Published in final edited form as:

Cancer Lett. 2009 May 8; 277(1): 1–7. doi:10.1016/j.canlet.2008.08.005.

Cancer stem cells in multiple myeloma

Nilanjan Ghosh and William Matsui*

Division of Hematologic Malignancies, Department of Oncology and The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, CRB245, 1650 Orleans Street, Baltimore, MD 21231, USA

Abstract

Several key observations providing evidence for the cancer stem cell hypothesis and insights into the unique biology of these cells have come from the study of multiple myeloma. These include evidence that cancer cells may be functionally heterogeneous in spite of their genetic homogeneity and that malignant progenitors share many biological features with normal adult stem cells including drug resistance and regulatory processes governing self-renewal. We review studies that have examined clonogenic cells in multiple myeloma, highlight controversies regarding the cell of origin in multiple myeloma, and discuss potential targeting strategies.

Keywords

Multiple myeloma; Stem cells; B cells; Hedgehog; Immune therapy

1. Background

Increasing data from several human cancers suggest that neoplastic cells within individual tumors are functionally heterogeneous cells despite their clonal origins. In particular, the potential for long-term proliferation appears to be restricted to subpopulations of “cancer stem cells” functionally defined by their capacity to undergo self-renewal and give rise to differentiated cells that phenotypically recapitulate the original tumor [1-6]. Furthermore, these functional properties have implicated cancer stem cells in mediating disease initiation, relapse and progression. In multiple myeloma, the existence of cancer stem cells has long been proposed since early experiments examining the growth potential of mouse plasma cell tumors were carried out over four decades ago. However, the exact nature of the myeloma stem cell and its relationship to normal plasma cell and B cell development is unclear. We will review the evidence that multiple myeloma consists of heterogeneous cell types and that biologically distinct cancer stem cells exist within the disease. We will also discuss potential strategies that may target myeloma stem cells and prevent disease relapse and progression.

2. Cellular heterogeneity in multiple myeloma

2.1. Functional heterogeneity

Normal plasma cells are terminally differentiated and lack significant replicative potential. Similarly, multiple myeloma plasma cells appear quiescent [7], and early evidence that clonogenic growth potential was not a feature shared by all tumor cells was provided by studies carried out by Bergsagel and Valeriote [8]. These investigators studied a mouse plasma cell

*Corresponding author. Tel.: +1 410 955 2808; fax: +1 410 614 7279. E-mail address: matsui@jhmi.edu (W. Matsui).

Conflict of Interest Statement The authors indicated no potential conflicts of interest.

tumor, Adj. PC5, derived from Balb/C mice in which chronic peritoneal inflammation was induced by incomplete Freud's adjuvant and inactivated *Staphylococci*. Building upon the pioneering work by Till and McCullough who demonstrated the existence of multi-potent hematopoietic stem cells [9], these investigators studied tumor cell engraftment within syngeneic recipient animals following intravenous injection. Using spleen colony formation as an indicator of clonogenic growth potential, the tumorigenic cell frequency of Adj. PC5 cells was estimated at 1 in 1000-4000 cells, relatively low compared to mouse models of acute leukemia and aggressive lymphoma. Furthermore, the resulting colonies were capable of engrafting secondary recipients demonstrating that these clonogenic cells could undergo self-renewal. These investigators subsequently developed an *in vitro* clonogenic assay and confirmed the functional heterogeneity of Adj. PC-5 cells since only a minority of cells was capable of producing tumor colonies [10]. The applicability of these studies to human multiple myeloma was later demonstrated following the development of an *in vitro* clonogenic assay capable of supporting the growth of human primary tumors by Salmon and Hamburger [11, 12]. This assay allowed the clonogenic frequency of a variety of human tumors to be determined, and they estimated that the tumor colony forming potential of primary bone marrow samples derived from multiple myeloma patients was approximately 1 in 100-100,000 cells. Human myeloma colonies could also be dissociated and undergo further colony formation in secondary cultures suggesting that these clonogenic cells had the capacity for self-renewal. Therefore, these studies demonstrate that the clonogenic potential of multiple myeloma cells either *in vitro* or *in vivo* is restricted to a minority of tumor cells.

2.2. Phenotypic heterogeneity

Plasma cells normally arise from the differentiation of B cells, and the potential that B cells are involved in the pathogenesis of multiple myeloma has been investigated by a number of groups. The immunoglobulin gene rearrangement and resulting antibody idiotype provides a highly specific means of determining clonal relationships in B cell malignancies that is unique among human cancers. Initial studies utilizing anti-idiotype antibodies generated against the circulating monoclonal immunoglobulin (M protein) produced by myeloma cells identified cells in both the bone marrow and blood [13]. Furthermore, these antibodies identified idiotypes at the cell surface similar to the staining of B cells rather than within the cytoplasm typical of plasma cells, and subsequent phenotyping confirmed that they expressed surface antigens reminiscent of B cells. These clonal relationships were later confirmed by studies demonstrating that the identical immunoglobulin gene sequences or chromosomal abnormalities contained within myeloma plasma cells could be found in B cells isolated from the peripheral blood and bone marrow [14-21]. Additionally, these clonotypic B cells have been implicated as precursor cells in multiple myeloma since they have been found to differentiate into antibody secreting mature plasma cells *in vitro* [22,23].

2.3. Multiple myeloma cell of origin

Analysis of the immunoglobulin gene sequence itself has provided insights into the stage of normal B cell development that gives rise to multiple myeloma. Early B cell development is marked by VDJ rearrangement within the immunoglobulin heavy-chain gene [24]. Following antigenic stimulation during the late stages of the primary and secondary immune responses, further antibody diversification is generated by somatic hypermutation within the germinal center. The B cell progeny which have high antigen avidity undergo extensive proliferation and clonal expansion whereas those with poor specificity undergo apoptosis. After this selection process, somatic hypermutation ceases and memory B cells and plasma cells lack the ability for further antibody diversification. Sequencing of the immunoglobulin genes from myeloma plasma cells has demonstrated the presence of extensive somatic hypermutation without intraclonal variation [25-27]. This suggests that the disease arises from a post-germinal

center compartment, and subsequent studies of circulating clonotypic cells have revealed that they express CD19 and CD27 consistent with a memory B cell phenotype [28,29].

3. Cancer stem cells in multiple myeloma

Cellular heterogeneity is a feature of most human tumors, and the association between distinct phenotypes and specific functional growth properties has been highlighted by the identification of cancer stem cells in an increasing number of human malignancies. These studies have relied upon the engraftment of human tumors in immunodeficient mice, and the first model to support xenotransplantation of primary human multiple myeloma specimens was developed by Yaccoby and Epstein using SCID mice implanted with human fetal bone fragments (SCID-hu mice) [30]. In this model, the majority of primary multiple myeloma bone marrow specimens engraft when injected directly into the human bone fragment, and the several clinical aspects of multiple myeloma including circulating M protein, hypercalcemia, and resorption of the human bone are observed. Subsequent studies demonstrated that growth within the human bone fragment of SCID-hu mice was restricted to plasma cells defined by a CD38^{high}CD45^{neg} surface phenotype, whereas cell fractions containing CD19⁺ B cells were incapable of growth [31]. In addition, the capacity of plasma cells to self-renew was demonstrated by engrafting secondary SCID-hu recipient mice.

In contrast to these studies, Pilarski and Belch demonstrated that cells from the peripheral blood of patients with late-stage disease or G-CSF mobilized peripheral blood from patients with minimal disease were capable of engrafting immunodeficient NOD/SCID mice lacking any human elements following intraosseous or intracardiac injection [32]. Engrafting animals demonstrated circulating M protein as well as lytic bone lesions typical of myeloma, and tumor cells could be transplanted into secondary recipients indicative of self-renewal. Furthermore, engrafting tumor cells consisted of both CD38^{hi} plasma cells as well as CD19⁺ B cells, but the capacity of each of these cell types to achieve primary or secondary engraftment was not determined. A subsequent study examining a single patient with progressive disease found that circulating clonotypic cells expressing the B cell marker CD19 and lacking the plasma cell surface antigen CD138 could engraft NOD/SCID mice and give rise to both CD19⁺ and CD138⁺ tumor cells [33]. These results suggest that clonotypic B cells are myelomagenic in NOD/SCID mice.

Our group studied the engraftment of primary myeloma specimens in NOD/SCID mice and found that plasma cells defined by surface expression of CD138 were incapable of engraftment following intravenous injection [34]. In contrast, cells lacking CD138 were able to engraft mice and give rise to mature CD138⁺ plasma cells functionally capable of producing circulating M protein. Using an *in vitro* clonogenic assay in which CD138^{neg}, but not CD138⁺ cells, gave rise to myeloma colonies similar to our *in vivo* results, we found that clonogenic myeloma cells expressed the B cell surface antigens, CD45, CD19, CD20, and CD22. Therefore, clonogenic myeloma growth appears to be restricted to phenotypic B cells.

Adaptive immune recall over the lifetime of an individual is mediated by memory B and T cells. Although relatively differentiated and incapable of maturation into multiple tissue types, both memory B and T cells are thought to share biologic properties with normal stem cells that contribute to their long-term persistence. Comparative genetic analysis of memory B and T cells have demonstrated that these cells highly express genes critical for the self-renewal of hematopoietic and embryonic stem cells [35,36]. Furthermore, memory T cells have been found to undergo asymmetrical cell division, a process generally thought to be restricted to true stem cells [37]. Although asymmetric cell division has not been demonstrated for memory B cells, careful studies examining the kinetics of memory B cells in response to specific antigen exposure coupled with the maintenance of their specific phenotype and long-term proliferative

capacity strongly suggests that the process of self-renewal maintains life-long antigen recognition [38-41]. Other human cancers in which stem cells have been well defined include acute myeloid leukemia and brain tumors. Interestingly, cancer stem cells in these two diseases phenotypically resemble normal hematopoietic and neural stem cells, respectively, suggesting that human cancers may arise from cells that normally have the capacity for self-renewal [4, 42,43]. The finding that clonotypic myeloma B cells phenotypically resemble normal memory B cells and evidence that these cells are capable of self-renewal during the maintenance of long-term immunologic memory suggests that myeloma arises from this compartment. We recently further studied the CD138^{neg} clonogenic myeloma cells and found that *in vitro* colony formation was mediated by cells that co-express CD19 and CD27 surface antigens that define memory B cells [44,45]. We also found that CD19⁺CD27⁺CD138^{neg} cells isolated from the peripheral blood of multiple myeloma patients were able to engraft NOD/SCID mice and generate mature CD138⁺ myeloma plasma cells. Moreover, CD19⁺ B cells isolated from engrafted animals had the ability to recapitulate disease in secondary recipients. Therefore, our studies suggest that clonogenic myeloma cells are not mature CD138⁺ plasma cells but instead resemble memory B cells that have the capacity to undergo self-renewal and give rise to differentiated tumor cells.

The discrepancies between these reports have generated controversy surrounding the phenotype of cancer stem cells in multiple myeloma. However, similar inconsistencies have emerged in studies of clonogenic cell populations in other human cancers, such as colon and pancreatic carcinomas [5,6,46-48]. These results are likely to be influenced by the nature of the specific functional assay used to study clonogenic myeloma growth. The bone marrow microenvironment has been found to play an important role in the proliferation, survival, and drug resistance of multiple myeloma plasma cells, and the presence of fetal human bone fragments within SCID-hu mice may contribute to the engraftment of mature plasma cells in this setting. However, it is unclear whether the engraftment of myeloma plasma cells in these animals fully establishes their role as the myeloma stem cell since SCID-hu mice can engraft with acute myeloid leukemic blasts expressing markers of lineage commitment that are thought to lack long-term proliferative potential [49]. It is also possible that the precise methods used to isolate candidate cell populations or the biological heterogeneity among patients at different clinical stages of disease progression may have also influenced the observed results. Therefore, a comparison of clonogenic assays using precisely defined cellular subsets and primary samples derived from clinically similar patient populations may better define the stem cell in myeloma.

4. Therapeutic implications of myeloma cancer stem cells

4.1. Drug resistance

A variety of clinical approaches are capable of producing responses that include corticosteroids and conventional cytotoxic chemotherapeutic agents administered at normal levels or at high-doses in combination with stem cell rescue [50]. Furthermore, several novel agents with unique mechanisms of action of been recently approved for clinical use [50]. This wide range of therapeutic options has steadily improved the ability to induce clinical responses detected as decreased plasma cells in the bone marrow or circulating M protein. However, multiple myeloma remains incurable for the vast majority of patients suggesting that cancer stem cells with the growth capacity to mediate relapse are relatively resistant to these clinical strategies. Several studies have found that circulating clonotypic B cells may persist following systemic treatment and their frequency increases during clinical relapse [51-54]. These results suggest that these cells are drug resistant and mediate tumor regrowth, and clonotypic B cells have been found to express the multi-drug transporter P-glycoprotein [55].

We compared the effects of several anti-myeloma agents on distinct cellular compartments and found that CD138^{neg} clonogenic precursors are relatively resistant to most of these agents at

concentrations that inhibit mature CD138⁺ plasma cells [44]. These agents included glucocorticoids, alkylators, the thalidomide analogue lenalidomide, the proteasome inhibitor bortezomib that are currently used in the treatment of myeloma patients, and it is likely that these results explain the why these agents are effective at tumor debulking but lack curative potential as single agents. The cellular processes that mediate drug resistance by cancer stem cells are poorly understood, but normal stem cells are similarly resistant to a wide variety of toxic compounds through multiple mechanisms. Interestingly, several flow cytometric assays with the capacity to identify both normal and cancer stem cells, such as the side population and Aldefluor assays, measure distinct cellular properties that can confer drug resistance [56, 57]. We found that these assays can identify memory B cells within the circulation of multiple myeloma patients clonally related to the malignant plasma cells [44]. Therefore, the drug resistance exhibited by myeloma stem cells may be mediated by similar processes that protect normal stem cells.

4.2. Targeting self-renewal pathways

Cancer stem cells have been defined by two critical properties, the capacity to undergo self-renewal and give rise to differentiated tumor cells [58]. Similar to the shared properties that may promote drug resistance, it is likely that the pathways regulating the self-renewal of normal stem cells play a similar role in cancer stem cells. Highly conserved signaling pathways, such as Notch, Wnt, and Hedgehog regulate stem cell fate decisions during normal development, and each of these is active in a wide range of human cancers, including multiple myeloma [59,60]. We investigated the role of Hedgehog signaling in multiple myeloma, and found that key regulators of Hedgehog signal transduction were over-expressed in both multiple myeloma cell lines and clinical specimens [61]. Furthermore, Hedgehog pathway activation appeared to be increased in the CD138^{neg} cells we previously identified as myeloma cancer stem cells. We examined the functional role of Hedgehog signaling on multiple myeloma stem cells and found that pathway activation by Hedgehog ligand induced the expansion of less differentiated CD138^{neg} cells, whereas pathway inhibition using a monoclonal neutralizing antibody against Hedgehog ligands or the naturally occurring small molecule inhibitor cyclopamine limited subsequent clonogenic growth. The inhibition of clonogenic growth was coupled with the accelerated differentiation of these cells into mature CD138⁺ cells suggesting that Hedgehog signaling plays a critical role in dictating multiple myeloma stem cell fate decisions. Therefore, conserved stem cell regulatory processes, such as developmental signaling pathways may provide therapeutic targets within drug resistant cancer stem cells.

4.3. Immunological targeting of myeloma stem cells

Monoclonal antibodies have emerged as effective agents for the treatment of a wide variety of human cancers. In particular, the humanized antibody rituximab can improve clinical outcomes in a wide variety of B cell malignancies [62,63], and findings that myeloma stem cells phenotypically resemble normal B cells have suggested that it may be able to target these cells. Examining primary myeloma specimens, we found that treatment with rituximab in combination with complement to induce antibody-dependent cellular lysis limited tumor colony formation *in vitro* [44]. Rituximab has been clinically studied in multiple myeloma and demonstrated limited effects on outcomes in a small single-armed clinical trial [64]. However, the disparity between these clinical results and our *in vitro* data may be explained by recently correlative laboratory studies suggesting that rituximab may bind to myeloma stem cells, but lacks the capacity to eliminate these cells *in vivo* (C.A. Huff, personal communication).

The graft versus host reaction generated during allogeneic bone marrow transplantation is associated with long-term remissions and suggests that immune based approaches may be able to eliminate multiple myeloma cancer stem cells [65,66]. The morbidity and mortality associated with allogeneic transplantation has limited the use of this procedure use in the

majority of multiple myeloma patients, but its curative potential has generated great interest in developing tumor specific immunotherapeutic approaches. The ideal target antigen in multiple myeloma is unknown, but the high degree of tumor specificity associated with the M protein idiotype has led to its use in clinical studies. One such study involving vaccination with purified M protein led to a reduction in the levels of circulating clonotypic B cells in 4 of 6 evaluable patients [67]. Little effect was observed on the M protein levels indicating that this approach had little impact upon overall disease burden. However, the prolonged life-span of multiple myeloma plasma cells may have prevented detection of clinical benefit during the 30 week evaluation period even if clonogenic multiple myeloma stem cells had been targeted. Furthermore, several small clinical trials have utilized dendritic cells pulsed with M protein as a vaccination strategy either alone or following high-dose therapy [68-71]. These studies clearly indicate that anti-idiotype immunity can be generated using these approaches, but the capacity to target myeloma stem cells has yet to be addressed. The embryonic stem cell-associated antigen SOX2 may represent another potential antigen expressed by multiple myeloma stem cells [72]. SOX2 was identified during the examination of tumor antigens serologically recognized by patients with monoclonal gammopathy of unknown significance but absent in those with multiple myeloma. Intriguingly, SOX2 is expressed by clonogenic myeloma cells and stimulation of anti-SOX2 immunity limited the clonogenic growth of primary myeloma samples *in vitro*. Autologous lymphocytes infiltrating tumors have been found to recognize cancer cells in several diseases. In multiple myeloma the bone marrow represents the tumor microenvironment and this compartment has been found to have a significantly greater frequency of T cells recognizing myeloma antigens compared to the peripheral blood. Following *in vitro* activation and expansion using anti-CD3/CD28 beads, marrow derived T cells have been found to be capable of inhibiting both CD138⁺ and CD138^{neg} cells *in vitro* suggesting that they are active against both mature tumor cells and cancer stem cells [73].

5. Persistent questions and future directions

Cancer stem cells have been identified in an increasing number of human malignancies, but many questions remain. For example, the precise relationship between cancer stem cells and their normal counterparts is unknown. In myeloid leukemias and brain tumors shared antigenic profiles suggest that cancer stem cells arise from the transformation of normal adult stem cells. Similarly, our data suggest that clonogenic multiple myeloma cells phenotypically resemble normal memory B cells that may be self-renewing. However, it is unclear whether all human cancers arise from normal self-renewing cells since studies in chronic myeloid leukemia and model systems of AML have demonstrated that specific genetic or epigenetic alterations may induce self-renewal in more differentiated cells that normally have limited replicative potential [74,75]. Furthermore, it is unclear what role cancer stem cells play during disease progression or whether the functional cellular hierarchy characterizing tumors at initial diagnosis is maintained during this process.

The processes that regulate cancer stem cells are also poorly understood. In several cancers, specific genetic lesions carry prognostic significance. This suggests that these alterations directly influence the biology of clonogenic cells which dictate long-term outcomes, but the functional consequences of specific lesions on cancer stem cells are just beginning to emerge [76,77]. Extrinsic regulation of normal adult stem cells is mediated by the stem cell niche that maintains quiescence and self-renewal potential [78]. But there is little data suggesting that cancer stem cells similarly require or are regulated by the microenvironment. Furthermore, several signaling pathways that regulate normal stem cells are active in cancer stem cells, but their potential to serve as anti-cancer targets is unknown.

In most human cancers studied to date, stem cells have been found to be relatively rare and restricted to phenotypically defined subpopulations of tumor cells. However, recent findings demonstrate that the cells responsible for *in vivo* clonogenic growth of murine tumors in syngeneic recipients may be less phenotypically restricted and relatively frequent [79]. Therefore, the identification and characterization of cancer stem cells may be influenced by xenotransplantation of human cancers in immunodeficient mice. It is likely that this issue as well as the relevance of cancer stem cells will be more definitively resolved through carefully executed and analyzed therapeutic trials and correlative laboratory studies.

Multiple myeloma may provide the ideal setting for investigating these questions. Several advances have been made in understanding basic myeloma biology, such as the role of the microenvironment in therapeutic resistance and identification of specific genetic lesions that can dictate long-term outcomes or are associated with disease progression [80–83]. Additionally, ready access to primary clinical samples along the entire disease continuum that spans asymptomatic monoclonal gammopathy of unknown significance to aggressive myeloma allows clonogenic cell populations to be serially investigated within individual patients. Therefore, the integration of disease-specific biology with more definitive studies defining the multiple myeloma stem cell may provide insights that are applicable to many human tumors.

Acknowledgements

This work was supported by grants from the National Institutes of Health, The International Myeloma Foundation, The Sidney Kimmel Foundation for Cancer Research, and the American Society of Clinical Oncology.

References

- [1]. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang TC-CJ, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;367:645–648. [PubMed: 7509044]
- [2]. Al Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* 2003;100:3983–3988. [PubMed: 12629218]
- [3]. Zucchi I, Sanzone S, Astigiano S, Pelucchi P, Scotti M, Valsecchi V, Barbieri O, Bertoli G, Albertini A, Reinbold RA, Dulbecco R. The properties of a mammary gland cancer stem cell. *Proc. Natl. Acad. Sci. USA* 2007;104:10476–10481. [PubMed: 17566110]
- [4]. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004;432:396–401. [PubMed: 15549107]
- [5]. Dalerba P, Dylla SJ, Park I.k, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. *Proc. Natl. Acad. Sci. USA* 2007;104:10158–10163. [PubMed: 17548814]
- [6]. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106–110. [PubMed: 17122772]
- [7]. Drewinko B, Alexanian R, Boyer H, Barlogie B, Rubinow SI. The growth fraction of human myeloma cells. *Blood* 1981;57:333–338. [PubMed: 7448427]
- [8]. Bergsagel DE, Valeriote FA. Growth characteristics of a mouse plasma cell tumor. *Cancer Res* 1968;28:2187–2196. [PubMed: 5723963]
- [9]. TILL JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res* 1961;14:213–222. [PubMed: 13776896]
- [10]. Park CH, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. *J. Natl. Cancer Inst* 1971;46:411–422. [PubMed: 5115909]

- [11]. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977;197:461–463. [PubMed: 560061]
- [12]. Hamburger A, Salmon SE. Primary bioassay of human myeloma stem cells. *J. Clin. Invest* 1977;60:846–854. [PubMed: 302265]
- [13]. Mellstedt H, Hammarstrom S, Holm G. Monoclonal lymphocyte population in human plasma cell myeloma. *Clin. Exp. Immunol* 1974;17:371–384. [PubMed: 4219599]
- [14]. Billadeau D, Ahmann G, Greipp P, Van Ness B. 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;178:1023–1031. [PubMed: 8350044]
- [15]. Bakkus MH, Van R, Van Camp IB, Thielemans K. Evidence that the clonogenic cell in multiple myeloma originates from a pre-switched but somatically mutated B cell. *Br. J. Haematol* 1994;87:68–74. [PubMed: 7947257]
- [16]. Bergsagel PL, Smith AM, Szczepek A, Mant MJ, Belch AR, Pilarski LM. In multiple myeloma, clonotypic B lymphocytes are detectable among CD19⁺ peripheral blood cells expressing CD38, CD56, and monotypic Ig light chain. *Blood* 1995;85:436–447. [PubMed: 7529064]
- [17]. Chen BJ, Epstein J. Circulating clonal lymphocytes in myeloma constitute a minor subpopulation of B cells. *Blood* 1996;87:1972–1976. [PubMed: 8634446]
- [18]. Szczepek AJ, Seeberger K, Wizniak J, Mant MJ, Belch AR, Pilarski LM. A high frequency of circulating B cells share clonotypic Ig heavy-chain VDJ rearrangements with autologous bone marrow plasma cells in multiple myeloma, as measured by single-cell and in situ reverse transcriptase-polymerase chain reaction. *Blood* 1998;92:2844–2855. [PubMed: 9763569]
- [19]. Rasmussen T, Kastrup J, Knudsen LM, Johnsen HE. High numbers of clonal CD19⁺ cells in the peripheral blood of a patient with multiple myeloma. *Br. J. Haematol* 1999;105:265–267. [PubMed: 10233390]
- [20]. Pilarski LM, Giannakopoulos NV, Szczepek AJ, Masellis AM, Mant MJ, Belch AR. In multiple myeloma, circulating hyperdiploid B cells have clonotypic immunoglobulin heavy chain rearrangements and may mediate spread of disease. *Clin. Cancer Res* 2000;6:585–596. [PubMed: 10690543]
- [21]. Zojer N, Schuster-Kolbe J, Assmann I, Ackermann J, Strasser K, Hubl W, Drach J, Ludwig H. Chromosomal aberrations are shared by malignant plasma cells and a small fraction of circulating CD19⁺ cells in patients with myeloma and monoclonal gammopathy of undetermined significance. *Br. J. Haematol* 2002;117:852–859. [PubMed: 12060120]
- [22]. Bergui L, Schena M, Gaidano G, Riva M, Caligaris-Cappio F. Interleukin 3 and interleukin 6 synergistically promote the proliferation and differentiation of malignant plasma cell precursors in multiple myeloma. *J. Exp. Med* 1989;170:613–618. [PubMed: 2787832]
- [23]. Caligaris-Cappio F, Bergui L, Tesio L, Pizzolo G, Malavasi F, Chilosi M, Campana D, van CB, Janossy G. Identification of malignant plasma cell precursors in the bone marrow of multiple myeloma. *J. Clin. Invest* 1985;76:1243–1251. [PubMed: 2931452]
- [24]. Kuppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N. Engl. J. Med* 1999;341:1520–1529. [PubMed: 10559454]
- [25]. Bakkus MH, Heirman C, Van Riet I, Van Camp B, Thielemans K. Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. *Blood* 1992;80:2326–2335. [PubMed: 1421403]
- [26]. Sahota S, Hamblin T, Oscier DG, Stevenson FK. Assessment of the role of clonogenic B lymphocytes in the pathogenesis of multiple myeloma. *Leukemia* 1994;8:1285–1289. [PubMed: 8057663]
- [27]. Vescio RA, Cao J, Hong CH, Lee JC, Wu CH, Der DM, Wu V, Newman R, Lichtenstein AK, Berenson JR. Myeloma Ig heavy chain V region sequences reveal prior antigenic selection and marked somatic mutation but no intraclonal diversity. *J. Immunol* 1995;155:2487–2497. [PubMed: 7650379]
- [28]. Rasmussen T, Jensen L, Johnsen HE. The clonal hierarchy in multiple myeloma. *Acta Oncol* 2000;39:765–770. [PubMed: 11145430]
- [29]. Rasmussen T, Lodahl M, Hancke S, Johnsen HE. In multiple myeloma clonotypic. *Leuk. Lymphoma* 2004;45:1413–1417. [PubMed: 15359642]

- [30]. Yaccoby S, Barlogie B, Epstein J. Primary myeloma cells growing in SCID-hu mice. A model for studying the biology and treatment of myeloma and its manifestations. *Blood* 1998;92:2908–2913. [PubMed: 9763577]
- [31]. Yaccoby S, Epstein J. The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. *Blood* 1999;94:3576–3582. [PubMed: 10552969]
- [32]. Pilarski LM, Hipperson G, Seeberger K, Pruski E, Coupland RW, Belch AR. 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;95:1056–1065. [PubMed: 10648422]
- [33]. Pilarski LM, Seeberger K, Coupland RW, Eshpeter A, Keats JJ, Taylor BJ, Belch AR. Leukemic B cells clonally identical to myeloma plasma cells are myelomagenic in NOD/SCID mice. *Exp. Hematol* 2002;30:221–228. [PubMed: 11882359]
- [34]. Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, Smith BD, Civin CI, Jones RJ. Characterization of clonogenic multiple myeloma cells. *Blood* 2004;103:2332–2336. [PubMed: 14630803]
- [35]. Luckey CJ, Bhattacharya D, Goldrath AW, Weissman IL, Benoist C, Mathis D. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* 2006;103:3304–3309. [PubMed: 16492737]
- [36]. Tomayko MM, Anderson SM, Brayton CE, Sadanand S, Steinell NC, Behrens TW, Shlomchik MJ. Systematic comparison of gene expression between murine memory and naive B cells demonstrates that memory B cells have unique signaling capabilities. *J. Immunol* 2008;181:27–38. [PubMed: 18566367]
- [37]. Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, Banerjee A, Longworth SA, Vinup KE, Mrass P, Oliaro J, Killeen N, Orange JS, Russell SM, Weninger W, Reiner SL. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* 2007;315:1687–1691. [PubMed: 17332376]
- [38]. Schitteck B, Rajewsky K. Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. *Nature* 1990;346:749–751. [PubMed: 2388695]
- [39]. Decker DJ, Linton PJ, Zaharevitz S, Biery M, Gingeras TR, Klinman NR. Defining subsets of naive and memory B cells based on the ability of their progeny to somatically mutate in vitro. *Immunity* 1995;2:195–203. [PubMed: 7534621]
- [40]. Heyzer-Williams LJ, Cool M, Heyzer-Williams MG. Antigen-specific B cell memory: expression and replenishment of a novel B220-memory B cell compartment. *J. Exp. Med* 2000;191:1149–1166. [PubMed: 10748233]
- [41]. Aviszus K, Zhang X, Wysocki LJ. Silent development of memory progenitor B cells. *J. Immunol* 2007;179:5181–5190. [PubMed: 17911603]
- [42]. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med* 1997;3:730–737. [PubMed: 9212098]
- [43]. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. Cancerous stem cells can arise from pediatric brain tumors. *Proc. Natl. Acad. Sci. USA* 2003;100:15178–15183. [PubMed: 14645703]
- [44]. Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, McNiece I, Lin L, Ambinder RF, Peacock C, Watkins DN, Huff CA, Jones RJ. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. *Cancer Res* 2008;68:190–197. [PubMed: 18172311]
- [45]. Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. *Immunol. Today* 2000;21:204–206. [PubMed: 10782048]
- [46]. Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St CR, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D, Rafii S. CD133 expression is not restricted to stem cells, and both CD133 and CD133 metastatic colon cancer cells initiate tumors. *J. Clin. Invest* 2003;112:1–2.
- [47]. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67:1030–1037. [PubMed: 17283135]

- [48]. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1:313–323. [PubMed: 18371365]
- [49]. Namikawa R, Ueda R, Kyoizumi S. Growth of human myeloid leukemias in the human marrow environment of SCID-hu mice. *Blood* 1993;82:2526–2536. [PubMed: 8104540]
- [50]. Kyle RA, Rajkumar SV. Multiple myeloma. *N. Engl. J. Med* 2004;351:1860–1873. [PubMed: 15509819]
- [51]. Pilarski LM, Belch AR. Circulating monoclonal B cells expressing P glycoprotein may be a reservoir of multidrug-resistant disease in multiple myeloma. *Blood* 1994;83:724–736. [PubMed: 7507731]
- [52]. Kiel K, Cremer FW, Rottenburger C, Kallmeyer C, Ehrbrecht E, Atzberger A, Hegenbart U, Goldschmidt H, Moos M. Analysis of circulating tumor cells in patients with multiple myeloma during the course of high-dose therapy with peripheral blood stem cell transplantation. *Bone Marrow Transpl* 1999;23:1019–1027.
- [53]. Rottenburger C, Kiel K, Bosing T, Cremer FW, Moldenhauer G, Ho AD, Goldschmidt H, Moos M. Clonotypic CD20⁺ and CD19⁺ B cells in peripheral blood of patients with multiple myeloma post high-dose therapy and peripheral blood stem cell transplantation. *Br. J. Haematol* 1999;106:545–552. [PubMed: 10460621]
- [54]. Rasmussen T, Jensen L, Honore 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;96:4357–4359. [PubMed: 11110713]
- [55]. Pilarski LM, Szczepek AJ, Belch AR. Deficient drug transporter function of bone marrow-localized and leukemic plasma cells in multiple myeloma. *Blood* 1997;90:3751–3759. [PubMed: 9345062]
- [56]. Goodell MA. Multipotential stem cells and ‘side population’ cells. *Cytotherapy* 2002;4:507–508. [PubMed: 12568983]
- [57]. Kastan MB, Schlaffer E, Russo JE, Colvin OM, Civin CI, Hilton J. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood* 1990;75:1947–1950. [PubMed: 2337669]
- [58]. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu. Rev. Cell Dev. Biol* 2007;23:675–699. [PubMed: 17645413]
- [59]. Taipale J, Beachy PA. The hedgehog and Wnt signalling pathways in cancer. *Nature* 2001;411:349–354. [PubMed: 11357142]
- [60]. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105–111. [PubMed: 11689955]
- [61]. Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, Devereux WL, Rhodes JT, Huff CA, Beachy PA, Watkins DN, Matsui W. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc. Natl. Acad. Sci. USA* 2007;104:4048–4053. [PubMed: 17360475]
- [62]. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den NE, Salles G, Gaulard P, Reyes F, Lederlin P, Gisselbrecht C. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N. Engl. J. Med* 2002;346:235–242. [PubMed: 11807147]
- [63]. Schulz H, Bohlius JF, Trelle S, Skoetz N, Reiser M, Kober T, Schwarzer G, Herold M, Dreyling M, Hallek M, Engert A. Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. *J. Natl. Cancer Inst* 2007;99:706–714. [PubMed: 17470738]
- [64]. Treon SP, Pilarski LM, Belch AR, Kelliher A, Preffer FI, Shima Y, Mitsiades CS, Mitsiades NS, Szczepek AJ, Ellman L, Harmon D, Grossbard ML, Anderson KC. CD20-directed serotherapy in patients with multiple myeloma: biologic considerations and therapeutic applications. *J. Immunother* 2002;25:72–81. [PubMed: 11924912]
- [65]. Bensinger WI, Gahrton G. Allogeneic hematopoietic cell transplantation for multiple myeloma. In: Thomas ED, Blume KG, Forman, editors. *Hematopoietic Stem Cell Transplantation*. Blackwell Scientific Publications; Boston: p. 887–891.
- [66]. Huff CA, Fuchs EJ, Noga SJ, O'Donnell PV, Ambinder RF, Diehl L, Borrello I, Vogelsang GB, Miller CB, Flinn IA, Brodsky RA, Marcellus D, Jones RJ. Long-term follow-up of T cell-depleted

- allogeneic bone marrow transplantation in refractory multiple myeloma: importance of allogeneic T cells. *Biol. Blood Marrow Transpl* 2003;9:312–319.
- [67]. Rasmussen T, Hansson L, Osterborg A, Johnsen HE, Mellstedt H. Idiotypic vaccination in multiple myeloma induced a reduction of circulating clonal tumor B cells. *Blood* 2003;101:4607–4610. [PubMed: 12576327]
- [68]. Lim SH, Bailey-Wood R. Idiotypic protein-pulsed dendritic cell vaccination in multiple myeloma. *Int. J. Cancer* 1999;83:215–222. [PubMed: 10471530]
- [69]. Reichardt VL, Milazzo C, Brugger W, Einsele H, Kanz L, Brossart P. Idiotypic vaccination of multiple myeloma patients using monocyte-derived dendritic cells. *Haematologica* 2003;88:1139–1149. [PubMed: 14555310]
- [70]. Bendandi M, Rodriguez-Calvillo M, Inoges S, Lopez-Diaz de CA, Perez-Simon JA, Rodriguez-Caballero A, Garcia-Montero A, Almeida J, Zabalegui N, Giraldo P, San MJ, Orfao A. Combined vaccination with idiotype-pulsed allogeneic dendritic cells and soluble protein idiotype for multiple myeloma patients relapsing after reduced-intensity conditioning allogeneic stem cell transplantation. *Leuk. Lymphoma* 2006;47:29–37. [PubMed: 16321824]
- [71]. Curti A, Tosi P, Comoli P, Terragna C, Ferri E, Cellini C, Massaia M, D'Addio A, Giudice V, Di BC, Cavo M, Conte R, Gugliotta G, Baccarani M, Lemoli RM. Phase I/II clinical trial of sequential subcutaneous and intravenous delivery of dendritic cell vaccination for refractory multiple myeloma using patient-specific tumour idiotype protein or idiotype (VDJ)-derived class I-restricted peptides. *Br. J. Haematol* 2007;139:415–424. [PubMed: 17910631]
- [72]. Spisek R, Kukreja A, Chen LC, Matthews P, Mazumder A, Vesole D, Jagannath S, Zebroski HA, Simpson AJG, Ritter G, Durie B, Crowley J, Shaughnessy JD Jr, Scanlan MJ, Gure AO, Barlogie B, Dhodapkar MV. Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. *J. Exp. Med* 2007;204:831–840. [PubMed: 17389240]
- [73]. Noonan K, Matsui W, Serafini P, Carbley R, Tan G, Khalili J, Bonyhadi M, Levitsky H, Whartenby K, Borrello I. Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. *Cancer Res* 2005;65:2026–2034. [PubMed: 15753403]
- [74]. Jamieson CHM, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL, Weissman IL. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N. Engl. J. Med* 2004;351:657–667. [PubMed: 15306667]
- [75]. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR, Armstrong SA. Transformation from committed progenitor to leukaemia stem cell initiated by MLLG₂AF9. *Nature* 2006;442:818–822. [PubMed: 16862118]
- [76]. Liu S, Ginestier C, Charafe-Jauffret E, Foco H, Kleer CG, Merajver SD, Dontu G, Wicha MS. BRCA1 regulates human mammary stem/progenitor cell fate. *Proc. Natl. Acad. Sci* 2008;105:1680–1685. [PubMed: 18230721]
- [77]. Barabe F, Kennedy JA, Hope KJ, Dick JE. Modeling the initiation and progression of human acute leukemia in mice. *Science* 2007;316:600–604. [PubMed: 17463288]
- [78]. Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006;441:1075–1079. [PubMed: 16810242]
- [79]. Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science* 2007;317:337. [PubMed: 17641192]
- [80]. Hideshima T, Anderson KC. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. *Nat. Rev. Cancer* 2002;2:927–937. [PubMed: 12459731]
- [81]. Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, Dewald GW, Van Ness B, Van Wier SA, Henderson KJ, Bailey RJ, Greipp PR. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003;101:4569–4575. [PubMed: 12576322]
- [82]. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, Epstein J, Yaccoby S, Sawyer J, Burington B, Anaissie E, Hollmig K, Pineda-Roman M, Tricot G, van Rhee F, Walker R, Zangari M, Crowley J, Barlogie B, Shaughnessy JD Jr. The molecular classification of multiple myeloma. *Blood* 2006;108:2020–2028. [PubMed: 16728703]
- [83]. Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, Sawyer JR, Hollmig K, Zangari M, Pineda-Roman M, van Rhee F, Cavallo F, Burington B, Crowley J, Tricot G, Barlogie B, Shaughnessy JD

Jr. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood* 2006;108:1724–1732. [PubMed: 16705089]