HHS Public Access

Author manuscript

Exp Hematol. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

Exp Hematol. 2017 October ; 54: 17–25. doi:10.1016/j.exphem.2017.07.004.

Retinoic acid, CYP26, and drug resistance in the stem cell niche

Salvador Alonso, **Richard J Jones**, and **Gabriel Ghiaur**

Sidney Kimmel Comprehensive Cancer Center, Department of Oncology, Johns Hopkins University, Baltimore, Maryland

Abstract

The bone marrow niche is essential for hematopoietic stem cells to maintain lifelong blood production, by balancing their self-renewal and differentiation. Hematologic malignancies have a similar hierarchical organization to their normal counterparts, with rare populations of cancer stem cells that rely on the microenvironment to survive and propagate their differentiated malignant progenitor cells. Cancer cells alter their microenvironment to create a supportive niche where they endure chemotherapy, survive as minimal residual disease, and eventually prevail at relapse. Powerful morphogens such as retinoids, Wnt/βcatenin, Notch and Hedgehog control stem cell fates across tissues including normal and malignant hematopoiesis. The molecular conversations between these pathways as well as the mechanisms that control their activity and create gradients at cellular scale remain a mystery. Here, we discuss accumulating evidence that suggests that CYP26, the primary retinoid-inactivating enzyme, plays a critical role in the integration of two of these molecular programs: the retinoid and Hedgehog pathways. Induction of stromal CYP26 by either one of these pathways limits retinoic acid concentration in the stem cell niche with profound effects on tissue homeostasis and drug resistance. Bypassing this gatekeeping mechanism holds promise for overcoming drug resistance and improving clinical outcomes in hematological malignancies and cancer in general.

Keywords

drug resistance; stem cell niche; Hedgehog; retinoic acid

Introduction

Hematopoietic stem cells (HSCs) are responsible for hematological homeostasis and regeneration, and are the only cells capable of giving rise to all blood lineages [1, 2]. Because HSCs self-renew throughout the lifespan to maintain blood production, they are prone to accumulate mutations, and thus, to undergo malignant transformation. Analogous to the normal counterparts from which they originate, these emergent cancer stem cells

*Corresponding author. 1650 Orleans St. Room 243, Baltimore, MD, 21231, Work phone: 410-502-3183, gghiaur1@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have declared that no conflict of interest exists.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(CSCs) have the capacity to self-renew, and are responsible for maintaining the bulk of the tumor. Within the bone marrow (BM) microenvironment, HSCs and CSCs compete to establish communications with surrounding cells through cell-cell contacts and soluble factors. These interactions maintain stem cells and their progeny by regulating their quiescence, self-renewal and differentiation [3, 4]. In the case of CSCs, the interactions with the BM niche are essential to maintain their tumor initiation capacity and protect them from threats posed by the immune system and chemotherapy [5, 6].

Perhaps the first definitive evidence for the existence of stem cells was provided in the hematopoietic system, when Till and McCulloch reported the presence of hematopoietic colonies in the spleen of irradiated mice after transplantation with BM cells [1]. It was also in the hematopoietic system where the term "stem cell niche" was described for the first time, after Schofield found that stem cells from the bone marrow had the ability to reconstitute hematopoiesis indefinitely, while spleen-derived stem cells previously reported by Till and McCulloch did not have this ability [7]. He postulated the existence of a distinctive niche within the BM necessary to maintain a stem cell phenotype and function.

Analogous to the role of normal stem cells in maintaining lifelong tissue homeostasis, evidence gathered over the past two decades suggests that a rare population of cancer cells with the ability to self-renew and differentiate is responsible for initiating and propagating cancer [8–10]. Though initial evidence supporting the cancer stem cell (CSC) model was provided by Fialkow at al. five decades ago[11], strong support of the CSC concept came from the observation that only a rare subset of leukemia cells, comprising less than 0.01% of the tumor, were able to engraft and produce acute myeloid leukemia (AML) in immunodeficient mice [12]. Many studies have now provided further evidence that AML maintains a hierarchical organization similar to normal hematopoiesis, with HSCs as the target of malignant transformation and the bulk of the disease comprised of differentiated progeny [9, 10]. Accordingly, the term leukemia stem cells (LSCs) is now used for the cells that initiate the disease since they fulfill the two most important criteria that define stemness: 1) differentiation, as demonstrated by their ability to generate the disease in immunocompromised; and 2) self-renewal, evidenced by their ability to propagate the disease upon transfer into secondary recipients.

Recent advances in cancer treatment have significantly improved the prognosis of patients with certain malignancies. In multiple myeloma (MM) for instance, novel agents such as proteasome inhibitors in combination with induction chemotherapy are able to achieve remission in over 70% of patients [13]. However, similar to patients with other hematological malignancies and solid cancers, most patients in remission eventually relapse and die of their disease [14, 15]. Increasing evidence demonstrates the existence of an immature MM population that resembles B cells, with decreased or absent expression of the classic plasma cell marker CD138 and decreased production of Ig [16, 17]. Similar to LSCs, MM B cells are able to give rise to differentiated progenitor cells and recapitulate the primary disease in immunodeficient mice [16]. Most importantly, this population of MM cells is resistant to various chemotherapy agents [17, 18].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The bone marrow niche and cancer

The BM niche is broadly subdivided into a perivascular area, located close to the central vein of the BM, and an endosteal area, located close to the internal lining of the bone. Both areas harbor a variety of stromal cells, including mesenchymal stromal cells (MSCs), adipocytes, fibroblasts, endothelial cells and osteoblasts [3, 4]. The BM provides a unique niche for malignant hematopoietic cells as well as a common metastatic site for solid tumors.

Indeed, many of the mechanisms by which the BM microenvironment maintains HSCs are also used by cancer cells to survive chemotherapy and maintain stem cell features[5]. Primitive human AML cells transplanted into immunodeficient mice appear to initially concentrate in the endosteal regions of mouse long bones, followed by migration, expansion, and accumulation of more mature AML cells in the central region of the BM [19]. Other malignancies, including MM [20] and even solid tumors [21, 22], also appear to preferably reside in the endosteal niche of the BM. In this region, they establish interactions with surrounding stromal cells via direct cell-cell contacts and soluble factors that facilitate maintenance of stem-like features, including quiescence and chemotherapy resistance [19–23]. As might be expected if LSCs and their progeny occupied different areas of the BM, apoptosis was most evident in the central BM cavity after chemotherapy while cancer cells near the endosteum were relatively spared [19]. Physical interactions are facilitated by CXCL12, which is highly expressed by mesenchymal cells and binds to CXCR4 on AML [24], MM [25] and micrometastatic solid tumors including prostate cancer [26–28]. Interestingly, CXCL12 expression by normal mesenchymal cells is significantly higher in patients with MM compared to healthy controls [29]. This increased CXCL12 expression appears to reorganize the cytoskeleton of MM cells promoting adherence to stroma cells and drug resistance [29].

Recognizing the role of the BM microenvironment in chemoprotection, therapeutic strategies have focused on displacement of malignant cells from the BM niche by targeting adhesion molecules or chemokines. Mouse studies have demonstrated that inhibition of the CXCR4-CXCL12 axis mobilizes cancer cells from the BM into the peripheral circulation, where they are more sensitive to chemotherapy [29–31]. Similarly, blocking VLA-4 results in a dramatic increase of AML cells in the peripheral circulation, and the combination of this antibody with cytarabine delays disease progression in mice [32]. Expression of CD44 on cancer cells is also associated with maintenance of a stem cell phenotype and drug resistance, and its inhibition reduces the size of the stem cell compartment and enhances chemotherapy efficacy in MM, AML, and CML [33–35]. Clinical trials exploring such mobilization strategies are ongoing [36–39]. In addition, a phase I/II study using the CXCR4 inhibitor plerixafor in combination with bortezomib in patients with relapsed/refractory MM showed an overall response rate of 51%, suggesting that mobilization can sensitize resistant cells to bortezomib [36]. These studies have increased the understanding of how cancer cells are retained within the BM, and have proposed therapeutic approaches to overcome this problem. They do not however, explain what molecular mechanisms are responsible for maintaining stemness in the BM microenvironment and how these signals promote drug resistance in the niche and thus, contribute to minimal residual disease.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Regulation of stemness by the microenvironment's control of RA

Early observations during the nineteenth century recognized that anemia often occurred in individuals with night blindness, unknowingly establishing the first association between retinoic acid (RA) and hematopoiesis [40]. Definitive evidence emerged almost 100 years ago, when it was noted that vitamin A-deficient rats displayed a reduction in hematopoietic cells in the BM [41]. Subsequent clinical studies demonstrated that vitamin A supplementation improved anemia and reduced susceptibility to infections in children [42]. More recently, studies showing the ability of RA to differentiate promyelocytes [43, 44], together with the dramatic clinical responses of patients with acute promyelocytic leukemia (APL) treated with all-trans RA (ATRA) [45, 46], suggested a role for RA in myeloid differentiation.

Growing evidence suggest that retinoic acid signaling may be differentially regulated between human and mouse HSCs and between HSCs and more differentiated progenitor cells. To this end, several groups have confirmed the pro-differentiation role of retinoids on human HSCs [47, 48] while elegant studies in murine models have shown the opposite effects [49, 50]. While in-depth studies to explain these interspecies differences are lacking, analysis of published data hint towards potential explanations for observed differences. For once, the stem cell populations analyzed in human vs. mouse studies are significantly different given the state of the art definitions of HSCs in these animal models: long term, label retaining extremely quiescent, high quality HSCs in mouse and SCID repopulating cells in humans. Thus, it is conceivable that the HSC population analyzed in mouse vs. human is functionally different. This distinction is particularly important when it comes to retinoid signaling as it was shown to have pleiotropic effects depending on the hematopoietic stem/progenitor population analyzed. Another potential difference between mouse and human HSCs is expression of different retinoic acid receptors. Data from Purton's as well as Trumpp's groups suggest that RARγ plays a dominant role in HSC homeostasis in murine models. While expression analysis of human HSCs suggest that RARα is the predominant receptor expressed. While the differential roles of RARα and RARγ are just beginning to be evaluated, there is enough evidence to suggest that maybe RARα is associated with differentiation while RARγ may induce self-renewal. These biological differences corroborated with the differential sensitivity of various RARs to retinoic acid (Ghiaur G – unpublished data) may account for the apparent opposing effects of retinoids on murine vs. human HSCs. Further studies to evaluate these biological differences are warranted. As it pertains to human HSCs, they are intrinsically programmed to respond to RA as demonstrated by high expression of RARα and ALDH1A1 but the downstream targets of RA signaling are down-regulated. Conversely, HPCs show an active RA pathway, with strong expression of downstream target genes. These data suggested that HSCs are intrinsically programmed and able to respond to RA, but are prevented from doing so within the BM microenvironment [48]. Indeed, when HSCs are removed from their niche and cultured *in vitro*, they promptly upregulate downstream components of the RA pathway and differentiate [47, 48]. However, when HSCs are co-cultured with BM mesenchymal cells, they maintain both their stem cell phenotype and ability to reconstitute immunodeficient mice for many weeks. Mesenchymal cells within the BM prevent retinoid-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

induced differentiation of HSCs by maintaining a low-RA concentration via CYP26-mediated degradation [48]. This mechanism of stem cell protection appears conserved across multiple tissues. Accordingly, Sertoli stroma cells also express high levels of CYP26 and protect the male germ cells from exposure to mesonephros-produced RA [51]. Thus, at least in the BM and the developing gonad, local control of RA levels is essential to maintain stem cell function and homeostasis.

The use of all-trans RA (ATRA) in the treatment of acute promyelocytic leukemia (APL) has fundamentally changed the outcome in this disease. The PML/RARα fusion protein that contributes to the pathophysiology of APL, also renders malignant blasts highly sensitive to ATRA-induced differentiation [52, 53]. Non-APL AML blasts are also sensitive to pharmacological concentrations (up to 10^{-6}M) of ATRA *in vitro*, though only some subsets (i.e. NPM1 mutated) are as sensitive as APL blasts (IC50 $<10^{-7}$M). Nevertheless, clinical studies failed to demonstrate any benefit from addition of ATRA to treatment regimens in non APL-AML [54–59]. Moreover even in APL when used as single agent, ATRA induces remission without cure and cannot eliminate MRD except perhaps if used in special liposomal formulations [46, 60]. Stromal CYP26 can metabolize pharmacological concentrations of ATRA [61] and render this drug ineffective in inducing differentiation in APL and non-APL AML blasts in the presence of BM stroma. In addition, ATRA can induce stromal CYP26 and thus, create even more protective niches in the BM marrow[62] (Figure 1). Of note, the addition of CYP26 inhibitors or the use of CYP26 resistant retinoids is able to overcome ATRA-resistance mediated by the microenvironment [61, 62].

Regulation of plasma cell and MM stemness by RA

In normal biology, plasma cells originate from B cells that have encountered a B-cell receptor (BCR) specific antigen in the germinal center [63, 64]. B-cells with high affinity receptors are selected to differentiate into memory B cells, and memory B cells are epigenetically programmed to rapidly differentiate into plasma cells upon re-exposure to their BCR-specific antigen [64]. One of the factors implicated on the transition of memory B cells to plasma cells is RA. Accordingly, mice that are fed a vitamin A deficient diet have impaired plasma cell differentiation and Ig production [65]. Conversely, activation of the retinoid pathway favors differentiation of memory B cells into plasma cells and potentiates Ig secretion [66, 67]. The differentiation of B cells into plasma cells is accompanied by significant molecular and structural changes. The most important functional change is the development of a robust synthetic machinery, including a prominent endoplasmic reticulum (ER) to accommodate Ig production and secretion [68]. As a result of their abundant Ig synthesis, plasma cells are more prone to toxic buildup of unfolded proteins, which are degraded in the proteasome to prevent ER stress [69, 70]. The unfolded protein response (UPR) leads to apoptosis when protein degradation by the proteasome is insufficient and ER stress is high [70]. This results in higher sensitivity of plasma cells to proteasome inhibition, compared to B lymphocytes [17, 18].

Data from our group and others suggest that MM follows normal B cell differentiation with populations of cells that differ in morphology, cell surface expression profile, and sensitivity to chemotherapy [17, 18, 71]. The predominant cell population in MM consists of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

archetypical CD138^{+} plasma cells with high secretory activity, and thus they are sensitive to proteasome inhibition. Conversely, there is also a small population of CD138^{-} MM cells that resemble memory B cells, do not secrete Ig, and are resistant to bortezomib. Moreover, in patients who have received bortezomib and are in remission, CD138^{-} MM cells increase in frequency demonstrating that MM plasma cells have been selectively targeted [17]. In addition, in patients with progressive disease on bortezomib, the MM cells often express the memory B cell marker CD27 [72].

Initial findings in normal hematopoiesis raised the question of whether stromal CYP26 not only protected HSCs and CSCs from endogenous retinoid-induced differentiation, but also whether it promotes stemness, and thus, drug resistance. Using MM as proof of concept, it is now clear that proximity to the mesenchymal stroma induces epigenetic changes in the malignant cells (Figure 2)[71]. In this case, MM cells acquire a B cell phenotype, with down-regulation of the plasma cell markers CD138, Blimp1, XBP1s (spliced isoform of X-box binding protein 1), and CHOP (C/EBP homologous protein), and up-regulation of the B cell marker BCL-6 (B-Cell CLL/Lymphoma 6) [71]. These changes depend on stroma's ability to metabolize endogenous retinoids and can be reproduced in stroma-free conditions by blocking RA signaling [71]. Thus, while the bulk of MM cells is exposed to endogenous retinoids and has high ER stress, a minute population may occupy retinoid low niches, have low ER stress, and survive treatment with proteasome inhibitors [71]. It is tempting to hypothesize that protection from endogenous retinoids may explain chemoresistance not only during treatment with proteasome inhibitors but also more broadly to chemotherapy and even immunotherapy agents. To this end, Daratumumab, an anti-CD38 monoclonal antibody that is FDA approved for treatment of MM patients has improved efficacy in vitro when combined with ATRA [73]; this effect relies on ATRA-induced upregulation of CD38 on MM plasma cells. Since proximity to mesenchymal stroma creates retinoid low niches and downregulates CD38 expression on malignant plasma cells [71], treatment with Daratumumab should be less effective in the stem cell niche compared to the bulk of the tumor. Concomitant treatment with ATRA, while it may further sensitize the bulk of the MM to Daratumumab, potentially could increase minimal residual disease by creating more protective niches through upregulation of CYP26 changing local retinoids kinetics (see Figure 1)[74]. Moreover, retinoids induce differentiation of various cancer types with a tendency to metastasize and become dormant in the BM cavity[21, 22, 75–80]. Thus, as further discussed in the next section, this mechanism of niche-mediated chemoprotection may apply to other hematological malignancies and even solid cancers metastatic to bone.

As mentioned earlier, approaches to overcome niche-chemoprotection have focused on mobilization strategies using inhibitors of chemokines or adhesion molecules [37–39]. In MM, the CXCR4 inhibitor plerixafor mobilizes MM cells from the BM into the circulation, reaching a plateau within two to three days of treatment [25]. Using an *in vitro* model, we tested the ability of tumor mobilization strategies to sensitize MM cells to chemotherapy [71]. We found that MM cells remained partially resistant to bortezomib for at least 48 hours following separation from the BM stroma cells, suggesting that stromal-induced change in phenotype and subsequent drug resistance may not be immediately reversed by mobilization of cancer cells. Conversely, CYP26 inhibition in stroma co-cultures increased bortezomib sensitivity to the same degree regardless of whether MM cells were subsequently mobilized

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

or not [71]. Thus, mobilization strategies sever important cell-cell and cell-matrix interactions which should sensitize malignant cells to chemotherapeutic agents. In addition, since bone marrow niches have altered drug pharmacokinetics [81], removing the cancer cells from these niches has the added benefit of improved exposure to chemotherapy. Nevertheless, the epigenetic changes induced by the BM microenvironment (decreased Ig production and ER stress in the case of MM, or cell cycle quiescence for instance) may continue to render the malignant cells resistant to chemotherapy even while in circulation. Thus, the timing of administration of mobilizing agents and chemotherapy has to be carefully considered and the effects of these strategies not only on mobilization but also on drug sensitivity should be measured to ensure success.

Since retinoids regulate differentiation in various malignancies [78–80, 82], these findings raised the question of whether stromal CYP26 provides a general mechanism of chemoresistance in cancer. In this regard, retinoids synergize with FLT3 tyrosine kinase inhibitors (TKIs) to induce apoptosis of leukemic blasts expressing FLT3/ITD [83]. However, this combination is rendered inefficient in the presence of BM mesenchymal cells [84]. Inhibition of stromal CYP26 or treatment with a CYP26 resistant retinoid differentiates FLT3/ITD AML blasts and resensitizes them to FLT3 TKIs. Similarly, Churchman et al. recently reported that the synthetic retinoid bexarotene differentiates acute lymphoblastic leukemia (ALL) cells *in vitro* and *in vivo*, and synergizes with the TKI dasatinib to significantly prolong survival in a BCR-ABL1 ALL mouse model, despite having limited activity as monotherapy[85].

Implications for solid cancers

The BM microenvironment provides a protective niche not only to cells from hematologic malignancies but also solid cancers. Indeed, breast, prostate, lung, and renal cancer cells, as well as other malignancies, frequently metastasize to the endosteal region of the bone early during the disease [21, 22, 75, 77]. In the bone marrow, by directly competing with HSCs for their niches, metastatic cells become quiescent and survive chemotherapy [21, 22]. This explains why patients sometimes present with bone metastases after having been in remission for years from a localized primary tumor. Thus, it is tempting to hypothesize that stromal CYP26 promotes acquisition of a dormant, stem cell phenotype in malignancies in general. To this end, multiple preclinical studies demonstrated activity of retinoids as differentiation therapy in solid cancers, including breast, colon, lung, and prostate [78–80, 82], however, for the most part, clinical studies failed to show any benefit. It is plausible that similar to observations in MM and AML, local inactivation of retinoids by cancer associated fibroblasts renders them clinically ineffective in solid tumors. Indeed, expression of CYP26 in the stromal compartment has recently been reported in solid cancers. Specifically, in pancreatic cancer, increased expression of stromal CYP26 is associated with an "activated stromal subtype" and significantly decreased survival [86]. Moreover, the presence of an activated stroma was associated with higher xenograft success rate, suggestive of a more stem-like activity [86]. Similarly, CYP26 is expressed in the stromal compartment of a "stem/mesenchymal" subtype of colon cancer, associated with a particularly poor prognosis [87]. These studies suggest that similar to findings in leukemia and MM, expression of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

stromal CYP26 may be associated with a stem-like, drug resistant phenotype in solid tumors as well.

Recent studies have suggested that niche-chemoprotection is not a unidirectional process but rather there is cross-talk in which cancer cells signal to their surrounding stroma to build a reinforced microenvironment [88]. For instance, the release of exosomes by chronic lymphocytic leukemia (CLL) cells induces an inflammatory phenotype in stroma cells and promotes cancer growth [89]. Myeloid leukemia cells similarly stimulate stroma cells via paracrine thrombopoietin and CCL3, increasing their ability to support cancer cells [90]. In solid cancers, Yauch et al. reported that pancreatic and colon cancer cells highly expressed the Hedgehog (Hh) pathway ligands Sonic and Indian, but were unable to autonomously activate the pathway since the tumor cells did not express the receptor Smoothened [91]. Stroma cells on the other hand did not express the ligands but exhibited Hh pathway activation in response to ligands secreted by cancer cells, promoting tumor growth and chemoresistance. Similarly, in breast cancer, high expression of Hh ligands by malignant cells is associated with activation of the Hh pathway in stroma cells, a poorly differentiated phenotype, and decreased survival [92].

These studies further support the notion that an “activated” niche is beneficial to tumor cells. However, the mechanisms by which the “activated” stroma signals interact with cancer cells remain incompletely understood. We have shown that the MM plasma cells augment bone marrow stroma’s ability to metabolize retinoids via induction of CYP26 [71]. Among the soluble factors secreted during this interaction, Sonic Hh can upregulate stromal CYP26, a process mediated via Smoothened. It has long been observed that MM cells secret Hh ligands [93] but the target cell of these ligands remains controversial. Since activation of Hh pathway in the BM stroma led to overexpression of stromal CYP26 in MM [71], the microenvironment in pancreatic cancer may similarly overexpress CYP26 as the malignant clone signals to the surrounding microenvironment via Hh [94].

Conclusion

It is clear now that cross-talk between MM and the BM microenvironment produces an “activated” niche that augments tumor maintenance [71]. CYP26 is expressed by stroma cells in the BM, where it maintains a low-retinoid niche that supports stemness and drug resistance. Moreover, paracrine Hh secretion by MM cells upregulated stromal CYP26 and further reinforced a protective microenvironment [71]. CYP26 is also expressed in the stromal compartment of aggressive subtypes of pancreatic [86] and colon cancers [87]. Cells from pancreatic, colon, breast, and other solid tumors are known to communicate with neighboring stroma cells via paracrine Hh [91, 92, 95, 96]. Since both stromal CYP26 and paracrine Hh signaling have been associated with worse prognoses in solid tumors, it is plausible that crosstalk between malignant cells and their microenvironment involving the retinoid and Hh pathways similarly promotes stemness and drug resistance in solid tumors. If confirmed, CYP26-resistant synthetic retinoids could bypass these cancer-stroma communications as we have shown in MM [71], and demonstrate therapeutic activity in solid tumors metastatic to bone.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Acknowledgments

This work was supported by the following funding entities: National Heart, Lung, and Blood Institute Grant 1K08 HL127269-01 (to G.G.); Commonwealth Foundation Pilot Project (to G.G.); Leukemia and Lymphoma Society Grant 14086277 (to R.J.J. and G.G.); National Cancer Institute Grant P30 CA006973 (to R.J.J.); and National Institutes of Health Grant P01 CA015396 (to R.J.J.).

References

1. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. 1961. Radiat Res. 2012; 178:AV3–7. [PubMed: 22870977]
2. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell. 2000; 100:157–168. [PubMed: 10647940]
3. Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat Med. 2014; 20:833–846. [PubMed: 25100529]
4. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014; 505:327–334. [PubMed: 24429631]
5. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009; 114:1150–1157. [PubMed: 19401558]
6. Schepers K, Campbell TB, Passegue E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell. 2015; 16:254–267. [PubMed: 25748932]
7. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood cells. 1978; 4:7–25. [PubMed: 747780]
8. 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]
9. Haase D, Feuring-Buske M, Konemann S, et al. Evidence for malignant transformation in acute myeloid leukemia at the level of early hematopoietic stem cells by cytogenetic analysis of CD34+ subpopulations. Blood. 1995; 86:2906–2912. [PubMed: 7579382]
10. Ghiaur G, Gerber J, Jones RJ. Concise review: Cancer stem cells and minimal residual disease. Stem Cells. 2012; 30:89–93. [PubMed: 22045578]
11. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. Proceedings of the National Academy of Sciences of the United States of America. 1967; 58:1468–1471. [PubMed: 5237880]
12. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994; 367:645–648. [PubMed: 7509044]
13. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/GMMG-HD4 trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:2946–2955. [PubMed: 22802322]
14. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005; 352:2487–2498. [PubMed: 15958804]
15. Kumar SK, Lee JH, Lahuerta JJ, et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. Leukemia. 2012; 26:149–157. [PubMed: 21799510]
16. Matsui W, Huff CA, Wang Q, et al. Characterization of clonogenic multiple myeloma cells. Blood. 2004; 103:2332–2336. [PubMed: 14630803]
17. Leung-Hagesteijn C, Erdmann N, Cheung G, et al. Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. Cancer Cell. 2013; 24:289–304. [PubMed: 24029229]
18. Matsui W, Wang Q, Barber JP, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Res. 2008; 68:190–197. [PubMed: 18172311]
19. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007; 25:1315–1321. [PubMed: 17952057]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

20. Chen Z, Orlowski RZ, Wang M, Kwak L, McCarty N. Osteoblastic niche supports the growth of quiescent multiple myeloma cells. Blood. 2014; 123:2204–2208. [PubMed: 24425802]
21. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. Nat Rev Cancer. 2011; 11:411–425. [PubMed: 21593787]
22. Shiozawa Y, Pedersen EA, Havens AM, et al. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. The Journal of clinical investigation. 2011; 121:1298–1312. [PubMed: 21436587]
23. Saito Y, Uchida N, Tanaka S, et al. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. Nat Biotechnol. 2010; 28:275–280. [PubMed: 20160717]
24. Tavor S, Petit I, Porozov S, et al. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. Cancer Res. 2004; 64:2817–2824. [PubMed: 15087398]
25. Azab AK, Runnels JM, Pitsillides C, et al. CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. Blood. 2009; 113:4341–4351. [PubMed: 19139079]
26. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res. 2002; 62:1832–1837. [PubMed: 11912162]
27. Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res. 2010; 16:2927–2931. [PubMed: 20484021]
28. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001; 410:50–56. [PubMed: 11242036]
29. Alsayed Y, Ngo H, Runnels J, et al. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. Blood. 2007; 109:2708–2717. [PubMed: 17119115]
30. Burger JA, Peled A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. Leukemia. 2009; 23:43–52. [PubMed: 18987663]
31. Uy GL, Rettig MP, Motabi IH, et al. A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. Blood. 2012; 119:3917–3924. [PubMed: 22308295]
32. Matsunaga T, Takemoto N, Sato T, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nat Med. 2003; 9:1158–1165. [PubMed: 12897778]
33. Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. Nat Med. 2006; 12:1175–1180. [PubMed: 16998483]
34. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nature medicine. 2006; 12:1167–1174.
35. Bjorklund CC, Baladandayuthapani V, Lin HY, et al. Evidence of a role for CD44 and cell adhesion in mediating resistance to lenalidomide in multiple myeloma: therapeutic implications. Leukemia. 2014; 28:373–383. [PubMed: 23760401]
36. Ghobrial IM, Shain K, Hanlon C, et al. Phase I/II trial of plerixafor and bortezomib as a chemosensitization strategy in relapsed or relapsed/refractory multiple myeloma. Blood. 2013; 122:1947–1947.
37. Andritsos LBJ, Jones JA, Hewes B, Kipps TJ, Hsu FJ, Burger JA. Preliminary Results From A Phase I Dose Escalation Study to Determine the Maximum Tolerated Dose of Plerixafor In Combination with Rituximab In Patients with Relapsed Chronic Lymphocytic Leukemia. Blood. 2010; 116:2450.
38. Uy GLAD, Cortes JE, Becker PS, Chen RW, Liesveld JL, Hewes B, Johns D, Erba HP. Safety and Tolerability of Plerixafor in Combination with Cytarabine and Daunorubicin in Patients with Newly Diagnosed Acute Myeloid Leukemia-Preliminary Results From a Phase I Study. Blood. 2011; 118:82.
39. Michael Andreeff ZZ, Kelly Mary A, Wang Rui-yu, McQueen Teresa, Duvvuri Seshagiri, Nowshad Gul, Borthakur Gautam, Burger Jan A, Kadia Tapan M, Jabbour Elias, Cortes Jorge E,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Kantarjian Hagop M, Konopleva Marina. Mobilization and Elimination of FLT3-ITD+ Acute Myelogenous Leukemia (AML) Stem/Progenitor Cells by Plerixafor/G-CSF/Sorafenib: Results From a Phase I Trial in Relapsed/Refractory AML Patients. Blood. 2012; 120:142.

40. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr. 2002; 56:271–281. [PubMed: 11965502]
41. Findlay GMMR. The Bone Marrow in Deficiency Diseases. J Pathol Bacteriol. 1922; 25:402–403.
42. Abbott ODAC, Overstreet MR. Effect of avitaminosis A on the human blood picture. American Journal of Physiology. 1939; 126:254–260.
43. Tobler A, Dawson MI, Koeffler HP. Retinoids. Structure-function relationship in normal and leukemic hematopoiesis in vitro. J Clin Invest. 1986; 78:303–309. [PubMed: 3459735]
44. Gratas C, Menot ML, Dresch C, Chomienne C. Retinoid acid supports granulocytic but not erythroid differentiation of myeloid progenitors in normal bone marrow cells. Leukemia. 1993; 7:1156–1162. [PubMed: 8350615]
45. Huang ME, Ye YC, Chen SR, et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood. 1988; 72:567–572. [PubMed: 3165295]
46. Warrell RP Jr, Frankel SR, Miller WH Jr, et al. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). N Engl J Med. 1991; 324:1385–1393. [PubMed: 1850498]
47. Chute JP, Muramoto GG, Whitesides J, et al. Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:11707–11712. [PubMed: 16857736]
48. Ghiaur G, Yegnasubramanian S, Perkins B, Gucwa JL, Gerber JM, Jones RJ. Regulation of human hematopoietic stem cell self-renewal by the microenvironment's control of retinoic acid signaling. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:16121–16126. [PubMed: 24043786]
49. Cabezas-Wallscheid N, Buettner F, Sommerkamp P, et al. Vitamin A-Retinoic Acid Signaling Regulates Hematopoietic Stem Cell Dormancy. Cell. 2017; 169:807–823. e819. [PubMed: 28479188]
50. Purton LE, Bernstein ID, Collins SJ. All-trans retinoic acid enhances the long-term repopulating activity of cultured hematopoietic stem cells. Blood. 2000; 95:470–477. [PubMed: 10627451]
51. Bowles J, Knight D, Smith C, et al. Retinoid signaling determines germ cell fate in mice. Science. 2006; 312:596–600. [PubMed: 16574820]
52. Weis K, Rambaud S, Lavau C, et al. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. Cell. 1994; 76:345–356. [PubMed: 8293468]
53. Raelson JV, Nervi C, Rosenauer A, et al. The PML/RAR alpha oncoprotein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells. Blood. 1996; 88:2826–2832. [PubMed: 8874178]
54. Schlenk RF, Dohner K, Kneba M, et al. Gene mutations and response to treatment with all-trans retinoic acid in elderly patients with acute myeloid leukemia. Results from the AMLSG Trial AML HD98B. Haematologica. 2009; 94:54–60. [PubMed: 19059939]
55. Milligan DW, Wheatley K, Littlewood T, Craig JI, Burnett AK. Group NHOCS. Fludarabine and cytosine are less effective than standard ADE chemotherapy in high-risk acute myeloid leukemia, and addition of G-CSF and ATRA are not beneficial: results of the MRC AML-HR randomized trial. Blood. 2006; 107:4614–4622. [PubMed: 16484584]
56. Estey EH, Thall PF, Pierce S, et al. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin +/− all-trans retinoic acid +/− granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. Blood. 1999; 93:2478–2484. [PubMed: 10194425]
57. Balusu R, Fiskus W, Rao R, et al. Targeting levels or oligomerization of nucleophosmin 1 induces differentiation and loss of survival of human AML cells with mutant NPM1. Blood. 2011; 118:3096–3106. [PubMed: 21719597]
58. Burnett AK, Hills RK, Green C, et al. The impact on outcome of the addition of all-trans retinoic acid to intensive chemotherapy in younger patients with nonacute promyelocytic acute myeloid

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

leukemia: overall results and results in genotypic subgroups defined by mutations in NPM1, FLT3, and CEBPA. Blood. 2010; 115:948–956. [PubMed: 19965647]

59. Schlenk RF, Lubbert M, Benner A, et al. All-trans retinoic acid as adjunct to intensive treatment in younger adult patients with acute myeloid leukemia: results of the randomized AMLSG 07-04 study. Annals of hematology. 2016; 95:1931–1942. [PubMed: 27696203]
60. Wang ZY, Sun GL, Lu JX, Gu LJ, Huang ME, Chen SR. Treatment of acute promyelocytic leukemia with all-trans retinoic acid in China. Nouv Rev Fr Hematol. 1990; 32:34–36. [PubMed: 2349080]
61. Su M, Alonso S, Jones JW, et al. All-Trans Retinoic Acid Activity in Acute Myeloid Leukemia: Role of Cytochrome P450 Enzyme Expression by the Microenvironment. PLoS One. 2015; 10:e0127790. [PubMed: 26047326]
62. Norsworthy KJ, Hernandez D, Su M, et al. RAR-Alpha Targeting Compounds Overcome Bone Marrow (BM) Stromal Protection of AML By CYP26. Blood. 2015; 126
63. Shlomchik MJ, Weisel F. Germinal center selection and the development of memory B and plasma cells. Immunol Rev. 2012; 247:52–63. [PubMed: 22500831]
64. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol. 2005; 5:230–242. [PubMed: 15738953]
65. Mora JR, Iwata M, Eksteen B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science. 2006; 314:1157–1160. [PubMed: 17110582]
66. Ertesvag A, Aasheim HC, Naderi S, Blomhoff HK. Vitamin A potentiates CpG-mediated memory B-cell proliferation and differentiation: involvement of early activation of p38MAPK. Blood. 2007; 109:3865–3872. [PubMed: 17209053]
67. Indrevaer RL, Moskaug JO, Paur I, et al. IRF4 Is a Critical Gene in Retinoic Acid-Mediated Plasma Cell Formation and Is Deregulated in Common Variable Immunodeficiency-Derived B Cells. J Immunol. 2015; 195:2601–2611. [PubMed: 26276871]
68. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol. 2015; 15:160–171. [PubMed: 25698678]
69. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol. 2003; 4:321–329. [PubMed: 12612580]
70. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011; 334:1081–1086. [PubMed: 22116877]
71. Alonso S, Hernandez D, Chang YT, et al. Hedgehog and retinoid signaling alters multiple myeloma microenvironment and generates bortezomib resistance. J Clin Invest. 2016; 126:4460–4468. [PubMed: 27775549]
72. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med. 1998; 188:1679–1689. [PubMed: 9802980]
73. Nijhof IS, Groen RW, Lokhorst HM, et al. Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of daratumumab. Leukemia. 2015; 29:2039–2049. [PubMed: 25975191]
74. Chen AC, Gudas LJ. An analysis of retinoic acid-induced gene expression and metabolism in AB1 embryonic stem cells. The Journal of biological chemistry. 1996; 271:14971–14980. [PubMed: 8663043]
75. Catena R, Luis-Ravelo D, Anton I, et al. PDGFR signaling blockade in marrow stroma impairs lung cancer bone metastasis. Cancer Res. 2011; 71:164–174. [PubMed: 21097719]
76. Ginestier C, Wicinski J, Cervera N, et al. Retinoid signaling regulates breast cancer stem cell differentiation. Cell cycle. 2009; 8:3297–3302. [PubMed: 19806016]
77. Lim PK, Bliss SA, Patel SA, et al. Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. Cancer Res. 2011; 71:1550–1560. [PubMed: 21343399]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

78. Rosewicz S, Stier U, Brembeck F, et al. Retinoids: effects on growth, differentiation, and nuclear receptor expression in human pancreatic carcinoma cell lines. Gastroenterology. 1995; 109:1646–1660. [PubMed: 7557150]
79. Sidell N. Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells in vitro. J Natl Cancer Inst. 1982; 68:589–596. [PubMed: 7040765]
80. Tang XH, Gudas LJ. Retinoids, retinoic acid receptors, and cancer. Annu Rev Pathol. 2011; 6:345–364. [PubMed: 21073338]
81. Alonso S, Su M, Jones JW, et al. Human bone marrow niche chemoprotection mediated by cytochrome P450 enzymes. Oncotarget. 2015; 6:14905–14912. [PubMed: 25915157]
82. Ginestier C, Wicinski J, Cervera N, et al. Retinoid signaling regulates breast cancer stem cell differentiation. Cell Cycle. 2009; 8:3297–3302. [PubMed: 19806016]
83. Ma HS, Greenblatt SM, Shirley CM, et al. All-trans retinoic acid synergizes with FLT3 inhibition to eliminate FLT3/ITD+ leukemia stem cells in vitro and in vivo. Blood. 2016; 127:2867–2878. [PubMed: 27103744]
84. Ma MEM, Hayley S., Shirley, Courtney M., Duffield, Amy S., Bruner, J Kyle, Li, Li, Nguyen, Bao, Greenblatt, Sarah, Jung, Eric, Aplan, Peter D., Jones, Richard J., Small, Donald, Ghiaur, Gabriel. FLT3 Inhibition and Retinoid Signaling Overcome Stromal Protection to Target FLT3/ITD-Expressing Leukemia Stem Cells in the Bone Marrow Microenvironment. Blood. 2015; 126:790. [PubMed: 26124496]
85. Churchman ML, Low J, Qu C, et al. Efficacy of Retinoids in IKZF1-Mutated BCR-ABL1 Acute Lymphoblastic Leukemia. Cancer Cell. 2015; 28:343–356. [PubMed: 26321221]
86. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015; 47:1168–1178. [PubMed: 26343385]
87. Isella C, Terrasi A, Bellomo SE, et al. Stromal contribution to the colorectal cancer transcriptome. Nat Genet. 2015; 47:312–319. [PubMed: 25706627]
88. Ghiaur G, Wroblewski M, Loges S. Acute Myelogenous Leukemia and its Microenvironment: A Molecular Conversation. Seminars in hematology. 2015; 52:200–206. [PubMed: 26111467]
89. Paggetti J, Haderk F, Seiffert M, et al. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood. 2015; 126:1106–1117. [PubMed: 26100252]
90. Schepers K, Pietras EM, Reynaud D, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell. 2013; 13:285–299. [PubMed: 23850243]
91. Yauch RL, Gould SE, Scales SJ, et al. A paracrine requirement for hedgehog signalling in cancer. Nature. 2008; 455:406–410. [PubMed: 18754008]
92. O'Toole SA, Machalek DA, Shearer RF, et al. Hedgehog overexpression is associated with stromal interactions and predicts for poor outcome in breast cancer. Cancer Res. 2011; 71:4002–4014. [PubMed: 21632555]
93. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:4048–4053. [PubMed: 17360475]
94. Tian H, Callahan CA, DuPree KJ, et al. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:4254–4259. [PubMed: 19246386]
95. Shaw A, Gipp J, Bushman W. The Sonic Hedgehog pathway stimulates prostate tumor growth by paracrine signaling and recapitulates embryonic gene expression in tumor myofibroblasts. Oncogene. 2009; 28:4480–4490. [PubMed: 19784071]
96. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009; 324:1457–1461. [PubMed: 19460966]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Highlights

- Bone marrow microenvironment metabolizes retinoids via CYP26 and creates retinoid low niches
- Normal hematopoietic stem cells are protected in these retinoid low bone marrow niches
- Malignant hematopoietic cells hijack these niches and evade chemotherapy
- Malignant clones reinforces these niches via increased expression of CYP26
- Similar mechanisms protect solid tumor cells metastatic to the bone

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

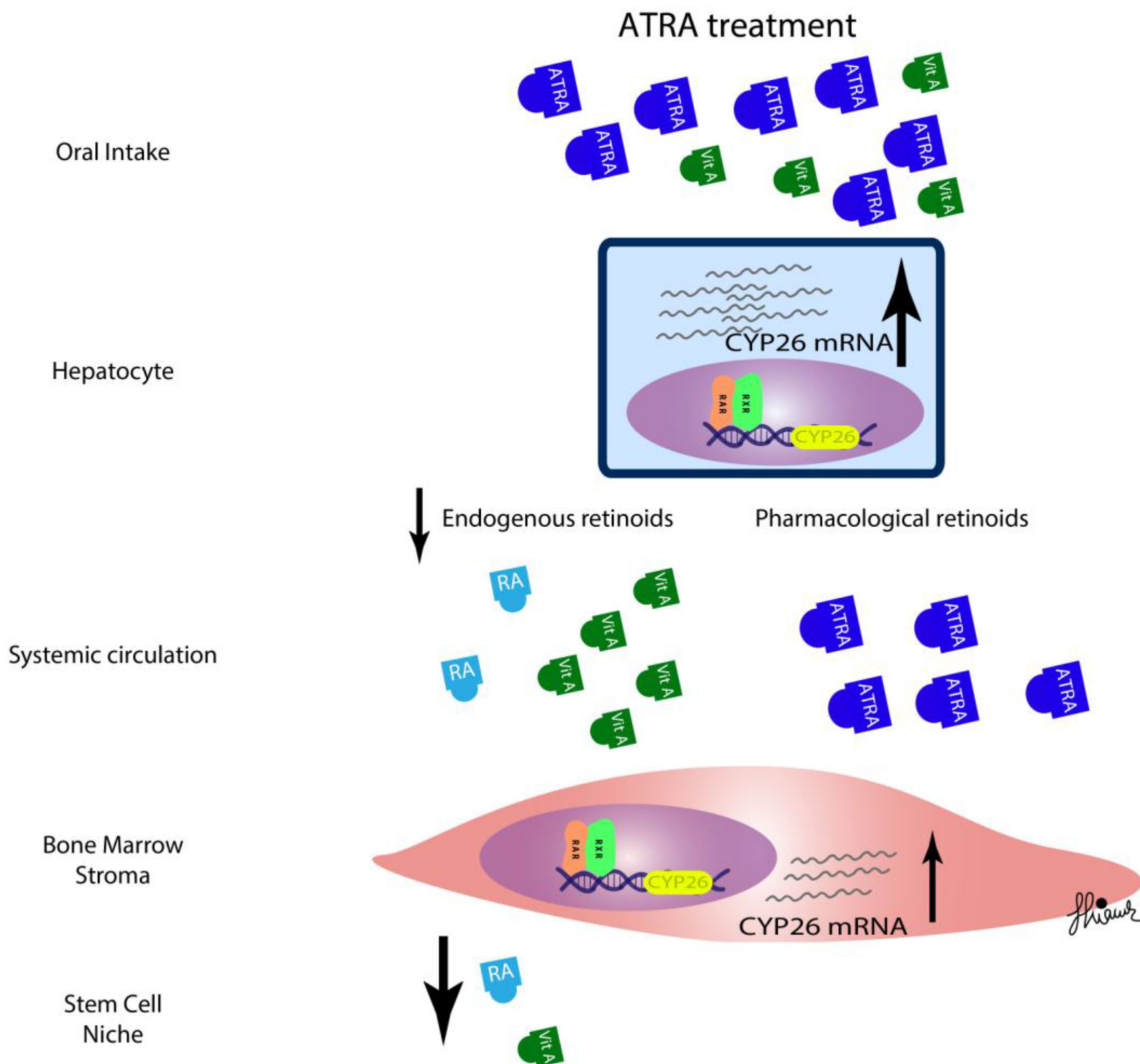

Figure 1. Proposed model of retinoid homeostasis during treatment with all-trans retinoic acid (ATRA)

Oral retinoids are delivered via portal circulation to the liver where they get processed to endogenous retinoids [Vit A – retinol and small amounts of retinoic acid (10^{-9}M)]. Upon oral uptake, ATRA is similarly delivered via portal circulation to the liver prior to entering the systemic circulation. Hepatic CYP26 enzymes are rapidly induced in an effort to maintain systemic retinoid homeostasis. In spite of this mechanism, initial administration of ATRA results in systemic concentrations in 10^{-6}M range. Subsequent administrations of ATRA results in sustained upregulation of hepatic CYP26 and lower and lower plasma concentrations (hence the on-off schedule of therapy with ATRA in APL). Relatively higher plasma concentrations during initial treatment with ATRA may also induce local CYP26 enzymes such as those in the bone marrow stroma. They subsequently create even lower

retinoid concentrations locally. Thus, for niches that are biochemically shielded from systemic circulation (for instance the bone marrow niche), administration of ATRA may result in paradoxically lower local levels of retinoids. Abbreviations: RA – endogenous retinoid acid, ATRA – pharmacological all-trans retinoic acid, VitA – endogenous retinol, RAR – retinoic acid receptor, RXR – retinoid X receptor, CYP26 – Cytochrome P450 retinoid inactivating enzyme.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

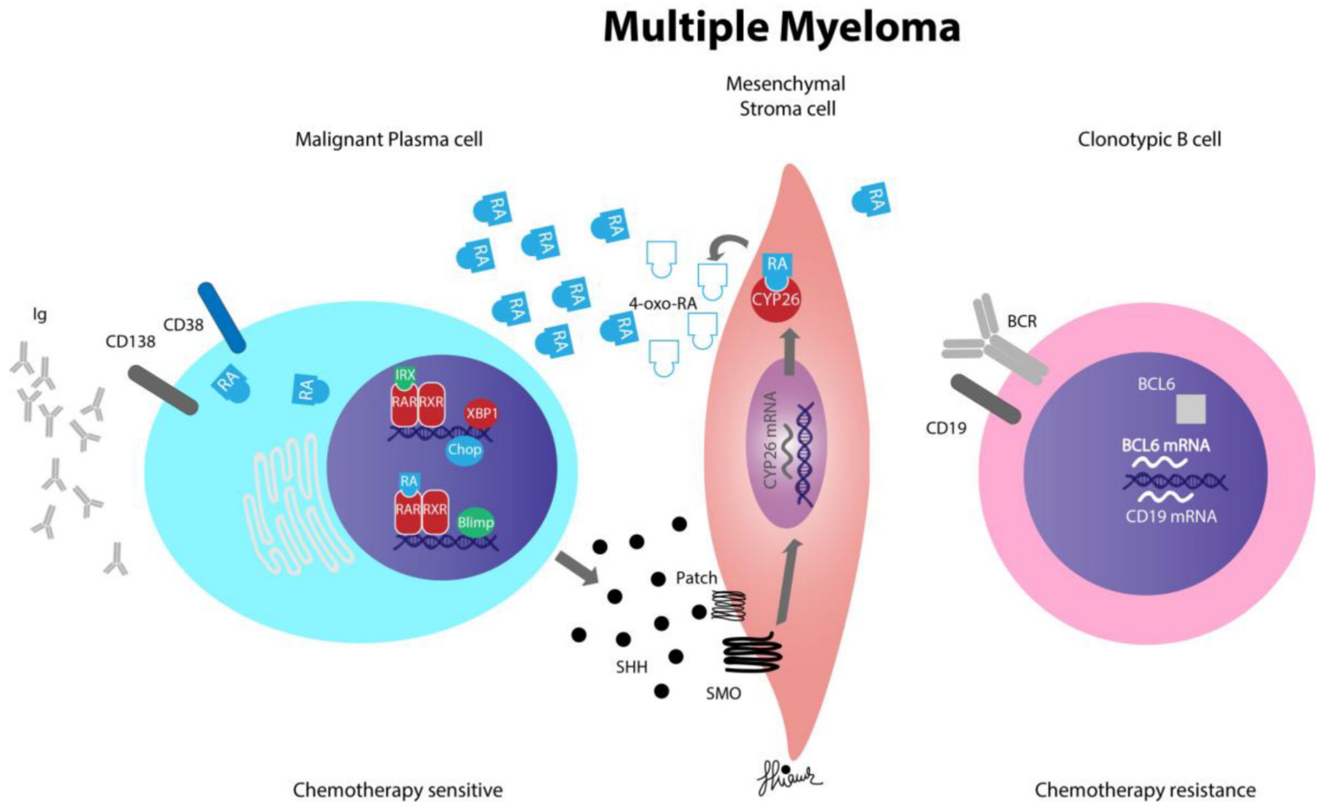

Figure 2. Model of niche dependent chemoresistance in multiple myeloma

In multiple myeloma, the bulk of the tumor is represented by CD138 expressing malignant plasma cells. These cells are exposed to systemic chemokines and morphogens, including retinoids. Retinoids contribute to the relatively high expression of CD38 and CD138 (two retinoid acid target genes), as well as abundant secretion of immunoglobulins and subsequent *increased ER stress*. These MM plasma cells are sensitive to various therapeutic agents including *proteasome inhibitors* and some targeted antibodies [e.g., Daratumumab (Nijhof IS et al., Leukemia 2015)]. However, a minute population of multiple myeloma cells occupy defined, retinoid low niches in the bone marrow. The lack of retinoids results in relatively low CD38 levels, absence of CD138 and decreased secretion of immunoglobulins. These cells appear to resemble the clonotypic B cells described in multiple myeloma, be resistant to therapy, and be responsible for disease relapse. This protective niche is reinforced by the malignant clone which secretes Hedgehog thus upregulating mesenchymal CYP26 (Alonso S et al., JCI 2016). Abbreviations: RA – retinoid acid, RAR – retinoic acid receptor, RXR – retinoid X receptor, SHH – Sonic Hedgehog, SMO – Smoothened, Ig – immunoglobulins, CYP26 – Cytochrome P450 retinoid inactivating enzyme, BCR – B-cell receptor.