Drug resistance remains an important obstacle to the cure of most cancers today. Most studies investigating this problem have focused on acquired drug resistance, or resistance which occurs following treatment that reduces initial tumor burden, but fail to eradicate a sufficient number of cancer cells that would prevent clinical recurrence. Although this approach has revealed mechanisms associated with tumor cell survival following previous, and frequently prolonged, exposure to drugs, for the most part, these mechanisms do not explain the phenomenon of minimal residual disease (MRD). Most widely used diagnostic techniques require approximately $10^9$ tumor cells for early detection; therefore, any treatment that reduces the tumor burden below this level will result in a clinical “complete remission.” Tumor that recurs following a complete remission is believed to be derived from MRD following initial therapy. Factors that allow for tumor cell survival and MRD following initial treatment may be considered either intrinsic or extrinsic. Intrinsic factors are believed to be related to preexisting cancer cell mutations that select for drug resistance and are consistent with the Luria-Delbruck hypothesis of acquired resistance (1). Alternatively, factors that promote tumor cell survival and drug resistance might exist that are external to the tumor cell itself. These factors theoretically could promote cell survival despite toxic insults such as chemotherapy or radiation. In this review, we will discuss how the tumor microenvironment may be a source for factors that promote tumor cell survival and result in MRD leading to the eventual emergence of drug-resistant cancer.

### Background

As a model for how the tumor microenvironment might influence drug response and the emergence of drug resistance, we will focus on common pathways that lead to drug resistance for both hematologic malignancies and solid tumors that metastasize to the bone marrow. The bone marrow contains candidate components that could contribute to reduced drug activity and the emergence of drug resistance including soluble factors, such as interleukins, stromal cells, and extracellular matrix components, such as fibronectin (see Fig. 1). This review will focus on three phases of drug resistance development: tumor cell homing to the protective bone marrow microenvironment, the initial environment-mediated drug resistance (EM-DR; see Table 1 for a summary of references discussed in this review that describe the EM-DR phenotype), and finally, the development of microenvironment-independent resistance or acquired drug resistance. We will also review therapeutic implications of targeting the first two phases of environmentally induced drug resistance that may prevent the eventual emergence of acquired drug resistance. Identification of targets during these initial phases will enable the development of drugs that can be used in combination with traditional agents to mitigate EM-DR, thereby making the primary chemotherapy

### Abstract

The bone marrow microenvironment facilitates the survival, differentiation, and proliferation of hematopoietic cells. These cells are supported by fibroblast-like bone marrow stromal cells, osteoblasts, and osteoclasts which secrete soluble factors and extracellular matrix proteins that mediate these functions. This rich environment serves as a safe haven not only for normal and malignant hematopoietic cells, but also for epithelial tumor cells that metastasize to bone, offering protection from chemotherapeutic agents by common mechanisms. Soluble factors produced in the bone marrow, such as stromal cell–derived factor-1 and interleukin-6, mediate homing, survival, and proliferation of tumor cells, and integrin-mediated adhesion sequesters tumor cells to this protective niche. Environment-mediated drug resistance includes a combination of soluble factors and adhesion, and can be subdivided into soluble factor–mediated drug resistance and cell adhesion–mediated drug resistance. Because it is induced immediately by the microenvironment and is independent of epigenetic or genetic changes caused by the selective pressure of drug exposure, environment-mediated drug resistance is a form of *de novo* drug resistance. In this form of drug resistance, tumor cells are transiently and reversibly protected from apoptosis induced by both chemotherapy and physiologic mediators of cell death. This protection allows tumor cells to survive the insult of chemotherapy, leading to minimal residual disease, and thereby increases the probability for the development of acquired drug resistance.

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adhesion to BMS cells in response to SDF-1 and exhibited increased invasion of basement membranes and did not (12). These cells also migrated across BMS monolayers, bone marrow express CXCR4, whereas nonmetastatic cell lines cancer cell lines derived from tumors that had metastasized to manner (11). For example, Taichman et al. found that prostate the receptor and metastasize to bone in an SDF-1–dependent prostate, rhabdomyosarcoma, and neuroblastoma also express acute and chronic leukemias (5, 9, 10).

Tumor cell homing to the bone marrow microenvironment (CXCR4/SDF-1 axis). Chemokines are known to be important for hematopoietic cell homing within the bone marrow. Stromal cell–derived factor-1 (SDF-1 or CXCL12) is constitutively expressed by bone marrow stroma (BMS) cells, which are considered to be the main source of the chemokine in adults (2–4). This high concentration retains hematopoietic stem cells and progenitors for growth and differentiation, and sequesters mature B cells to the bone marrow. CXCR4 (CD184), a G protein–coupled cell surface receptor, is its primary receptor and is expressed by both normal and malignant hematopoietic cells, including B cell and T cell non–Hodgkin lymphoma, multiple myeloma, and acute and chronic leukemias (2, 5, 6). A new chemokine receptor, CXCR7, has recently been described for SDF-1 that mediates survival and adhesion (but not cell migration) in a variety of tumor cell lines, and contributes to tumor progression in animal models (7). Alsayed et al. showed that inhibition of CXCR4 blocked myeloma migration and homing to bone marrow by in vitro and in vivo methods (8). CXCR4-mediated tumor cell homing to bone marrow has also been observed in acute and chronic leukemias (5, 9, 10).

Additionally, several solid tumors including breast, ovarian, prostate, rhabdomyosarcoma, and neuroblastoma also express the receptor and metastasize to bone in an SDF-1–dependent manner (11). For example, Taichman et al. found that prostate cancer cell lines derived from tumors that had metastasized to bone marrow express CXCR4, whereas nonmetastatic cell lines did not (12). These cells also migrated across BMS monolayers, and exhibited increased invasion of basement membranes and adhesion to BMS cells in response to SDF-1 in vitro (12). Later work by the same group showed that blocking SDF-1 binding with CXCR4 antagonistic antibody prevented the skeletal metastasis of a human prostate cancer cell line introduced into nude mice by cardiac injection (4). They also noted a positive correlation between SDF-1 expression levels and tissues to which prostate cancer cells localized in mice. This axis is also important for the metastasis of other epithelial tumors. Yoon et al. showed that treatment with antagonist anti-CXCR4 blocked primary tumor growth and lung metastasis of a highly metastatic mouse model of squamous cell carcinoma of the head and neck (13). Interestingly, Uchida et al. showed that autocrine production of SDF-1 by squamous cell carcinoma cells enhanced metastasis to distant sites (14).

SDF-1 may not only attract tumor cells to bone marrow, but also stimulate cell survival. Burger et al. found that the viability of chronic lymphocytic leukemia B cells was enhanced by exogenous SDF-1 in vitro in the absence of supportive BMS cells (15). More recently, the same group and others have shown that small peptide inhibitors of CXCR4 could overcome BMS-mediated resistance to drug-induced apoptosis in primary chronic lymphocytic leukemia and acute myelogenous leukemia (AML; refs. 5, 16). Although this important chemokine has not been shown to mediate drug resistance directly, it does, at least, contribute to drug resistance by an indirect mechanism. Several groups have shown that it enhances VLA-4–mediated adhesion to the extracellular matrix components fibronectin and collagen in hematologic and solid tumors. This phenomenon has been shown in multiple myeloma (17) and induces etoposide resistance in small cell lung cancer (18), which has a very high rate of metastasis to bone marrow (2). Therefore, inhibitors of this pathway may not only block tumor homing and engraftment, but may also reverse the cell adhesion–mediated drug resistance (CAM-DR) phenotype of tumor...
IL-6 production by BMS then stimulates myeloma cells, which, in turn, stimulates IL-6 production by BMS. Increased vascular endothelial growth factor and fibroblast growth factor by soluble intermediaries enhances the secretion of growth factors and paracrine interaction of tumor cells with BMS through bone marrow, are known to produce high levels of IL-6 (26), their sensitivity to bortezomib (25). Supportive cells in the myeloma cell lines in a tissue culture model of EM-DR increases et al. showed that specific blockage of IL-6 signaling in multiple secreting clones were sensitive (24). More recently, Voorhees (dexamethasone)-induced apoptosis, whereas non–IL-6– were found to be resistant to both spontaneous and drug that produced autocrine IL-6 derived from patient samples

Diverse tumors exhibit the EM-DR phenotype by common mechanisms

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Abbreviations: SFM-DR, soluble factor–mediated drug resistance; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; SCLC, small cell lung cancer; MM, multiple myeloma; FN, fibronectin.
Cell adhesion-mediated drug resistance (de novo drug resistance). Integrin-mediated adhesion to extracellular matrix (ECM) components fibronectin, collagen, vitronectin, osteopontin, laminin, or stromal cells via cell receptors such as vascular cell adhesion molecule-1 (35) induces signaling critical for the regulation of proliferation, migration, and survival of normal hematopoietic and epithelial cells. Integrins are also important in the tumorigenesis of both cell types, and integrin expression patterns are altered in tumor cells. The expression of integrins known to enhance proliferation, migration, and survival are often increased, whereas integrins associated with the normal phenotype have been shown to decrease in tumor cells (36). For example, primary prostate cancer epithelial cells, but not normal prostate epithelial cells, express high levels of αvβ3 integrin and adhere to the ECM component vitronectin (37, 38). Interestingly, αvβ3 integrin also binds to ECM components fibronectin and osteopontin, which along with vitronectin, are highly expressed in bone marrow. Although the expression of this integrin is normally associated with leukocytes and osteoclasts (36, 38, 39), it is up-regulated in several malignancies of epithelial origin, including those of the breast, lung, and cervix, as well as glioblastoma and melanoma (36, 38, 40, 41).

This integrin expression pattern in tumor cells is associated with metastasis. Attachment of metastatic human prostate cancer cell lines to ECM components was found to be mediated by αvβ3 integrin and integrins containing the α3 and β1 components, whereas nonmetastatic parental cell lines used integrins containing α6 and β4 for adhesion (42). Advanced prostate cancer metastasizes to bone with a high frequency of ~80% (43). Experiments using a murine model of bone metastatic prostate cancer in which tumor cells express either wild-type, mutant, or no αvβ3 integrin expression show her requirement for tumor formation in the bone matrix (44). It is also important in the metastasis of breast and lung cancer to the bone marrow (41), and is associated with metastasis and decreased progression-free survival in cervical cancer (40).

Although αvβ3 is the primary integrin associated with solid tumors, β1 integrin expression is also associated with disease progression in these malignancies. In invasive breast cancer and small cell lung cancer, a negative correlation exists between increased β1 expression and survival (45, 46). White et al. showed the requirement of β1 integrin for tumor induction in breast cancer in a transgenic murine model of breast cancer using a mammary-specific β1 knockout mouse that spontaneously develops breast cancer (47). Furthermore, in epithelial tumors, expression of β1-containing integrins is associated with metastasis to bone. For example, α4β1 (VLA-4) expression in melanoma correlates with metastasis (48), and transfection of α4β1 in Chinese hamster ovary cells mediates bone metastasis (49).

Collectively, these data show that tumor cell expression of integrins α4β1 and αvβ3, like the chemokine receptor CXCR4, are important for epithelial tumor cell engraftment in the bone marrow. Importantly, expression of αvβ3 and β1-containing integrins in epithelial cancers is associated with drug resistance. Adhesion to αvβ3 and β1-containing integrins led to the inhibition of etoposide-induced DNA strand breaks in murine tumors, with metastasis to bone. For example, increased progression in these malignancies. In invasive breast cancer and epithelial tumors, expression of integrins containing αvβ1 and αvβ3 is associated with metastasis (49).

The role VLA-4 plays in mediating de novo drug resistance has been more extensively studied in hematopoietic cancers. In these malignancies, adhesion of tumor cells to ECM components or stromal cells via integrin α4β1 leads to CAM-DR (53–58). Damiano et al. showed that selection for drug resistance in myeloma cells led to the up-regulation of the integrin components αv, β1, and β3 (53). Furthermore, when otherwise drug-sensitive myeloma cells were adhered to fibronectin, a reversible de novo drug resistance phenotype was observed that was not due to reduced drug accumulation in cells or up-regulation of bcl-2 family members. This phenotype, called CAM-DR, has been observed in multiple myeloma and leukemias to mechanistically distinct types of chemotoxic agents, including melphalan (a alkylating agent), doxorubicin (anthracycline, topoisomerase II inhibitor), and mitoxantrone (anthracyclinedione, topoisomerase II inhibitor; refs. 53, 55, 56, 59). Importantly, this phenotype was also shown to mediate paclitaxel and etoposide resistance in epithelial tumors (50–52, 60).

Adhesion of RPMI 8226 myeloma cells to fibronectin via β1 integrin leads to G1 cell cycle arrest associated with increased p27Kip1 protein levels and decreased cyclin A and cyclin E–associated kinase activity (56). Cell cycle arrest and p27Kip1 up-regulation were reversed when adhesion was disrupted. p27Kip1 knockout experiments did not affect adhesion, but did reverse drug resistance, causally linking up-regulation of this protein with the CAM-DR phenotype.

Adhesion of tumor cells to BMS is more complex than adhesion of integrin to fibronectin alone because it involves other adhesion molecules and signaling events. For example, Nefedova et al. found that Notch1 receptors expressed on multiple myeloma cell lines were stimulated by adhesion to BMS, which express the membrane-bound Notch ligand Jagged (61). Notch receptors are normally expressed by hematopoietic stem cells and are expressed in primary myeloma cells, but not in normal plasma cells (62). The signaling induced by this interaction up-regulated p21Cip1/WAF1, leading to growth inhibition and protection from drug-induced apoptosis. Lwin et al. extended these findings in non–Hodgkin B cell lymphoma cell lines. They found that adhesion of these cells to BMS was associated with posttranslational up-regulation of both p27Kip1 and p21Cip1/WAF1 via down-regulation of Skp2, a subunit of SCFSkp2 ubiquitin ligase (63). Skp2 down-regulation, in turn, was found to be mediated by up-regulation of Cdh1, an activating subunit of anaphase-promoting complex ubiquitin ligase. Taken together, these data suggest that the adhesion of hematopoietic tumors to fibronectin or BMS induces a reversible quiescent state that favors cell survival because the majority of standard cytotoxics target rapidly dividing cells. Therefore, adhesion-mediated quiescence may contribute to difficulties in eradicating tumor cells adhered to BMS or ECM in the bone marrow microenvironment.

β1 integrin adhesion also regulates the stability and trafficking of mediators and inhibitors of apoptosis. In leukemia and lymphoma cell lines, β1 integrin adhesion leads to a decrease in the stability of Bim, or Bcl-2–interacting mediator of cell death, by a posttranslational mechanism that is dependent on proteasomal-mediated degradation (64). This reduction in Bim levels in a human chronic myelogenous leukemia cell line mediates resistance to mitoxantrone and imatinib. β1 integrin adhesion to fibronectin also renders...
hematologic tumor cell lines resistant to apoptosis induced by the physiologic mediator of programmed cell death Fas (CD95; ref. 65). This resistance correlated with increased subcellular redistribution of c-Fas–associated death domain–like IL-1–converting enzyme-like inhibitory protein-long (c-FLIPl) from a preexisting insoluble membrane compartment to the soluble cytoplasmic compartment. The increased solubility allows c-FLIPl to associate with and inhibit the death-inducing signal complex that forms after CD95 ligation, blocking the resultant activation of the effector phase of apoptosis. Like the CAM-DR mechanisms mentioned above, this cell adhesion–mediated cell survival pathway is not mediated by transcriptional regulation. Importantly, resistance to Fas-mediated apoptosis in epithelial cells is also mediated by modulating c-FLIP, but its expression is up-regulated in these cells (66).

Nontranscriptional mechanisms may be required to mediate the rapid and transient induction characteristic of the CAM-DR phenotype. Adhesion-mediated survival and drug resistance pathways, in combination with soluble factor–mediated pathways, may contribute to MRD, allowing the development of more complex drug resistance mechanisms caused by the selective pressure of chemotherapy. Lessons learned about transient, extrinsic mechanisms of de novo drug resistance from the CAM-DR model will increase our understanding of the mechanisms by which the tumor microenvironment facilitates the emergence of more complex intrinsic drug resistance mechanisms.

**Acquired drug resistance.** The acquisition of drug resistance is the cause of treatment failure for many types of cancer. The bone marrow microenvironment is a safe haven in which tumor cells can survive the insult of chemotoxic agents, resulting in MRD. Tumor cell survival in the context of continued selective pressure of chemotherapy leads to the development of intrinsic genetic and epigenetic cellular changes, and ultimately, the more complex acquired drug resistance phenotype.

Hazlehurst et al. compared cellular changes in acquired and de novo melphalan resistance in the 8226 multiple myeloma cell line (59), and verified the clinical relevance of CAM-DR by showing de novo drug resistance in myeloma patient specimens. De novo resistance was induced by adhesion to fibronectin for 24 h (CAM-DR), whereas acquired resistance was developed over time by chronic exposure to melphalan in suspension, generating the 8226 subline LR5 (67). Adhesion of parental cells inhibited melphalan-induced apoptosis to a degree comparable to that observed in LR5 cells, and although LR5 cells showed a reduction in melphalan-induced DNA cross-links compared with the parental cell line in suspension, adhered parental cells did not.

This observation suggests that different mechanisms are responsible for de novo and acquired melphalan resistance. Oligonucleotide microarray analysis found that expression of 1,479 unique genes was changed in LR5 cells compared with the drug-sensitive parental cell line. By comparison, the expression of only 69 genes was changed by adhesion of the parental cell line to fibronectin. Only 21 genes were common to both groups. These results suggest that whereas acquired drug resistance is mediated by profound changes at the transcriptional level, de novo drug resistance is primarily mediated by posttranscriptional mechanisms. This work identified several molecular mechanisms of both acquired and de novo drug resistance that have since been validated in our in vitro acquired drug resistance and CAM-DR models (55, 56, 63, 68). It did not, however, address the influence of the bone marrow microenvironment on the development of acquired drug resistance. This consideration is important because the development of acquired drug resistance is likely even more complex in vivo, where tumor cells interact with their protective microenvironment.

In order to address this issue, Hazlehurst et al. explored the effects of fibronectin adhesion–mediated de novo drug resistance on the development of acquired resistance to the DNA-intercalating agent mitoxantrone in the human histiocytic lymphoma cell line U937 (55). The primary finding of this work was that both suspension and CAM-DR cell culture models of acquired drug resistance showed a correlation between reduced double-strand DNA breaks and reduced topoisomerase IIβ levels, but the mechanisms by which its expression was down-regulated in the two models were different. Although topoisomerase IIβ levels were decreased by a posttranscriptional mechanism in the CAM-DR model, they were down-regulated at the transcriptional level in the suspension model. Importantly, the level of resistance acquired in each of the two models was significantly different. Cells that developed resistance while adhered to fibronectin were 2.3-fold more resistant to mitoxantrone compared with cells that developed resistance in suspension. Resistance levels for both models were measured in suspension, and prior adhesion of cells in the absence of drug selection did not result in subsequent drug resistance in suspension; therefore, adhesion to fibronectin itself did not cause the increase in resistance. Rather, it was the adhesion of cells during selection that caused the dramatic increase in drug resistance. Furthermore, when common gene expression changes of at least 1.8-fold in the same direction for both models of acquired drug resistance were compared, the increase in resistance in the CAM-DR model correlated with much more dramatic changes than the suspension model (55). Therefore, tumor cell adhesion in the microenvironment not only causes CAM-DR, a form of de novo drug resistance, but also increases the level of acquired drug resistance upon subsequent exposure to drug in the absence of adhesion.

**Preclinical/Translational Advances**

Well-described cell culture models have been developed to study the mechanisms that lead to de novo and acquired drug resistance in hematologic malignancies. Therefore, these diseases are ideally suited to explore the efficacy of targeting bone marrow EM-DR pathways as a means of mitigating the development of acquired drug resistance. Insights into the molecular mechanisms that lead to the development of de novo and acquired drug resistance in bone marrow will lead to the identification of rational drug targets common to multiple types of cancer, and ultimately improve the treatment of both hematopoietic and solid epithelial tumors.

**Inhibitors of the CXCR4/SDF-1 axis.** The CXCR4/SDF-1 axis is important in bone marrow homing of acute lymphoblastic leukemia, chronic myeloid leukemia, and chronic lymphocytic leukemia cells (5). Although it would seem obvious that blocking tumor cell homing with CXCR4 antagonists would also have a negative effect on normal hematopoiesis, work in a
murine severe combined immunodeficiency model of AML showed that this is not the case. Surprisingly, Tavor et al. showed that although anti-CXCR4 treatment of mice previously engrafted with primary human AML caused a dramatic decrease in AML load in the blood, spleen, and bone marrow in a dose-dependent manner, the same treatment did not affect engrafted primary normal human progenitors in this system (69). Furthermore, blocking this axis by anti-CXCR4 or a small molecule CXCR4 antagonist (AMD3100) decreased AML cell survival in vitro, but SDF-1 treatment increased it. Importantly, peptide antagonists of CXCR4 have been shown to reverse BMS cell–mediated chemoresistance to fludarabine or cytarabine in primary chronic lymphocytic leukemia and AML cocultures, respectively (16, 70). Taken together, these results suggest that tumor cells, unlike normal cells, require CXCR4 signaling to maintain engraftment and tumor survival in the bone marrow microenvironment, and that this axis can be targeted to combat de novo drug resistance in hematologic malignancies.

In agreement with these findings, a number of groups have reported that short-term treatment of patients with the CXCR4 antagonist AMD3100 for stem cell mobilization did not result in significant toxicity (2, 5). Experiments should be done to determine the toxicity of similar treatments in combination with chemotherapeutic agents to normal hematopoiesis. This is because mobilized normal progenitors might also be susceptible to cytotoxic drugs without the protection of the bone marrow microenvironment. This work suggests that it may be possible to specifically target this pathway in tumors while sparing normal progenitor cells. A possible explanation of this phenomenon might be that tumor cells express higher levels of CXCR4 than normal cells (2). Therefore, treatment strategies that involve the blockage of the CXCR4/SDF-1 and/or integrin axis might avoid MRD by blocking tumor engraftment and limiting CAM-DR, and ultimately, mitigate the development of acquired drug resistance in a variety of tumors.

**Inhibitors of BMS-derived IL-6.** Because IL-6 is a pleiotropic cytokine that plays an important role in the homeostasis of a large variety of cell types, treatment strategies that target this soluble factor directly are likely to have adverse consequences. Therefore, recent work in cell culture models have focused on blocking increased IL-6 production by BMS, which results from myeloma and leukemic cell interactions with BMS. Bisbing et al. described an indolinone (BIBF 1000) inhibitor that specifically inhibits the kinase activity of fibroblast growth factor and vascular endothelial growth factor receptor tyrosine kinases by competitively binding to the ATP-binding pocket of their kinase domains, disrupting the tumor-BMS amplification loop that increases IL-6 production by the tumor microenvironment. This compound inhibited IL-6 production by BMS cells in coculture with either myeloma cell lines or patient myeloma cells, leading to both spontaneous and increased dexamethasone-induced apoptosis of myeloma cell lines and patient myeloma cells. Significantly, it did not induce apoptosis in normal B lymphocytes (71).

Using a similar strategy, Golay et al. tested the activity of the histone deacetylase inhibitor ITF2357 on myeloma and AML cells in cell culture models of the bone marrow microenvironment and in vivo. This compound induced apoptosis in several myeloma and AML cell lines and in freshly isolated myeloma specimens. Furthermore, it reduced IL-6 production by primary BMS cells by 80% to 95% and was cytotoxic for AML and myeloma cell lines cocultured with BMS, but was not toxic to BMS cells (72). Importantly, this drug also significantly prolonged survival in a severe combined immunodeficiency mouse model of AML. This work suggests that IL-6 production by the bone marrow microenvironment can be targeted in therapeutic strategies to treat hematologic malignancy. This strategy may also be useful in the treatment of bone metastatic prostate cancer. Decreasing IL-6 levels in metastatic hormone-independent prostate cancer cell lines by reducing nuclear factor κB signaling with an inhibitor of IKK (PS-1145), a key pathway intermediate, increased their sensitivity to docetaxel (34). Similarly, Borsellino et al. noted that inhibition of IL-6 signaling sensitized prostate cancer cells to etoposide and cisplatin (73). Future work in this area should continue to investigate the specificity of the two classes of inhibitors to tumor cells and their microenvironment to avoid possible toxicity.

**Integrin inhibitors.** To date, antibody, peptide, and peptide mimetic integrin antagonists have been used primarily to block angiogenesis in solid tumors. These antagonists have been used to target the αvβ3 integrin that is up-regulated on angiogenic epithelial cells. It is encouraging that clinical trials focused on targeting αvβ3 integrin with a synthetic cyclic peptide (Cilengitide, Merck) in glioblastoma showed that these drugs have very low toxicity at high doses (74). Because αvβ3 integrin is also important for epithelial tumor metastasis to bone and CAM-DR, antagonists that target this integrin might also prevent drug resistance in epithelial malignancies. More recent preclinical work suggests that this may be the case by showing that a nonpeptide antagonist of αvβ3 integrin (PSK14004) blocks skeletal metastasis in animal models of breast and ovarian cancer (75). Although a great deal of work has been done to develop peptide and nonpeptide antagonists for αvβ3 integrins expressed in solid tumors, very little work has been done to develop inhibitors to integrins as effective treatments for hematologic malignancies. Nevertheless, experiments with αv and VLA-4 antagonistic antibodies in in vivo models of epithelial tumor metastasis and hematologic malignancy, respectively, identified these molecules as rational drug targets to decrease tumor growth and prevent drug resistance. For example, Park et al. used an in vitro three-dimension laminin-rich ECM model to evaluate the requirement of breast cancer cells for αvβ1 adhesion. One nonmalignant and five malignant cell lines were treated with a β1 integrin inhibitory antibody using this system. Integrin inhibition of the malignant cell lines led to decreased proliferation and increased apoptosis, but had no effect on the nonmalignant breast cancer cell line. Importantly, this treatment also decreased tumor volume and increased tumor apoptosis in vivo and was not toxic to animals (76). Recently, Matsunaga et al. used a mouse model of AML to show that VLA-4 adhesion promotes MRD in mice treated with cytarabine (ArA). Furthermore, concurrent treatment with anti-VLA-4 antibodies negated this resistance and greatly increased survival (77). Others have shown that these antibodies, in combination with melphalan treatment, greatly reduced tumor burden in bone in a mouse model of multiple myeloma (78).

Collectively, these data suggest that small molecule inhibitors and antagonistic antibodies to integrins have low toxicity to normal cells and block multiple stages of tumor progression. The importance of integrins for both tumor...
metastasis and drug resistance makes these molecules promising drug targets for the prevention of drug resistance induced by the bone marrow microenvironment. Preclinical studies with small molecular VLA-4 inhibitors have been limited to rodent models of inflammatory and autoimmune diseases, including asthma, arthritis, and experimental autoimmune encephalitis (79–81). Similarly, studies with αvβ3 inhibitors have focused on angiogenesis in solid tumors. Future experiments should focus on testing the ability of currently available small molecule VLA-4 and αvβ3 inhibitors to overcome the CAM-DR phenotype and bone marrow tumor engraftment in cell culture and animal models of hematologic and epithelial malignancy. Treatment strategies that involve the blockade of the CXCR4/SDF-1, BMS-derived IL-6, and/or the integrin axis, may avoid MRD by blocking tumor engraftment and limiting CAM-DR, and ultimately, mitigate the development of acquired drug resistance in a variety of tumors.

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The Bone Marrow Microenvironment as a Tumor Sanctuary and Contributor to Drug Resistance

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