are defined by their ability to divide asymmetrically. Through this process, the division of a stem cell results in the formation of two daughter cells – one of which is another stem cell, and the other of which is a committed progenitor that is capable of further differentiation and proliferation but lacks the ability to self-renew. A CSC would act similarly to sustain the growth and spread of the disease by repopulating the distinct cell types represented within the tumour. However, it should also be expected that a CSC would not be subject to the same intrinsic and extrinsic controls as normal stem cells.

Self-renewal

Proliferation and self-renewal are not synonymous. Self-renewal is a unique division of a cell in which the capacity of one or both progeny to proliferate and differentiate is similar to those of the parental cell. Although a committed progenitor cell might have an extensive ability to proliferate, it is destined to eventually become terminally differentiated and stop dividing. On the other hand, a self-renewing cell division of a HSC results in a cell that maintains its proliferative capacity and can reconstitute the blood system for the life of an animal [12,13].

Most tumours develop over a period of months to years and like normal tissues consist of heterogeneous populations of cells. In previous models of cancer, the unregulated growth of tumours was attributed to the serial acquisition of genetic events that resulted in: turning on genes promoting proliferation, silencing genes involved in inhibiting proliferation and circumventing genes involved in programmed cell death. In the stem cell model for cancer, another key event in tumourigenesis is the disruption of genes involved in the regulation of stem cell self-renewal. Thus, some of the cancer cells within a tumour share with normal stem cells the ability to replicate without losing the capacity to proliferate.

It is not surprising then that a number of genes initially identified as oncogenes have been implicated in normal stem cell self-renewal decisions. Genes that have been demonstrated to be involved in regulation of self-renewal in normal stem cells from many tissues include Bmi-1, Notch and Wnt [14–22]. All of these genes were initially identified for their roles in tumour formation. Since both CSCs and their progeny share identical mutations that drive tumour formation, it is likely that epigenetic events are responsible for differentiation of at least some of the cancer cells [23].

The cancer stem cell concept

It seems that tumours are composed of a heterogeneous population of cells, within which resides a small population of cancer stem cells that are exclusively responsible for the growth and propagation potential of the whole tumour. However, an alternative hypothesis, the stochastic model, could also explain the heterogeneous potential of tumour cells to self-renew. This model predicts that all tumour cells have the potential to self-renew and recapitulate the tumour, but that the probability that any particular tumour cell enters the cell cycle and finds an environment permissive for growth in an assay of tumourigenesis is varying [24]. To differentiate between these two models it is necessary to define distinct populations of cells within tumours, based on surface immunophenotypic or functional characteristics, to purify these populations to homogeneity and to develop assays evaluating and dissecting i) the gene regulation of self-renewal capacity and ii) the differentiation ability. The functional assessment of a cancer stem cell requires not only the ability to form a new stem cell, but also to recapitulate precisely the phenotype of the initial disease i.e. to balance self-renewal and differentiation.

Stepwise oncogenesis and cellular hierarchy

Haematological malignancies are heterogeneous and believed to be a consequence of a stepwise process of oncogene activation and genetic deregulation in a complex genetic make-up resulting in recurrent

chromosomal abnormality or translocations that give no clue to the hierarchical origin at the cellular level.

Cancer initiating human stem cells have been studied by two strategies: First, in animal models analysing patient samples sorted into different subpopulations for engraftment capability by disease growth *in vivo* and thereby evaluation of the cell of origin. Second, *in vitro* by tracking detectable acquired mutations, oncogenes or normal lineage specific genetic markers (e.g. the B cell receptor) back in the cellular hierarchy at the single cell level. Both of these strategies have provided valuable information about the potential stem cell compartments in patient samples from different stages like medullar contra extra-medullary diseases.

These strategies are based on the idea that a clinical detectable rearrangement or mutation has been acquired in one single cell, which may be the germ for one of the subsequent clonally expanded and detectable end stage populations. In other words, any cell surviving an acquired genetic event, at any step of the disease, may be responsible for that specific step of progression and therefore defines an originating cell. However, in accordance with the definition above, this cell will only be a CSC if *self renewal is maintained* resulting in maintenance and clonal expansion, making the mutations detectable as a part of the genetic profile of end stage tumour cells. Furthermore, it is likely that the *frequency is low* due to the clonal expansion but it needs to be shown that such a cell has preserved multipotential capacity. Finally, the CSC itself does generate *cancer progenitors* with the possibility that mutations that affect symmetric cell division may lead to the fully transformed state and extra-medullary disease.

The lesson from acute and chronic myeloid leukaemia

A hierarchic model has been described for AML, suggesting that the malignant clone includes immature low frequent leukaemia progenitors or stem cells within a proliferating pool of leukaemia end stage cells in the bone marrow. These proliferating cells seem to originate from a non-dividing compartment of quiescent or dormant leukaemia stem cells, which are therapy resistant and responsible for disease recurrence following therapy. According to the "multistep-oncogenesis" theory, AML develops as the consequence of a series of genetic alterations in rare bone marrow cells believed to be stem cells or progenitors. It is unknown at which level(s) of haematopoietic differentiation the initial and/or leukaemic transforming genetic events occur, but the malignant process leads to a maturation stop and aggregation of immature, dedifferentiated cells of one or more lineages, easy to identify, characterise and quantitated by multiparametric flow cytometry.

Historically the pattern of inactivation in X-linked genes has documented CML [25] as well as AML [26] as clonal in origin and that early stem and progenitor cells are involved in the development of CML and AML [27]. However, it was not until advances in the identification, separation and characterisation of discrete subpopulations were available, that the existence of a leukaemic CSC was demonstrated e.g. by studies of diagnostic samples from patients representing each subtype of AML, which were separated into CD34+CD38+ and CD34+CD38– fractions and injected intravenously into sub-lethally irradiated NOD/SCID mice, that at the same time were supported by injections of human cytokines. Engraftment was assessed at 4–8 weeks by human-specific DNA sequences. Human cells from the bone marrow of transplant recipients were then isolated, based on the expression of the human form of CD45, and transplanted into secondary recipients. These experiments showed that the capacity to transfer human AML to recipient mice resided exclusively within the CD34+CD38– fraction. Furthermore, these cells had the same capacity to induce all subtypes of AML, except for M3 (the most differentiated subtype of AML, acute promyelocytic leukaemia). The leukaemias that developed in the secondary recipients closely resembled the human cancer, demonstrating that leukaemic CSCs have long-term self-renewal capabilities and also determine the stage of the differentiation block during leukaemogenesis [28–32]. Further experiments from the laboratories of Craig Jordan and Donna Hogge have refined the immunophenotype of the LSC in AML to be CD34+CD38–CD90– interleukin 3 receptor (IL-3R)+CD71– human leukocyte antigen (HLA)-DR-CD117– [33–36]. Similar functional studies have been performed using primary ALL cells that carry the *BCR–ABL* fusion gene; the CSC is also defined by the CD34+CD38– immunophenotype [37]. In B-cell precursor ALL, the CSC is CD34+ but lacks expression of the more mature B-lymphoid markers CD10 and CD19 [38]. The knowledge originating from such key observations is that in AML and ALL initial and/or transforming oncogenetic events may arise at different progenitor levels of haematopoietic differentiation within the myeloid hierarchy that is similar to normal haematopoiesis.

Another cancer type that is associated with the BCR–ABL fusion is CML, which is also considered to be a stem cell disorder. Expression of the *BCR–ABL* fusion transcript has been observed in

myeloid end-stage cells as well as the CD34+ stem cell compartment [39,40]. However, recent data indicates that the CML stem cell compartment might be dynamic when it progresses to blast crisis as self-renewal properties were observed *in vitro* within the granulocyte/monocyte progenitor (GMP) compartment that normally lacks the potential for self-renewal [41]. Such data demonstrate that the CSC may change during disease progression following acquisition of transforming mutations.

The lesson from multiple myeloma

In multiple myeloma (MM), which is an incurable B cell malignancy characterised by uncontrolled growth of plasma cells (PCs) in the bone marrow (BM), the observations are similar, as animal studies have revealed malignant PCs to contain cancer initiating cells not found among earlier B cell subsets. However, cells belonging to the myeloma clone (termed clonotypic cells) that precede the PC stage have been identified in peripheral blood, lymph nodes and bone marrow [42]. The earliest clonotypic cells identified showed a preswitch isotype and have exclusively been identified in the CD38- B cell compartment [43,44] suggesting that the clonotypic precursor in myeloma could be a memory B-cell [45]. The main question is whether the clonal precursor cells are intrinsic or not to the maintenance of the MM PC clone. Whether clonal precursor cells are fully transformed cells with a clonogenic potential remains a controversial issue with studies also supporting the idea of a MM "stem-cell" present in a plasmablastic compartment [45–48], contrasting with negative studies failing to identify a clonogenic potential in this compartment [49–51].

MM oncogenesis is a multistep transformation process which may be preceded by a pre-malignant state called monoclonal gammopathy of undetermined significance (MGUS) [52]. Two early pathways of MM oncogenesis have been identified: a non-hyperdiploid pathway characterised by the presence of translocations involving the immunoglobulin heavy chain (IGH) locus (14q32), and a hyperdiploid pathway that often has multiple trisomies [53] Translocations involving the 14q32 locus show an array of promiscuous target genes with CYCLIN D1 t(11;14) and FGFR3/MMSET t(4;14) being the most frequently involved [52]. IGH translocations are introduced at the MGUS state [52] with the majority of breakpoints falling within the switch regions of the *IGH* gene [53]. This suggests that the mechanisms and the timing of the translocation are that of normal IGH switch recombination. In the hyperdiploid pathway one of the most recurrent genetic alterations is trisomy of chromosome 11, considered an early event in MM oncogenesis [54].

MM cells show stable VDJ joining sequences and accumulation of somatic mutations in the complementary determined regions (CDR) [55] and an absence of intra-clonal diversity [55,56]. These findings support the idea that MM cells are derived from a germinal centre (GC) or post GC B cell. The GC B cell can differentiate into both a memory B-cell and a PC. We have previously identified a CD19+/CD27+/CD38– subset of clonotypic cells [54,57,58] and as all clonotypic cells have accumulated somatic mutations in the *VH* genes, these cells meet all the characteristics of a memory B-cell [59]

The presence of memory B-cells expressing the same oncogene as the aberrant PCs in both malignant MM and pre-malignant MGUS indicates that the dysregulation of the observed oncogenes (CYCLIN D1 high/low and IGH-MMSET) alone is not sufficient for transformation. Thus, the identified MM related memory B-cells with 14q32 translocation and CYCLIN D1 low phenotype must accumulate additional genetic alterations to become transformed MM cells. A similar situation has been described for human acute myeloid leukemia (AML) with t(8;21) translocations. In this case, the translocation is an initial event in the pathogenesis occurring in a HSC and is necessary, but not sufficient, for transformation, and the t(8;21) positive HSC must receive additional mutational events to transform [60].

Although we have been able to identify both clonotypic and translocation 14q32 positive cells within the memory B-cell compartment, we were unable to identify RAS mutated memory B-cells in MM patients with RAS mutated PCs (unpublished observations). Thus, in a MM patient with both early and late oncogenic events present in the MM PCs, early but not late oncogenic events were identified in memory B cells.

Thus, for at least the large group of MM patients harbouring RAS mutations, one can consider the clonotypic memory B-cells as a myeloma initiating cell compartment that coexists with the transformed clone, most likely initiated outside the memory compartment. In accordance with the classical stem cell concept, its involvement as germ cells maintaining the malignant clone is still an open question.

Conclusions

Due to bias, much of what we know may be wrong! This was pointed out by Potten and Loeffler, who

realised that a stem cell's characteristics relate to its future potential and can only be studied effectively by allowing the cell to express that potential. Therefore, to characterise a cell as a stem cell, allowing it to differentiate, the original cell is lost; at the same time one may only see a limited range of responses. The authors continued by drawing an analogy to the Heisenberg Uncertainty Principle. Originally formulated in quantum physics theory, it says that "the very act of measuring the properties of a certain body inevitably alters the characteristics of that body, thus giving rise to uncertainty in the evaluation of its properties". Stem cell assays all observe the response after a perturbation to the system. So far, no scientist has identified the HSC and it is still an open question as to how it should be defined? However, new technologies studying cancer stem cell compartments have given us the opportunity to describe in an objective way some of the characteristics of the early cellular and genetic events in haematological malignancies. However, we still need to study genes involved in the phenomenon of self renewal to fully understand the role of CSCs in pathogenesis.

Going forward, finding more effective treatments for haematological malignancies will certainly remain a major challenge, at least in the near future. Nonetheless, therapies designed to target CSCs specifically represent a fundamentally new and exciting approach, and hold great promise for the development of better therapeutic regimens for patients.

Authors' contributions

All authors listed below have approved submission for publication. Further information about the contributions of each partner who participated in this collaborative review is given below:
Malene Krag Kjeldsen assisted in data interpretation and manuscript preparation; *Thomas Urup* assisted in data interpretation and manuscript preparation; *Kirsten Fogd* assisted in data interpretation and manuscript preparation; *Linda Pilgaard* assisted in data interpretation and manuscript preparation; *Martin Boegsted* assisted in data interpretation and manuscript preparation; *Mette Nyegaard* assisted in data interpretation and manuscript preparation; *Ilse Christiansen* assisted in data interpretation and manuscript preparation; *Anne Bukh* assisted in data interpretation and manuscript preparation; *Karen Dybkaer* assisted in data interpretation and manuscript preparation; *Hans E Johnsen* is corresponding author/guarantor and coordinator, assisted in study design, provision of biomaterial and patient data, data collection, analysis and interpretation and manuscript preparation.

Conflict of interest statement

None declared.

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References

1 Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* 1990;**110**(4):1001–20.
2 Heisenberg W. *Z Phys* 1927;**43**:172. Translation in: Wheeler JA, Zurek WH, eds. *Quantum Theory and Measurement*. Princeton, NJ: Princeton University Press; 1983, pp. 62–84.
3 Ho AD, Wagner W. The beauty of asymmetry: asymmetric divisions and self-renewal in the haematopoietic system. *Curr Opin Hematol* 2007;**14**(4):330–6.
4 Forsberg EC, Bhattacharya D, Weissman IL. Hematopoietic stem cells: expression profiling and beyond. *Stem Cell Rev* 2006;**2**(1): 23–30.
5 Main JM, Prehn RT. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J Natl Cancer Inst* 1955;**15**:1023–9.
6 Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961;**14**:213–22.
7 Pappenheim A. Prinzipen der neuren Morphologischen Haematozytologie nach zytogenetischer Grundlage. *Folia Haematol* 1917;**21**:91–101.
8 Park CH, Bergsagel DE, McCulloch E. A. Mouse myeloma tumor stem cells: a primary cell culture assay. *J Natl Cancer Inst* 1971;**46**:411–22.
9 Bruce WR, Van Der Gaag H. A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. *Nature* 1963;**199**:79–80.

10 Sabbath KD, Ball ED, Larcom P, Davis RB, Griffin JD. Heterogeneity of clonogenic cells in acute myeloblastic leukemia. *J Clin Invest* 1985;**75**:746–53.
11 Griffin J, Lowenberg B. Clonogenic cells in acute myeloblastic leukemia. *Blood* 1986;**68**:1185–95.
12 Spangrude GJ, Heimfeld S, Weissman IL. *Science* 1988;**241**: 58–62.
13 Morrison SJ, Weissman IL. *Immunity* 1994;**1**:661–73.
14 Austin TW, Solar GP, Ziegler FC, Liem L, Matthews W. *Blood* 1997;**89**:3624–35.
15 Spink KE, Polakis P, Weis WI. *EMBO J* 2000;**19**:2270–9.
16 Bhardwaj G, Murdoch B, Wu D, et al. *Nat Immunol* 2001;**2**: 172–80.
17 Taipale J, Beachy PA. *Nature* 2001;**411**:349–54.
18 Calvi LM, Adams GB, Weibrecht KW, et al. *Nature* 2003;**425**: 841–6.
19 Lemischka IR, Moore KA. *Nature* 2003;**425**:778–9.
20 Lessard J, Sauvageau G. *Nature* 2003;**423**:255–60.
21 Reya T, Duncan AW, Ailles L, et al. *Nature* 2003;**423**:409–14.
22 Hanson RD, Hess JL, Yu BD, et al. *Proc Natl Acad Sci USA* 1999;**96**:14372–7.
23 Li L, Connelly MC, Wetmore C, Curran T, Morgan JI. *Cancer Res* 2003;**63**:2733–6.
24 Reya T, Morrison S, Clarke M, Weissman I. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**:105–11.
25 Fialkow P, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. *Proc Natl Acad Sci USA* 1967;**58**: 1468–71.
26 Fialkow P, Martin PJ, Najfeld V, et al. Acute nonlymphocytic leukemia: heterogeneity of stem cell origin. *Blood* 1981;**57**: 1068–73.
27 Fialkow PJ, Jacobson RJ, Papayannopoulou T. Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. *Am J Med* 1977;**63**:125–30.
28 Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;**3**:730–7.
29 McCune JM, Namikawa R, Kaneshima H, et al. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 1988;**241**:1632–9.
30 Kamel-Reid S, Letarte M, Sirard C, et al. A model of human acute lymphoblastic leukemia in immune-deficient SCID mice. *Science* 1989;**246**:1597–600.
31 Sirard C, Lapidot T, Vormoor J, et al. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. *Blood* 1996;**87**:1539–48.
32 Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;**367**:645–8.
33 Jordan CT, Upchurch D, Szilvassy SJ, et al. The interleukin-3 receptor chain is a unique marker for human acute myelogenous leukemia stem cells. *Leukemia* 2000;**14**:1777–84.
34 Blair A, Hogge DE, Ailles LE, Lansdorp PM, Sutherland HJ. Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. *Blood* 1997;**89**:3104–12.
35 Blair A, Hogge DE, Sutherland HJ. Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34+/CD71–/HLA–DR–. *Blood* 1998;**92**:4325–35.
36 Blair A, Sutherland HJ. Primitive acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo lack surface expression of c-kit (CD117). *Exp Hematol* 2000;**28**: 660–71.
37 Cobaleda C, Gutiérrez-Cianca N, Pérez-Losada J, et al. A primitive hematopoietic cell is the target for the leukemic transformation in human philadelphia-positive acute lymphoblastic leukemia. *Blood* 2000;**95**:1007–13.
38 Cox CV, Evely RS, Oakhill A, et al. Characterization of acute lymphoblastic leukemia progenitor cells. *Blood* 2004;**104**:2919–25.
39 Deininger M, Goldman J, Melo, J. The molecular biology of chronic myeloid leukemia. *Blood* 2000;**96**:3343–56.
40 Martin PJ, Najfeld V, Hansen JA, et al. Involvement of the B-lymphoid system in chronic myelogenous leukaemia. *Nature* 1980;**287**:49–50.
41 Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* 2004;**351**:657–67.
42 Rasmussen T, Lodahl M, Hancke S, Johnsen HE. In multiple myeloma clonotypic CD38-/CD19+/CD27+ memory B cells recirculate through bone marrow, peripheral blood and lymph nodes. *Leuk Lymphoma* 2004;**45**:1413–7.
43 Billadeau D, Ahmann G, Greipp P, Van NB. The bone marrow of multiple myeloma patients contains B cell populations at different stages of differentiation that are clonally related to the malignant plasma cell. *J Exp Med* 1993;**178**:1023–31.
44 Corradini P, Boccadoro M, Voena C, Pileri A. Evidence for a bone marrow B cell transcribing malignant plasma cell VDJ joined to C mu sequence in immunoglobulin (IgG)- and IgA-secreting multiple myelomas. *J Exp Med* 1993;**178**:1091–6.
45 Rasmussen T, Jensen L, Johnsen HE. The clonal hierachy in multiple myeloma. *Acta Oncol* 2001;**39**:765–70.
46 Reiman T, Seeberger K, Taylor BJ, et al. Persistent preswitch clonotypic myeloma cells correlate with decreased survival: evidence for isotype switching within the myeloma clone. *Blood* 2001;**98**:2791–9.
47 Pilarski LM, Hipperson G, Seeberger K, et al. Myeloma progenitors in the blood of patients with aggressive or minimal disease: engraftment and self-renewal of primary human myeloma in the bone marrow of NOD SCID mice. *Blood* 2000;**95**:1056–65.
48 Pilarski LM, Seeberger K, Coupland RW, et al. Leukemic B cells clonally identical to myeloma plasma cells are myelomagenic in NOD/SCID mice. *Exp Hematol* 2002;**30**:221–8.
49 Matsui W, Huff CA, Wang Q, et al. Characterization of clonogenic multiple myeloma cells. *Blood* 2004;**103**:2332–6.
50 Davies FE, Rawstron AC, Pratt G, et al. FICTION-TSA analysis of the B-cell compartment in myeloma shows no significant expansion of myeloma precursor cells. *Br J Haematol* 1999; **106**:40–6.
51 Yaccoby S, Epstein J. The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. *Blood* 1999;**94**: 3576–82.
52 Guikema JE, Vellenga E, Bakkus MH, Bos NA. Myeloma clonotypic B cells are hampered in their ability to undergo B-cell differentiation in vitro. *Br J Haematol* 2002;**119**:54–61.
53 Kuehl WM, Bergsagel PL. Multiple myeloma: Envolving genetic events and host interactions. *Nature Reviews Cancer* 2002;**2**: 175–87.
54 Bergsagel PL, Kuehl WM, Zhan F, et al. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood* 2005;**106**:296–303.

55 Bergsagel PL, Kuehl M. Chromosome translocation in multiple myeloma. *Oncogene* 2001;**20**:5611–22.
56 Vescio RA, Cao J, Hong CH, et al. Myeloma Ig heavy chain V region sequences reveal prior antigenic selection and marked somatic mutation but no intraclonal diversity. *J Immunol* 1995; **155**:2487–97.
57 Bakkus MH, Heirman C, Van RI, Van CB, Thielemans K. Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. *Blood* 1992;**80**:2326–35.
58 Rasmussen T, Jensen L, Honoré L, Johnsen HE. Frequency and kinetics of polyclonal and clonal B-cells in the peripheral blood of patients being treated for multiple myeloma. *Blood* 2000;**96**:4357–59.
59 Rasmussen T, Jensen L, Johnsen HE. Levels of circulating CD19+ cells in patients with multiple myeloma. *Blood* 2000;**95**:4020–1.
60 Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. *Immunol Today* 2000;**21**:204–6.