


REVIEW

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# Integrins as therapeutic targets in the organ-specific metastasis of human malignant melanoma

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## Abstract

Integrins are a large family of adhesion molecules that mediate cell-cell and cell-extracellular matrix interactions. Among the 24 integrin isoforms, many have been found to be associated with tumor angiogenesis, tumor cell migration and proliferation, and metastasis. Integrins, especially  $\alpha v\beta 3$ ,  $\alpha v\beta 5$  and  $\alpha 5\beta 1$ , participate in mediating tumor angiogenesis by interacting with the vascular endothelial growth factor and angiopoietin-Tie signaling pathways. Melanoma patients have a poor prognosis when the primary tumor has generated distant metastases, and the melanoma metastatic site is an independent predictor of the survival of these patients. Different integrins on the melanoma cell surface preferentially direct circulating melanoma cells to different organs and promote the development of metastases at specific organ sites. For instance, melanoma cells expressing integrin  $\beta 3$  tend to metastasize to the lungs, whereas those expressing integrin  $\beta 1$  preferentially generate lymph node metastases. Moreover, tumor cell-derived exosomes which contain different integrins may prepare a pre-metastatic niche in specific organs and promote organ-specific metastases. Because of the important role that integrins play in tumor angiogenesis and metastasis, they have become promising targets for the treatment of advanced cancer. In this paper, we review the integrin isoforms responsible for angiogenesis and organ-specific metastasis in malignant melanoma and the inhibitors that have been considered for the future treatment of metastatic disease.

**Keywords:** Integrin, Organ-specific metastasis, Malignant melanoma, Angiogenesis, Metastasis, Integrin inhibitors, Therapeutic target, Targeted therapies

## Background

Integrins are a large family of heterodimeric transmembrane glycoproteins that mediate cell-cell and cell-cellular environment interactions. They are widely expressed on many types of cells, including tumor cells, endothelial cells (ECs), pericytes, fibroblasts and immune cells [1]. Integrins are composed of two subunits: subunit  $\alpha$ , with a size of 120–170 kDa, and subunit  $\beta$ , with a size of 90–100 kDa [2]. In humans, there are 18  $\alpha$  subunits and 8  $\beta$  subunits that can assemble 24 different integrins with different binding properties, tissue distributions and biological functions [3, 4]. Both  $\alpha$  and  $\beta$  subunits possess a large extracellular domain, a transmembrane domain and usually a small cytoplasmic tail [3]. In cancer cells, the

extracellular domain serves as a receptor for adhesion proteins and growth factors in the extracellular matrix (ECM) [4], and the cytoplasmic tail is linked to the cytoskeleton and cellular signaling pathways, such as focal adhesion kinase (FAK) and Src family kinases [5], mediating tumor cell attachment, invasion, and migration, as well as tumor angiogenesis [1, 6–8]. Both integrin subunits are required for interactions with the cytoskeleton and ECM [9].

Malignant melanoma is a highly aggressive tumor and has a poor prognosis, especially when it has developed metastases [10]. Early cutaneous melanoma grows horizontally through the epidermis. This is called the radial growth phase (RGP) and is characterized as indolent with a lack of metastatic capability [11]. Over time, the vascular network is enriched, and melanoma cells replicate and increasingly express integrins and other adhesion molecules, which enables the melanoma cells to

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adhere to the vascular wall and invade the vessels [12]. At this point, melanoma begins the vertical growth phase (VGP), and the tumor begins to thicken and acquires the capability of metastasis. The expression levels of many integrins are changed when the melanoma growth is converted from the RGP to the VGP [13] to enable the tumor cells to interact with their environment, establishing an appropriate vascular network and acquiring a migratory and invasive phenotype. For instance,  $\beta 1$  and  $\beta 3$  integrins are up-regulated in many primary and metastatic melanoma cells in the VGP compared with those in the RGP, and the up-regulation is positively associated with the development of melanoma metastasis [14, 15]. The over-expression of integrin  $\alpha \beta 3$  by melanoma cells was also found to play an important role in converting melanoma from the non-tumorigenic RGP to the tumorigenic and invasive VGP [16].

The process of melanoma cell metastasis from a primary tumor to a secondary distant organ usually involves the following steps: detachment from the primary tumor, invasion into blood or lymphatic vessels (intravasation), survival in the circulation, adhesion to blood or lymphatic vessel wall, evasion from the vessels (extravasation), and arrest and colonization in a secondary organ. Integrin-mediated cell adhesion and migration are essential for the whole process of melanoma metastasis development. For example,  $\alpha \beta 3$  plays an important role in the transendothelial migration of melanoma cells by binding to adhesion molecule L1 on ECs in the tumor microenvironment (TME) [17]. In addition, in those melanoma cells that do not express  $\beta 3$  integrins,  $\beta 1$  integrins instead play a role in promoting the transendothelial migration of melanoma cells by binding to vascular cell adhesion molecule 1 (VCAM-1) on ECs in the TME [18].

The site of distant metastasis is an important and independent predictor of the survival of melanoma patients with metastatic disease [19, 20]. A recent large-scale cohort study showed that the median overall survival (OS) of melanoma patients with subcutaneous/lymph node metastasis (M1a) was 20.8 months, whereas the melanoma patients with metastasis to the lungs (M1b) had a median OS of 13 months, those with metastasis to the liver/digestive tract (M1c) showed an OS of 5.5 months, those with metastasis to the bone showed an OS of 3.6 months, and those with metastasis to the central nervous system (M1c) showed an OS of only 2.5 months [21]. Because of the large prognostic differences among types of organ-specific metastasis in melanoma, many studies have been focused on organ-specific metastasis with the aim of identifying not only the mediators responsible for directing metastatic melanoma cells to different organs but also therapeutic targets for metastatic melanoma. The distant metastatic sites of melanoma are influenced by communications

between the circulating tumor cells (CTCs) and the micro-environment of the targeted organ. One of the most important communications for manipulating organ-specific metastasis is the interaction between adhesion molecules and their receptors integrins. The expression of different integrins by melanoma cells facilitates the arrest and colonization of the melanoma cells in different organs [22]. For instance, melanoma cells expressing integrin  $\beta 3$  tend to metastasize to the lungs [23], whereas melanoma cells expressing integrin  $\beta 1$  preferentially develop lymph node metastases [23–25]. Furthermore, different integrins expressed on the ECs of the targeted organ may also regulate the integrity of its microvasculature, allowing extravasation of CTCs into that specific organ.

### **Integrins in angiogenesis and lymphangiogenesis**

Tumor cells metastasize to a distant organ by hematogenous and lymphogenous routes. Angiogenesis and lymphangiogenesis, which are mainly regulated by a series of growth factors and receptors, are critical in the above two metastatic routes. Among these growth factors and receptors, vascular endothelial growth factors (VEGFs) and vascular endothelial growth factor receptors (VEGFRs) are especially important to regulate the growth and maintenance of blood and lymphatic vessels. Additionally, angiopoietins (ANGs) and Tie receptors have been regarded in recent decades as the second important axis for regulating the maturation and plasticity of the vessels [26, 27]. Integrins, known as adhesion molecules that modulate cell-cell and cell-matrix interactions, have been identified to be expressed on ECs, lymphatic endothelial cells (LECs) and pericytes [28, 29] and participate in tumor angiogenesis by interacting with both the VEGF-VEGFR [30] and ANG-Tie [31–33] pathways. The roles that different integrin subunits play in angiogenesis have been reviewed elsewhere [28]. Briefly, integrin subunits  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 9$ ,  $\alpha v$ ,  $\beta 1$ ,  $\beta 3$  and  $\beta 5$  are involved in the process of physiological or pathological angiogenesis [28]. The most important subunits, to our current knowledge, that contribute to tumor angiogenesis and lymphangiogenesis will be discussed here.

Among the integrins on ECs,  $\alpha \beta 3$  is the most abundant and influential receptor regulating angiogenesis [34–36]. Activated  $\alpha \beta 3$  is co-localized with VEGFR-2 on the ECs of proliferating blood vessels [36]. The integrin  $\beta 3$  subunit on ECs, phosphorylated by VEGF-stimulated c-Src, in turn promotes the phosphorylation and activation of VEGFR-2 [30, 35, 36]. The parallel expression of  $\alpha 2 \beta 3$  and  $\alpha \beta 3$  integrins by melanoma cells could up-regulate the expression of basic fibroblast growth factor (bFGF) and promote the angiogenic phenotype [37]. Moreover, integrin  $\alpha \beta 3$  is required for the survival and maturation of newly formed blood vessels, and  $\alpha \beta 3$  antagonists have

been shown to induce the apoptosis of proliferative angiogenic ECs [38].

Another important member in the  $\alpha$  family is integrin  $\alpha$  $\beta$ 5, which is important for neuropilin 1 (NRP-1)-dependent angiogenesis and tumor aggressiveness in melanoma. Evidence has shown that when integrin  $\alpha$  $\beta$ 5 was inhibited, NRP-1 as a co-receptor of VEGF-A was also blocked, and thus the NRP-1-dependent angiogenesis and aggressiveness of melanoma was also reduced [39].

Integrin  $\beta$ 1 is the most abundantly expressed integrin subunit, and it heterodimerizes with at least 12  $\alpha$  subunits, forming 12 different isoforms [40]. Integrin  $\beta$ 1 could be directly activated by angiopoietin 2 (ANG-2), consequently leading to endothelial destabilization [41]. Integrin  $\beta$ 1 is important for B16 melanoma cells to adhere to ECs both in vivo and in vitro [42]. The activation of integrin  $\beta$ 1 in blood cells can also be regulated by “inside-out” signals, leading to metastatic tumor cell extravasation from the circulation into tissues [43]. In melanoma, activated integrin  $\beta$ 1 is required for the attachment of metastatic melanoma cells to the vascular basement membrane via regulating the downstream FAK/paxillin pathway [44], and this integrin helps the extravasation of metastatic melanoma cells into the liver [43] and lungs [45]. After the intravenous injection of melanoma cells, liver colonization was found to be significantly increased in animals with melanoma expressing activated integrin  $\beta$ 1 compared with that expressing wild-type integrin  $\beta$ 1 [43].

The dimerization of subunits  $\beta$ 1 and  $\alpha$ 5, integrin  $\alpha$ 5 $\beta$ 1, is the only known  $\alpha$ 5 integrin and has been clearly defined as a proangiogenic factor [46, 47]. Integrin  $\alpha$ 5 $\beta$ 1 directly interacts with Tie2 and regulates ANG-1-dependent angiogenesis through this interaction [33]. Additionally, another  $\beta$ 1 dimer,  $\alpha$ 9 $\beta$ 1, was found to directly bind to 121 isoforms of VEGF-A, and the blockade of  $\alpha$ 9 $\beta$ 1 specifically inhibited angiogenesis induced by VEGF-A165 and VEGF-A121 [48].

In addition, several integrins, including the previously mentioned  $\alpha$  $\beta$ 3,  $\alpha$  $\beta$ 5 and  $\alpha$ 5 $\beta$ 1, serve as receptors for ANG-2 in the absence of Tie2 [32, 49] and induce the enhancement of VEGF-mediated sprouting and FAK (Tyr397) phosphorylation [32, 50].

Less is known about the association between integrins and lymphangiogenesis. Integrin  $\beta$ 1, including the  $\alpha$ 4 $\beta$ 1,  $\alpha$ 2 $\beta$ 1,  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 9 $\beta$ 1 isoforms, may participate in the process of tumor-associated lymphangiogenesis [51, 52]. Studies have shown that all or 92% of human melanomas with pathologically positive lymph node involvement expressed integrin  $\beta$ 1, whereas only 26% or fewer of melanomas with pathologically negative lymph node involvement showed integrin  $\beta$ 1 expression [25, 53]. Integrin  $\alpha$ 9 $\beta$ 1 may contribute to lymphangiogenesis by

directly binding to the key lymphangiogenic factors VEGF-C and VEGF-D [52]. Additionally, the  $\alpha$ 4 integrin subunit may up-regulate VEGF-C expression and promote lymphangiogenesis together with VEGF-C in human colon cancer [54]. Furthermore, in melanoma,  $\alpha$ 4 expressed on melanoma cells binds to its counter receptor VCAM-1 expressed on LECs with a high affinity and induces the adhesion of melanoma cells to LECs [55], indicating that  $\alpha$ 4 may participate in lymphangiogenesis and lymphatic metastasis in melanoma.

### Integrins and the metastatic niche

Over 100 years ago, Steven Paget proposed the “seed and soil” hypothesis, stating that metastasis is dependent on the interactions between the “seed” (the cancer cells) and the “soil” (the host microenvironment) [56]. It is now established that cancer cells prepare a metastatic niche (also called a pre-metastatic niche) before leaving the primary tumor [57, 58]. Sowing the “seeds” of metastasis requires the action of tumor-secreted factors and tumor-shed extracellular vesicles that enable the “soil” at distant metastatic sites to encourage the outgrowth of incoming cancer cells [57]. In the pre-metastatic niche, immune cells, ECs and stromal cells, together with growth factors, chemokines, matrix-degrading factors and adhesion molecules, collaborate to accelerate assembly of the metastatic lesion [59]. Compelling evidence has shown that metastatic colonization can only successfully occur in certain organs, although tumor cells reach the vasculature of all organs [60, 61]. Indeed, the organotropic metastasis is rather determined by the specific metastatic niche established at different hosts [57].

Integrins in the metastatic niche participate to regulate immune cell activity and myeloid cell differentiation and function; most importantly, integrins are secreted by tumor cells and transported via exosomes to a distant organ to prepare the metastatic niche. Exosomes are small membranous extracellular vehicles (30–150 nm) that contain functional biomolecules (including proteins, lipids and nucleic acids) [62]. Tumor-derived exosomes bud off from tumor cells and transport different biomolecules to distant cells in the body, inducing vascular leakage, inflammation and bone marrow progenitor cell recruitment during pre-metastatic niche formation [63]. Indeed, tumor-derived exosomes play a vital role in developing organ-specific metastasis [57]. Different integrins on the surface of exosomes were recently found to play important roles in preparing favorable pre-metastatic niches in specific organs [64–66]. For instance, exosomal integrins  $\alpha$ 6 $\beta$ 4 and  $\alpha$ 6 $\beta$ 1 preferentially direct circulating melanoma cells to the lungs, whereas exosomal integrin  $\alpha$  $\beta$ 5 induces liver metastasis [65]. Targeting integrins  $\alpha$ 6 $\beta$ 4 and  $\alpha$  $\beta$ 5 resulted in decreased lung and liver metastasis, respectively [65].

### Integrins and organ-specific metastasis in malignant melanoma

As discussed above, integrins and their downstream signaling are vastly involved in regulating the vasculature, angiogenesis, the immune response and the stromal context of the metastatic niche, but different integrins are involved in inducing organ-specific metastasis. Different integrins that contribute to the organ-specific metastasis of malignant melanoma and promising inhibitors are summarized in Table 1.

#### Integrins and lymph node metastasis

Regional lymph node metastasis is an early sign of malignant spread and associated with a poor prognosis in melanoma patients. The development of lymph node metastasis starts with the following two steps: (1) the growth of lymphatic vessels (lymphangiogenesis) at the tumor periphery; and (2) the recruitment of melanoma cells into lymphatic vessels, which is defined as lymphatic invasion [67–69]. Some integrins, including  $\alpha 4\beta 1$ ,  $\alpha 2\beta 1$ ,  $\alpha 1\beta 1$  and  $\alpha 9\beta 1$ , participate in the process of lymphangiogenesis at the tumor periphery [51, 52], and some integrins, such as  $\alpha 9\beta 1$  and  $\alpha 4$ , help with the recruitment of melanoma cells into lymphatic vessels [52, 55].

Among those integrins, integrin  $\alpha 4$  is especially important in the processes leading to the lymph node metastasis of melanoma. It has been reported to be associated with tumorigenicity and lymph node metastasis in many

malignancies, including colon cancer [54], lung cancer [70], pancreatic ductal carcinoma [70] and melanoma [55]. Integrin  $\alpha 4$  can dimerize with  $\beta 1$  and  $\beta 7$  subunits, forming two isoforms,  $\alpha 4\beta 1$  (VLA-4) and  $\alpha 4\beta 7$ , in which  $\alpha 4\beta 1$  is especially important for the adhesion of melanoma cells to LECs via binding to VCAM-1 (Fig. 1) [18, 55]. On the one hand,  $\alpha 4\beta 1$  is expressed on some melanoma cells and helps them to attach to the VCAM-1<sup>+</sup> LECs [18, 55]. On the other hand, integrin  $\alpha 4\beta 1$  is also expressed on LECs in the lymph nodes, and activated  $\alpha 4\beta 1$  on LECs in lymph nodes plays a functional role in capturing VCAM-1<sup>+</sup> metastatic melanoma cells [29]. The lymphangiogenic growth factor VEGF-C, which is secreted by tumor cells and transported to the ECM, is supposed to induce the expression and activation of integrin  $\alpha 4\beta 1$  on LECs [29, 70]. The suppression of integrin  $\alpha 4\beta 1$  in LECs significantly prevents lymphangiogenesis at the tumor periphery and lymph node metastasis [70]. Taken together, antagonists of integrin  $\alpha 4\beta 1$  are promising for inhibiting the interactions of melanoma cells with the lymph node microenvironment and suppressing lymph node metastasis.

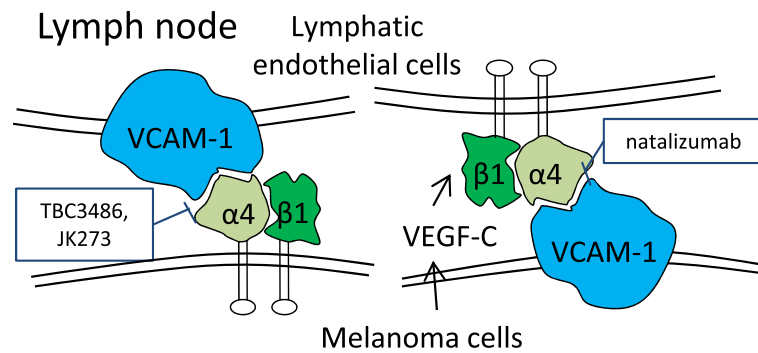
#### Integrins and lung metastasis

The lungs are the most commonly involved when melanoma metastasizes to a distant organ [21, 71]. Our previous study showed that lung metastasis in melanoma was mainly generated by the hematogenous route and was associated with angiogenic activity and pro-angiogenic genes [72]. Based on this aspect, the arrest of melanoma cells in the pulmonary microvasculature and the colonization of melanoma cells in the lungs are required for developing lung metastasis. Several integrins, such as  $\alpha v\beta 3$  and  $\alpha 2b$ , play important roles in helping circulating melanoma cells adhere to the vascular wall in the pulmonary microenvironment [73, 74], and some other integrins, such as  $\beta 1$ , intensify the colonization capability of metastatic melanoma cells in the lungs (Fig. 2) [75].

Integrin  $\alpha v\beta 3$ , a receptor for the ECM proteins vitronectin and fibronectin, is well characterized as a proangiogenic factor in melanoma. The expression of  $\alpha v\beta 3$  on breast cancer cells specifically directs tumor cells to the lungs and bones and promotes spontaneous metastasis to the lungs and bones from the mammary glands in a preclinical *in vivo* model [73]. Nevertheless,  $\alpha v\beta 3$  does not promote the proliferation of breast cancer cells *in vitro* or in the primary site *in vivo*, suggesting that  $\alpha v\beta 3$  may participate in enhancing tumor cell adherence to the pulmonary vasculature but not in promoting the proliferation of tumor cells [73]. Likewise, integrin  $\alpha v\beta 3$  in melanoma is required for the successful establishment of a pulmonary metastasis model by the tail vein injection of B16-F10 melanoma cells [76]. Treatment with the  $\alpha v\beta 3$  inhibitor MK-0429 after the tail vein injection significantly reduced the rate of metastasis in the lungs

**Table 1** The responsible integrins for the organ-specific metastasis of human melanoma and the inhibitors of these integrins

Integrin	Metastatic site	Inhibitor
$\alpha 4$	Lymph node [55]	TBC3486 [141] JK273 [144] Natalizumab (Antegren) [139, 140]
$\alpha 4\beta 1$	Lymph node [29, 53, 55, 138], bone [98]	TBC3486 [141] JK273 [144]
$\beta 1$	Lymph node [18, 53, 138]	AIIB2 [145]
$\alpha 2$	Liver [92]	–
$\alpha 2\beta 1$	Lung [82], liver [82]	BTT-3033, BTT-3034 [146]
$\alpha 5\beta 1$	Liver [96]	Volociximab (M200) [128] ATN-161 [95, 133–135] PF-04605412 [137]
$\beta 3$	Bone [101]	Abergrin (eteracizumab, MEDI-522) [121, 122] MK-0429 [76]
$\alpha v$	Brain [110]	Intetumumab (CNTO 95) [113] Cilengitide (EMD 121974) [120]
$\alpha v\beta 3$	Lung [76], bone [97], brain [108, 111],	MK-0429 [76] Abergrin (eteracizumab, MEDI-522) [121, 122] Cilengitide (EMD 121974) [120]

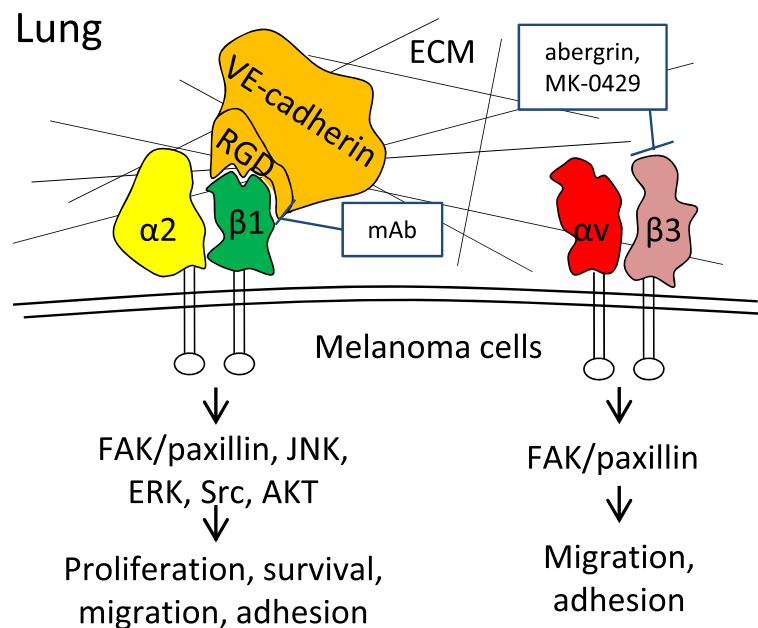


**Fig. 1** Integrin that is responsible for lymph node metastasis in melanoma and its inhibitors. Integrin  $\alpha 4$ , especially  $\alpha 4\beta 1$  isoform, promotes the adhesion of melanoma cells to the lymphatic endothelial cells (LECs) via binding to VCAM-1.  $\alpha 4\beta 1^+$  melanoma cells tend to adhere to VCAM-1 $^+$  LECs, and  $\alpha 4\beta 1^+$  LECs have high affinity to VCAM-1 $^+$  melanoma cells as well. The lymphangiogenic growth factor VEGF-C, which is secreted by tumor cells and transported to ECM, is supposed to induce the expression and activation of integrin  $\alpha 4\beta 1$  on LECs. Monoclonal antibody natalizumab and small molecules TBC3486 and JK273 are inhibitors of integrin  $\alpha 4\beta 1$

compared with the vehicle treatment, showing the important role of  $\alpha \nu \beta 3$  in specifically directing circulating melanoma cells to the lungs, eventually leading to pulmonary metastasis [76].

Integrin  $\alpha \nu \beta 5$ , another proangiogenic factor, is considered to induce carcinoma invasion and metastasis but not influence the proliferation of tumor cells in the primary site or in vitro [77]. It exerts this function at least partly by interacting with epidermal growth factor

(EGF) and EGF receptor (EGFR) [77–79]. Integrin  $\alpha \nu \beta 5$ , especially the  $\beta 5$  subunit, was found to be important for the development of pulmonary vascular permeability, mainly by regulating VEGF, transforming growth factor- $\beta$  (TGF- $\beta$ ) and thrombin [80]. Cilengitide, as an inhibitor of  $\alpha \nu \beta 3$  and  $\alpha \nu \beta 5$  integrins, was found to inhibit both the primary tumor growth and pulmonary metastasis of osteosarcoma [81]. However, no data have shown its impact on the pulmonary metastasis of melanoma.



**Fig. 2** Integrins that are responsible for the lung metastasis of melanoma and the inhibitors. VE-cadherin activates  $\alpha 2\beta 1$  integrin and the downstream signaling pathway by binding to the  $\beta 1$  subunit with the RGD motifs, and the activation of the  $\alpha 2\beta 1$  integrin pathway promotes tumor cell invasion and transendothelial migration, thus inducing lung and liver metastases. Blocking the interactions between integrin  $\alpha 2\beta 1$  and cadherin RGD motifs with highly selective monoclonal antibodies (mAb) significantly reduced the incidence of lung metastasis and improved the survival rate of the experimental mice. Integrins  $\alpha \nu \beta 3$ , as a proangiogenic factor, plays an important role in directing circulating melanoma cells to the lungs and eventually leading to pulmonary metastasis by enhancing the tumor cell adherence to the pulmonary vasculature. Integrins  $\alpha \nu \beta 3$  inhibitors abergrin and MK-0429 selectively bind to  $\beta 3$  subunit and reduce the incidence of pulmonary metastasis in melanoma mouse model

GPIIb is the protein produced by the integrin  $\alpha 2b$  (ITGA2B) gene. It plays a complex role in the development of lung metastasis in melanoma. Highly metastatic B16-D5 melanoma cells were injected intravenously into GPIIb-deficient (GPIIb<sup>-/-</sup>) or WT mice, and tumor cells rapidly accumulated in the pulmonary vasculature in the WT mice but not the GPIIb<sup>-/-</sup> mice, indicating that the presence of GPIIb in the pulmonary host is required for the arrest of melanoma cells in the lungs to develop lung metastasis [74]. However, surprisingly, the occurrence of lung metastasis was higher in the GPIIb<sup>-/-</sup> mice than the WT mice, indicating that the absence of integrin subunit  $\alpha 2b$  may accelerate the colonization of metastatic melanoma cells in the lungs [74]. Therefore, the role of integrin subunit  $\alpha 2b$  in the development of lung metastasis in melanoma is still controversial.

Integrin  $\beta 1$  and the downstream FAK signaling are considered important for the proliferation of metastatic cancer cells after they extravasate into the lungs [75]. Vascular endothelial-cadherin (VE-cadherin) is expressed in highly aggressive melanoma. VE-cadherin activates the  $\alpha 2\beta 1$  integrin pathway by binding to the  $\beta 1$  subunit with arginine-glycine-aspartate (RGD) motifs, and activation of the  $\alpha 2\beta 1$  integrin pathway promotes invasion and transendothelial migration, thus inducing lung and liver metastases in vivo [82]. A highly selective monoclonal antibody (mAb) specifically blocked the cadherin RGD-induced activation of  $\alpha 2\beta 1$ , significantly reduced the incidence of lung metastasis in melanoma and improved the survival rate of the experimental mice [83]. Consequently, integrin  $\beta 1$ , especially isoform  $\alpha 2\beta 1$ , may contribute to the later phase of pulmonary metastasis in melanoma and merits further investigations as a promising target for the treatment of metastatic melanoma.

A lung-specific EC adhesion molecule (Lu-ECAM-1, CLCA2) localized on endothelia of distinct branches of pulmonary blood vessels was identified in the 1990s as a factor that mediates the specific adherence of B16-F10 cells to the lungs [84]. The antibody blocking Lu-ECAM-1 reduced 90% of the lung colonies caused by B16-F10 cell injection [84]. In breast cancer, Lu-ECAM-1 (CLCA2) was capable of facilitating lung metastasis by interactions with integrin  $\alpha 6\beta 4$  expressed on breast cancer cells [85]. However, it is not known how Lu-ECAM-1 facilitated the formation of B16-F10 tumor cell colonies in the lungs. To the best of our knowledge, no data about the potential role of  $\alpha 6\beta 4$  on melanoma cells in leading to lung metastasis have been reported.

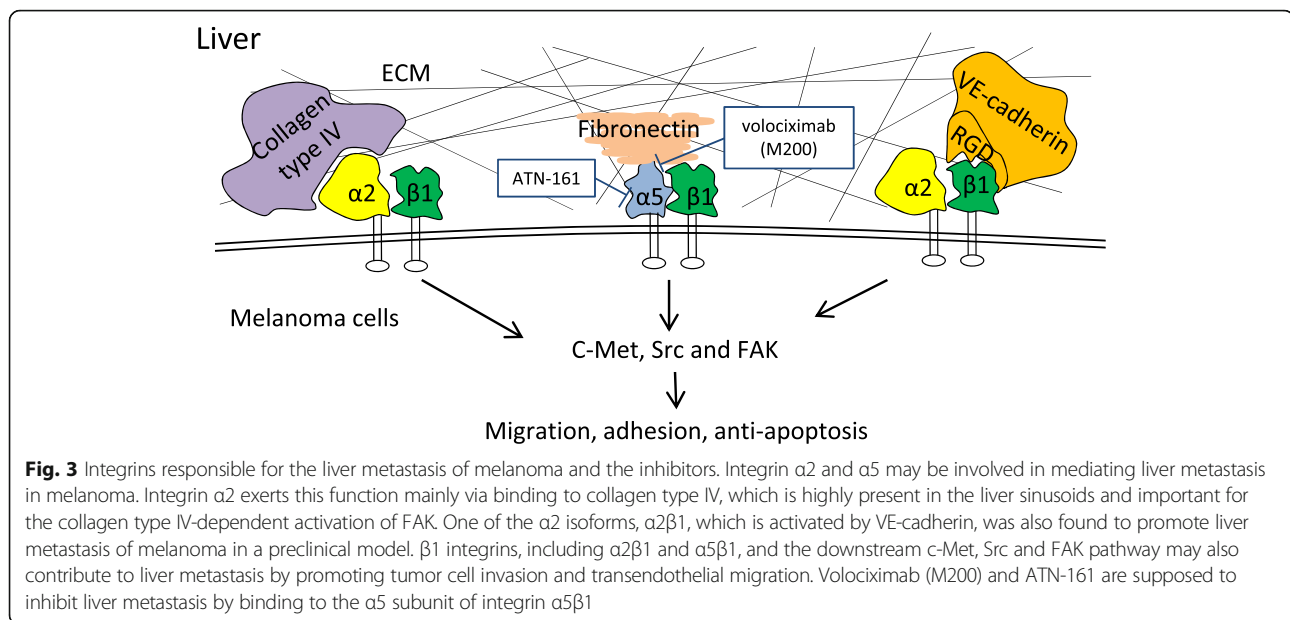
### Integrins and liver metastasis

The liver is another common organ for melanoma metastasis, in addition to the lymph nodes and lungs, because the liver receives a dual blood supply from the portal vein and hepatic arteries, and melanoma is a

hypervascular malignancy [86]. From a molecular perspective, this finding is additionally attributed to specific adhesive molecules, such as integrins, that direct metastatic melanoma cells to the liver. Many integrins, especially integrin  $\beta 1$ , play important roles in mediating the attachment of hepatocytes to the liver ECM and helping the proliferation and migration of myofibroblasts, leading to liver fibrosis [87–89]. In addition, liver fibrosis is closely linked to the preparation of the premalignant environment in the liver [90, 91].

Integrin  $\alpha 2$  (CD49b) is an important integrin that may contribute to the liver metastasis of melanoma. B16-KY8 is a cell line with a high propensity for forming hepatic nodules; the line was derived from B16-F0 melanoma cells by eight passages in a hepatic metastasis model. Out of a broad array of cell membrane molecules, integrin  $\alpha 2$  was uniquely up-regulated in the liver-metastasizing subline B16-KY8 versus the original line B16-F0 [92]. Cells with high and low integrin  $\alpha 2$  expression were selected from the B16-KY8 subline by cell sorting, and the cells with high  $\alpha 2$  expression caused significantly more hepatic nodules than those with low  $\alpha 2$  expression [92]. The selective role of integrin  $\alpha 2$  in liver metastasis was further demonstrated by functional studies in which integrin  $\alpha 2$  was inhibited and over-expressed in B16 melanoma cells [92]. Similarly, blocking integrin  $\alpha 2$  with anti-integrin  $\alpha 2$  antibody significantly prevented operation-induced liver metastasis in a rat colon cancer model [93]. Interestingly, blocking B16-KY8 melanoma cells with anti-integrin  $\alpha 2$  antibody reduced the number of liver metastases but increased the occurrence of peritoneal growth, indicating that integrin  $\alpha 2$  was rather specific for the development of liver metastasis in melanoma [92]. Integrin  $\alpha 2$  mediates liver metastasis mainly by binding to collagen type IV, which is highly present in the liver sinusoids and important for the collagen type IV-dependent activation of FAK [92]. One of the integrin  $\alpha 2$  isoforms,  $\alpha 2\beta 1$ , which is activated by VE-cadherin, was found to promote liver metastasis in preclinical melanoma and breast cancer models (Fig. 3) [82].

Integrin  $\alpha 5\beta 1$  is the only known  $\alpha 5$  integrin and seems to be an upstream factor of c-Met, Src and FAK [94]. The inhibition of integrin  $\alpha 5\beta 1$  has reduced the liver metastasis rate of ovarian cancer [94] and colorectal cancer [95] in mouse models. In a very recent study, integrin  $\alpha 5$  was reduced when the liver metastasis of melanoma was suppressed by treatment with the mitogen-activated protein (MEK) inhibitor selumetinib [96], suggesting the promise of integrin  $\alpha 5$  as a therapeutic target for liver metastasis in melanoma. However, limited investigations on melanoma have been performed to date.



### Integrins and bone metastasis

Integrins on both melanoma cells and host stromal cells (osteoclasts, vascular cells, inflammatory cells, platelets and bone marrow stromal cells) in bone play important roles in promoting bone metastasis [97].

Integrin signaling through  $\alpha 4\beta 1$  and  $\alpha v\beta 3$  on tumor cells may promote tumor cell metastasis to and proliferation in the bone microenvironment (Fig. 4) [97]. The over-expression of integrin  $\alpha 4\beta 1$  on primary melanoma cells was found to be associated with increased bone metastasis, probably via interaction with VCAM-1, which is constitutively expressed on bone marrow stromal cells [98]. Integrin  $\alpha v\beta 3$  plays an important role in generating new blood vessels, which is needed for tumor growth [38]. In addition, the  $\alpha v\beta 3$  ligand osteopontin on melanoma cells also promotes melanoma bone metastasis, probably through the ERK/MAPK pathway [99]. Osteopontin is an RGD-containing protein and promotes the attachment of melanoma cells to the bone microenvironment, where it is abundant [100]. Osteopontin in the bone marrow is needed for the growth of B16 melanoma cells implanted in the bone [100].

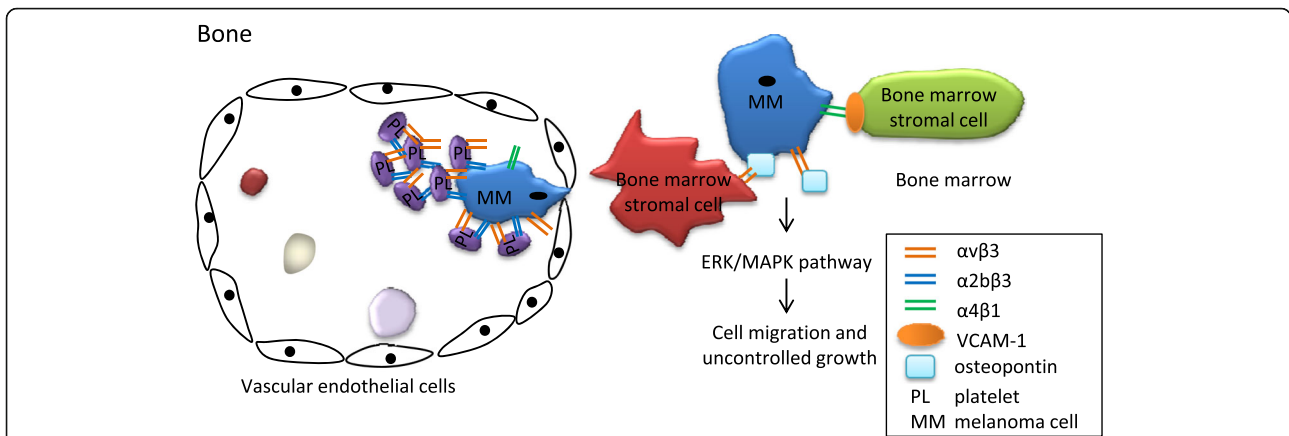
B16 melanoma cells were injected into the left cardiac ventricle of  $\beta 3^{+/+}$  and  $\beta 3^{-/-}$  mice, and 74% of the  $\beta 3^{+/+}$  mice developed osteolytic bone metastasis by 14 days, whereas only 4% of the  $\beta 3^{-/-}$  mice developed bone lesions [101], confirming the role of host cell  $\beta 3$  integrin expression during the process of melanoma bone metastasis. A functional study also showed that integrin  $\beta 3$  in the bone marrow was required for the functionality of newly formed blood vessels [102]. The  $\beta 3$  integrin subunit heterodimerizes with two  $\alpha$  subunits, forming isoforms  $\alpha 2\beta 3$  (GPIIb/IIIa)

and  $\alpha v\beta 3$  [103]. Both isoforms are required for the adhesion of melanoma cells to platelets [104] and platelet aggregation [97, 105], which are important for the capture of melanoma cells in the capillaries of bone.

### Integrins and brain metastasis

Due to the presence of the blood-brain barrier, the treatment of brain metastasis with recently developed targeted therapies and immunotherapies is not as effective as that of extracerebral metastases in melanoma. Thus, it remains a major challenge for the treatment of patients with malignant melanoma, and studies on brain-specific targeted therapeutics are warranted. The expression of  $\alpha v$  integrins was significantly up-regulated in the brain metastases of several solid tumors, including melanoma, compared with the corresponding primary tumors [106–109], indicating the role of  $\alpha v$  integrins in helping tumor cells penetrate the blood-brain barrier and colonize in the brain parenchyma (Fig. 5). The expression of several  $\alpha v$  integrins, such as  $\alpha v\beta 3$ ,  $\alpha v\beta 5$  and  $\alpha v\beta 8$ , on melanoma cells is up-regulated in the brain metastases compared with that in the primary tumor [108]. The over-expression of integrin  $\alpha v$  in melanoma cells was found to accelerate the cell migration rate in vitro and promote melanoma cells to adhere to the brain vasculature in vivo, consequently increasing the occurrence of brain metastasis in an athymic rat model [110]. These findings suggest that integrin  $\alpha v$  is promising as a therapeutic target for the brain-specific metastasis of melanoma.

Among these  $\alpha v$  integrins,  $\alpha v\beta 3$  may play a specific role in inducing the brain metastasis of melanoma. The

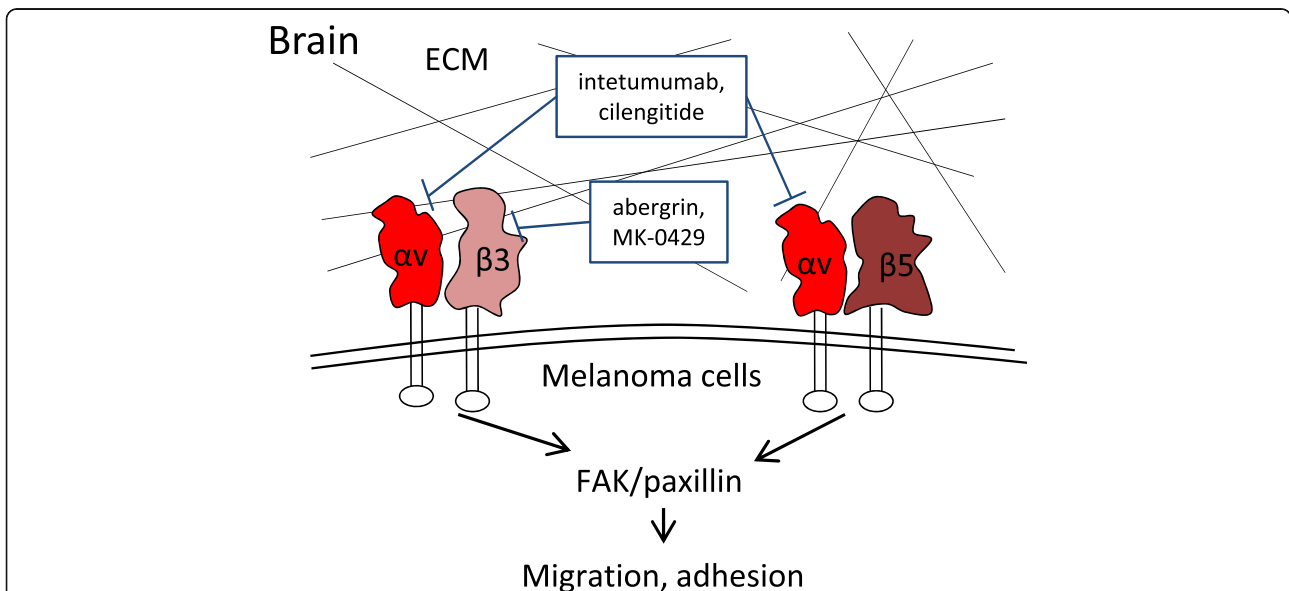


**Fig. 4** Integrins responsible for the bone metastasis of melanoma. Integrins  $\alpha 1 \beta 3$  and  $\alpha v \beta 3$  play important roles in the adhesion of melanoma cells to platelets and platelet aggregation, which are important for the adherence of circulating melanoma cells to the capillaries of bone. The over-expression of integrin  $\alpha 4 \beta 1$  on primary melanoma cells was found to be associated with increased bone metastasis, probably via the interaction with VCAM-1, which is constitutively expressed on bone marrow stromal cells. Integrin  $\alpha v \beta 3$  on melanoma cells is activated by binding to its ligand osteopontin, which is abundant in bone matrix. The activated integrin  $\alpha v \beta 3$  may enhance the ability of cell migration and thus induce bone metastasis probably via the downstream ERK/MAPK pathway

melanoma cell lines Mel57 and Zkr, which express  $\alpha v \beta 3$ , yielded metastasis reproducibly in the brain parenchyma, while other melanoma cell lines, MV3 and BLM, which do not express  $\alpha v \beta 3$ , only preferentially metastasized to the dura mater and leptomeninges instead of the parenchyma [111]. Nevertheless, a functional study by  $\alpha v \beta 3$  transfection in the BLM melanoma cell line did not show any differences in the metastatic pattern [111].

### Integrins as targets for melanoma therapies

Therapeutic drugs that target integrins mainly include three forms: therapeutic antibody-based drugs, peptide-based drugs and small molecule-based drugs; each form has its own advantages and limitations [112]. Antibody-based drugs have high target specificity and affinity and thus less toxicity. Additionally, they can be modified to be more specific and less toxic, but the high cost of production and the need for intravenous administration



**Fig. 5** Integrins responsible for the brain metastasis of melanoma and the inhibitors. Integrin  $\alpha v$ , including  $\alpha v \beta 3$  and  $\alpha v \beta 5$ , promotes the cell migration rate and the adherence of circulating melanoma cells to the brain vasculature, consequently increasing the occurrence of brain metastasis. Abergrin and MK-0429 selectively inhibit integrin  $\alpha v \beta 3$  by binding to the  $\beta 3$  subunit, whereas intetumumab and cilengitide inhibit both integrins by binding the  $\alpha v$  subunit



limit their production and clinical use. Peptide-based drugs are easier to design as most integrin ligands and their recognition consequences have been determined. They usually have moderate to high affinity but may lack specificity since the same ligand can be shared by many integrins. They also have additional disadvantages, such as the need for injection, a high production cost and limited stability. In addition to being readily synthesized, less costly, and more stable, small molecule-based drugs can be administered orally. Most of these drugs require high-throughput library screening. These small molecule antagonists generally have limitations in terms of bioavailability, serum protein binding and integrin selectivity. Many therapeutic drugs for melanoma have been under investigation in both preclinical studies and clinical trials (Table 2).

### Inhibitors of $\alpha v$ integrins

As discussed elsewhere in this paper,  $\alpha v$  integrins, especially  $\alpha v\beta 3$  and  $\alpha v\beta 5$ , play an important role in tumor angiogenesis by interacting with the VEGF-VEGFR and ANG-Tie systems. A fully human anti- $\alpha v$  integrin mAb, intetumumab (CNTO 95), was developed, and it has been shown to prevent angiogenesis and tumorigenesis in human melanoma xenografts in both nude mice and nude rats [113]. Interestingly, the effect of intetumumab on inhibiting tumor growth and tumor metastasis is more likely not dependent on its anti-angiogenic activity because this antibody only recognized  $\alpha v\beta 3$  and  $\alpha v\beta 5$  on human melanoma cells, not mouse angiogenic integrins [113]. Furthermore, intetumumab increased the sensitivity of radioresistant tumor cells, including M21 melanoma cells, to fractionated radiotherapy in an in vivo model [114]. Due to the promising results of preclinical studies, clinical studies have been designed to examine the efficacy of intetumumab for treating human metastatic melanoma. To date, it has been enrolled in phase I

[115] and phase II [116] clinical trials for treating melanoma and showed tolerable toxicity. Patients with stage IV melanoma were treated with dacarbazine and 10 mg/kg intetumumab compared with dacarbazine and a placebo. In terms of the clinical endpoint, no significant benefit was achieved from the regimen with intetumumab [116], possibly due to the limited number of patients enrolled; yet, health-related quality of life seemed to be improved in the patients treated with dacarbazine and intetumumab compared with those treated with dacarbazine and a placebo [117]. Larger-scaled studies on the promising efficacy of intetumumab in the treatment of melanoma and prostate cancer are warranted, but the development of the drug was discontinued by the original company, Centocor, Inc. [118].

Cilengitide (EMD 121974) is another inhibitor of integrins  $\alpha v\beta 3$  and  $\alpha v\beta 5$ . It has shown an anti-angiogenic effect and a promising antitumor effect in many cancers by inhibiting the binding of integrins  $\alpha v\beta 3$  and  $\alpha v\beta 5$  to the ECM [81, 119]. A randomized phase II clinical trial has been completed to evaluate the antitumor effect of cilengitide in patients with metastatic melanoma. The results showed that the drug was well tolerated but achieved minimal efficacy when used as a single-agent treatment [120]. Interestingly, the sole responder and one of two patients with stable disease had no  $\alpha v\beta 3$  expression at baseline, indicating that its clinical efficacy was independent of  $\alpha v\beta 3$  expression at baseline [120]. Likewise, in vitro studies found that cilengitide markedly decreased the invasiveness and angiogenic activity of melanoma cells by the inhibition of  $\alpha v\beta 5$  instead of  $\alpha v\beta 3$  [39]. To conclude, existing studies have shown that cilengitide exerts anti-angiogenic and anti-metastatic functions in an integrin  $\alpha v\beta 5$ -dependent and integrin  $\alpha v\beta 3$ -independent manner.

However, in addition to integrin  $\alpha v\beta 5$ , integrin  $\alpha v\beta 3$  is also important for tumor angiogenesis and tumorigenesis. Integrin  $\alpha v\beta 3$  is required for the survival and maturation

**Table 2** Integrin inhibitors and their effect on metastatic melanoma in preclinical studies and clinical trials

Integrin Inhibitor	Targeted integrin	Effect on metastatic melanoma	Clinical trial	Phase of the clinical trial
MK-0429	$\alpha v\beta 3$	Reduce lung metastasis of melanoma in mouse models [76]	–	–
Intetumumab (CNTO 95)	$\alpha v$	Inhibits melanoma cell adhesion, migration and invasion in vitro. Inhibit tumor growth in mouse xenografts of melanoma.	NCT00246012	Phase I/II [116]
Abergrin (eteracizumab, MEDI-522)	$\alpha v\beta 3$	Patients treated with Abergrin + decarbazine did not show survival benefit compared to decarbazine alone.	NCT00111696 NCT00066196	Phase I [147], Phase II [122]
Cilengitide (EMD 121974)	$\alpha v\beta 3$ and $\alpha v\beta 5$	It was well tolerated but achieved minimal efficacy when used as a single-agent treatment.	NCT00082875	Phase II [120]
Volociximab (M200)	$\alpha 5\beta 1$	It was well tolerated at 10 mg/kg Q2W, and achieved preliminary clinical effect: SD was observed in 32/37 (87%) of patients.	NCT00099970	Phase II [130]

Note: Q2W every 2 weeks, SD stable disease

of newly formed blood vessels, and an  $\alpha\beta3$  antagonist has been shown to induce the apoptosis of proliferative angiogenic ECs [38]. Several inhibitors that selectively target  $\alpha\beta3$  have been produced and have shown promising anti-tumor results in metastatic melanoma.

MK-0429 is a selective  $\alpha\beta3$  inhibitor, which was synthesized by Merck & Co., Inc. It was primarily used in prostate cancer and metastatic bone disease but was discontinued due to insufficient clinical benefits. Data from this company later reported promising results for the treatment of metastatic melanoma in preclinical studies, providing evidence that MK-0429 significantly reduced the lung metastasis of melanoma in a mouse model [76]. However, no clinical trials have been performed to date.

Another  $\alpha\beta3$  inhibitor, abergrin (etaracizumab, MEDI-522), manufactured by MedImmune, Inc., is a humanized mAb being investigated for the treatment of metastatic melanoma, prostate cancer, ovarian cancer and various other types of cancer. It has been used in metastatic melanoma in phase I [121] and phase II [122] clinical trials, showing tolerable side effects but unsatisfactory efficacy. Likewise, in metastatic melanoma, treatment with abergrin + dacarbazine did not achieve a relevant survival benefit compared with dacarbazine alone [123].

LM609 is a mouse mAb that specifically recognizes human integrin  $\alpha\beta3$  and inhibits angiogenesis [124]. It showed promising results for inhibiting angiogenesis, the tumor cell invasion ability and tumor growth in breast cancer and melanoma in the 1990s [38, 125]. Later, it was verified that the treatment of melanoma cell lines with LM609 or  $\alpha\beta3$  siRNA yielded similar results. Both strategies down-regulated the expression of  $\alpha\beta3$  and its downstream molecules FAK and PAK1, induced tumor cell apoptosis [126], and dramatically decreased the ability of tumor cell adhesion and invasion [127]. No in vivo studies or clinical trials have been performed.

### Integrin $\alpha5\beta1$ inhibitors

As the only known  $\alpha5$  integrin,  $\alpha5\beta1$  has been defined as a proangiogenic factor and may be associated with liver metastasis in melanoma [96]. Volociximab (M200) is a chimeric mAb that specifically binds integrin  $\alpha5\beta1$  and blocks the interaction between integrin  $\alpha5\beta1$  and its ligand, fibronectin [128]. It has shown promising activity in different types of cancer in preclinical and clinical studies, which has been reviewed elsewhere [128]. A phase I clinical trial using escalating doses of volociximab has shown that it can be administered at up to 15 mg/kg per week for solid tumors [129]. In the same year, a multicenter phase II study of volociximab in patients with relapsed metastatic melanoma revealed that weekly volociximab treatment at 15 mg/kg was well tolerated and achieved preliminary clinical efficacy [130]. More clinical studies using volociximab as a single-drug

treatment or combined with chemotherapy to treat other metastatic solid tumors have been performed since then to better understand its pharmacokinetics and clinical efficacy [131, 132].

ATN-161 is a small peptide that interacts with the N-terminus of the  $\beta1$ -domain of integrin  $\alpha5\beta1$  and inactivates integrin  $\alpha5\beta1$  [95]. ATN-161 inhibited VEGF-induced cell migration and capillary tube formation in ECs [133], suggesting that it has a potent anti-angiogenesis effect on tumors. Preclinical and clinical studies have shown that treatment with ATN-161 alone or together with chemotherapy not only reduces tumor angiogenesis and liver metastasis but also improves patient survival [95, 134, 135]. Intriguingly, it seems that ATN-161 more likely interacts with integrin  $\alpha5\beta1$  on activated ECs instead of integrin  $\alpha5\beta1$  on tumor cells because ATN-161 reduced the in vivo growth of xenograft human colon cancer cells (HT29) not expressing  $\alpha5\beta1$  [136].

A new  $\alpha5\beta1$  inhibitor, PF-04605412, which is a fully human, Fc-engineered IgG1 mAb, has failed in a phase I clinical trial on human patients with solid tumors because no antitumor effect was observed [137]. This finding may be due to the limited number of patients and the different solid tumors with variable stages included. In this case, modified regimens for heterogeneous patients with different tumors may be helpful.

### Inhibitors of $\alpha4$ integrins

There are two members in the  $\alpha4$  integrin family,  $\alpha4\beta1$  and  $\alpha4\beta7$ . Integrin  $\alpha4\beta1$  is specifically responsible for lymph node metastasis in melanoma [29, 53, 55, 138]. Natalizumab (Antegren, Tysabri) is a humanized mAb, which selectively blocks  $\alpha4$  integrins on the surface of lymphocytes, thereby preventing their adhesion to VCAM-1. It was approved internationally for the treatment of multiple sclerosis (MS). However, natalizumab seems to be associated with the development of melanoma in MS patients [139, 140]. The FDA's Adverse Event Reporting System (FAERS) (2004–2014) includes 137 natalizumab-associated melanoma reports in MS patients [140]. Among those patients, 34% were diagnosed with melanoma within 2 years of natalizumab treatment [140]. Consequently, natalizumab, which may lead to the occurrence of melanoma, is unlikely to be used as a therapeutic agent for metastatic melanoma.

However, natalizumab inhibits both  $\alpha4\beta1$  and  $\alpha4\beta7$ , which may make its effect slightly more complicated [141]. In contrast, TBC3486 is a small molecule that is 200-fold more potent in inhibiting  $\alpha4\beta1$  than  $\alpha4\beta7$  [141]. In addition, it is completely inactive against all other integrins tested, including  $\beta1$ ,  $\beta2$  and  $\beta3$  integrin family members [141]. Hsieh YT and colleagues have shown in recent years that TBC3486 can sensitize drug-resistant acute lymphoblastic leukemia to chemotherapy

[141, 142]. However, to date, no preclinical or clinical studies on the effect of TBC3486 on melanoma have been published.

JK273 is a small molecule inhibitor of integrin  $\alpha 4$ . It was identified by Lee J. and colleagues through a cell-based screen of small molecule libraries and has been shown to inhibit integrin  $\alpha 4$ -dependent cell migration [143]. Recently, the same group reported that this small molecule exerted a selective cytotoxic effect against non-small cell lung cancer NCI-H460 cells [144]. Further studies are warranted to determine its antitumor effect on other solid tumors, including melanoma.

## Conclusions

Integrins, especially  $\alpha v\beta 3$ ,  $\alpha v\beta 5$  and  $\alpha 5\beta 1$ , participate in mediating tumor angiogenesis by interacting with the VEGF and angiopoietin-Tie signaling pathways. Integrin subunits that show a clear association with the organ-specific metastasis of human malignant melanoma include  $\alpha 4$  and  $\beta 1$  for lymph node metastasis,  $\beta 3$  for lung and bone metastasis,  $\alpha 2$  for liver metastasis and  $\alpha v$  for brain metastasis. Although many different drugs targeting a variety of integrins have been developed, none of them have shown sufficient evidence for their clinical use in patients with metastatic melanoma. To conclude, the use of a single integrin as a therapeutic target for metastatic melanoma is not a promising approach because different integrins are responsible for angiogenesis and organ-specific metastasis in human malignant melanoma. However, toxicity is a challenging problem if several integrins are targeted simultaneously due to their prevalence and extensive involvement in maintaining normal biological and physiological functions.

## Abbreviations

ANG-2: Angiopoietin 2; bFGF: Basic fibroblast growth factor; CTCs: Circulating tumor cells; ECM: Extracellular matrix; ECs: Endothelial cells; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; FAERS: FDA's Adverse Event Reporting System; FAK: Focal adhesion kinase; LECs: Lymphatic endothelial cells; Lu-ECAM-1: Lung-specific EC adhesion molecule; mAb: Monoclonal antibody; MEK: Mitogen-activated protein; NRP-1: Neuropilin 1; OS: Overall survival; RGD motifs: Arginine-glycine-aspartate motifs; RGP: Radical growth phase; TGF- $\beta$ : Transforming growth factor- $\beta$ ; TME: Tumor microenvironment; VCAM-1: Vascular cell adhesion molecule 1; VE-cadherin: Vascular endothelial-cadherin; VEGFRs: Vascular endothelial growth factor receptors; VEGFs: Vascular endothelial growth factors; VGP: Vertical growth phase

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## Authors' contributions

RXH and EKR conceived the idea. RXH collected data and drafted the first manuscript and figures. EKR was responsible for overall revision of the entire manuscript and the figures. Both authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

- Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. 2010;10(1):9–22.
- Rivera-Nieves J, Gorfu G, Ley K. Leukocyte adhesion molecules in animal models of inflammatory bowel disease. *Inflamm Bowel Dis*. 2008;14(12):1715–35.
- Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. *Cold Spring Harb Perspect Biol*. 2011;3(3) <https://doi.org/10.1101/cshperspect.a004994>.
- Staunton DE, Lupher ML, Liddington R, Gallatin WM. Targeting integrin structure and function in disease. *Adv Immunol*. 2006;91:111–57.
- Bendas G, Borsig L. Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. *Int J Cell Biol*. 2012;2012:676731.
- Pan L, Zhao Y, Yuan ZJ, Qin GX. Research advances on structure and biological functions of integrins. *Spring*. 2016;5:1094.
- Plantefaber LC, Hynes RO. Changes in integrin receptors on Oncogenically transformed-cells. *Cell*. 1989;56(2):281–90.
- Pinon P, Wehrle-Haller B. Integrins: versatile receptors controlling melanocyte adhesion, migration and proliferation. *Pigm Cell Melanoma R*. 2011;24(2):282–94.
- Mousa SA. Cell adhesion molecules: potential therapeutic & diagnostic implications. *Mol Biotechnol*. 2008;38(1):33–40.
- Ali Z, Yousaf N, Larkin J. Melanoma epidemiology, biology and prognosis. *EJC Suppl*. 2013;11(2):81–91.
- Crowson AN, Magro CM, Mihm MC. Prognosticators of melanoma, the melanoma report, and the sentinel lymph node. *Mod Pathol*. 2006; 19(Suppl 2):S71–87.
- Vacca A, Ribatti D, Roncali L, Lospalluti M, Serio G, Carrel S, Dammacco F. Melanocyte tumor progression is associated with changes in angiogenesis and expression of the 67-kilodalton laminin receptor. *Cancer*. 1993;72(2):455–61.
- Vizkeleti L, Kiss T, Koroknai V, Ecsedi S, Papp O, Szasz I, Adany R, Balazs M. Altered integrin expression patterns shown by microarray in human cutaneous melanoma. *Melanoma Res*. 2017;27(3):180–8.
- Nikkola J, Vihinen P, Vlaykova T, Hahka-Kemppinen M, Heino J, Pyrhonen S. Integrin chains beta 1 and alpha v as prognostic factors in human metastatic melanoma. *Melanoma Res*. 2004;14(1):29–37.
- Van Belle PA, Elenitsas R, Satyamoorthy K, Wolfe JT, Guerry D, Schuchter L, Van Belle TJ, Albelda S, Tahin P, Herlyn M, et al. Progression-related expression of beta 3 integrin in melanomas and nevi. *Hum Pathol*. 1999; 30(5):562–7.
- Hsu MY, Shih DT, Meier FE, Van Belle P, Hsu JY, Elder DE, Buck CA, Herlyn M. Adenoviral gene transfer of beta 3 integrin subunit induces conversion from radial to vertical growth phase in primary human melanoma. *Am J Pathol*. 1998;153(5):1435–42.
- Voura EB, Ramjeesingh RA, Montgomery AMP, Siu CH. Involvement of integrin  $\alpha(v)\beta(3)$  and cell adhesion molecule L1 in transendothelial migration of melanoma cells. *Mol Biol Cell*. 2001;12(9):2699–710.
- Klemke M, Weschenfelder T, Konstandin MH, Samstag Y. High affinity interaction of integrin alpha 4 beta 1 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) enhances migration of human melanoma cells across activated endothelial cell layers. *J Cell Physiol*. 2007;212(2):368–74.
- Balch CM, Buzaid AC, Soong SJ, Atkins MB, Cascinelli N, Coit DG, Fleming ID, Gershenwald JE, Houghton A Jr, Kirkwood JM, et al. Final version of the American joint committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol*. 2001;19(16):3635–48.
- Tas F. Metastatic behavior in melanoma: timing, pattern, survival, and influencing factors. *J Oncol*. 2012;2012:647684.
- Sandru A, Voinea S, Panaitescu E, Blidaru A. Survival rates of patients with metastatic malignant melanoma. *J Med Life*. 2014;7(4):572–6.

22. Vink J, Thomas L, Etoh T, Bruijn JA, Mihm MC, Gattonicelli S, Byers HR. Role of Beta-1 Integrins in organ specific adhesion of melanoma-cells Invitro. *Lab Invest.* 1993;68(2):192–203.
23. Hieken TJ, Ronan SG, Farolan M, Shilkaitis AL, Das Gupta TK. Molecular prognostic markers in intermediate-thickness cutaneous malignant melanoma. *Cancer-Am Cancer Soc.* 1999;85(2):375–82.
24. Hieken TJ, Ronan SG, Farolan M, Shilkaitis AL, Das Gupta TK. Beta 1 integrin expression: a marker of lymphatic metastases in cutaneous malignant melanoma. *Anticancer Res.* 1996;16(4B):2321–4.
25. Hieken TJ, Ronan SG, Farolan M, Shilkaitis AL, Kim DK, Dasgupta TK. Beta(1) integrin expression in malignant-melanoma predicts occult lymph-node metastases. *Surgery.* 1995;118(4):669–75.
26. Helfrich I, Schadendorf D. Blood vessel maturation, vascular phenotype and angiogenic potential in malignant melanoma: one step forward for overcoming anti-angiogenic drug resistance? *Mol Oncol.* 2011;5(2):137–49.
27. Saharinen P, Eklund L, Pulkki K, Bono P, Alitalo K. VEGF and angiopoietin signaling in tumor angiogenesis and metastasis. *Trends Mol Med.* 2011;17(7):347–62.
28. Silva R, D'Amico G, Hodivala-Dilke KM, Reynolds LE. Integrins - the keys to unlocking angiogenesis. *Arterioscl Thromb Vas.* 2008;28(10):1703–13.
29. Garmy-Susini B, Avraamides CJ, Desrosellier JS, Schmid MC, Foubert P, Ellies LG, Lowy AM, Blair SL, Vandenberg SR, Datnow B, et al. PI3K alpha activates integrin alpha 4 beta 1 to establish a metastatic niche in lymph nodes. *P Natl Acad Sci USA.* 2013;110(22):9042–7.
30. Mahabeshwar GH, Feng WY, Reddy K, Plow EF, Byzova TV. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. *Circ Res.* 2007;101(6):570–80.
31. Lee J, Kim KE, Choi DK, Jang JY, Jung JJ, Kiyonari H, Shioi G, Chang W, Suda T, Mochizuki N, et al. Angiopoietin-1 guides directional angiogenesis through integrin alpha(v)beta(5) signaling for recovery of ischemic retinopathy. *Sci Transl Med.* 2013;5(203):203ra127.
32. Felcht M, Luck R, Schering A, Seidel P, Srivastava K, Hu JH, Bartol A, Kienast Y, Vettel C, Loos EK, et al. Angiopoietin-2 differentially regulates angiogenesis through TIE2 and integrin signaling. *J Clin Invest.* 2012;122(6):1991–2005.
33. Cascone I, Napione L, Maniero F, Serini G, Bussolino F. Stable interaction between alpha 5 beta 1 integrin and Tie2 tyrosine kinase receptor regulates endothelial cell response to Ang-1. *J Cell Biol.* 2005;170(6):993–1004.
34. Shattil SJ, Ginsberg MH. Integrin signaling in vascular biology. *J Clin Invest.* 1997;100(1):1–5.
35. De S, Razorenova O, McCabe NP, O'Toole T, Qin J, Byzova TV. VEGF-integrin interplay controls tumor growth and vascularization. *P Natl Acad Sci USA.* 2005;102(21):7589–94.
36. Mahabeshwar GH, Chen JH, Feng WY, Somanath PR, Razorenova OV, Byzova TV. Integrin affinity modulation in angiogenesis. *Cell Cycle.* 2008;7(3):335–47.
37. Dome B, Raso E, Dobos J, Meszaros L, Varga N, Puskas LG, Feher LZ, Lorincz T, Ladanyi A, Trikha M, et al. Parallel expression of alpha 11b beta 3 and alpha v beta 3 integrins in human melanoma cells upregulates bFGF expression and promotes their angiogenic phenotype. *Int J Cancer.* 2005;116(1):27–35.
38. Brooks PC, Montgomery AMP, Rosenfeld M, Reisfeld RA, Hu TH, Klier G, Cheresch DA. Integrin alpha(V)beta(3) antagonists promote tumor-regression by inducing apoptosis of Angiogenic blood-vessels. *Cell.* 1994;79(7):1157–64.
39. Ruffini F, Graziani G, Levati L, Tentori L, D'Atri S, Lacal PM. Cilengitide downmodulates invasiveness and vasculogenic mimicry of neuropilin 1 expressing melanoma cells through the inhibition of alpha v beta 5 integrin. *Int J Cancer.* 2015;136(6):E545–58.
40. Pozzi A, Jarad G, Moeckel GW, Coffa S, Zhang X, Gewin L, Eremina V, Hudson BG, Borza DB, Harris RC, et al. Beta 1 integrin expression by podocytes is required to maintain glomerular structural integrity. *Dev Biol.* 2008;316(2):288–301.
41. Hakanpaa L, Sipilä T, Leppanen VM, Gautam P, Nurmi H, Jacquemet G, Eklund L, Ivaska J, Alitalo K, Saharinen P. Endothelial destabilization by angiopoietin-2 via integrin beta 1 activation. *Nat Commun.* 2015;6
42. Cardones AR, Murakami T, Hwang ST. CXCR4 enhances adhesion of B16 tumor cells to endothelial cells in vitro and in vivo via beta(1) integrin. *Cancer Res.* 2003;63(20):6751–7.
43. Kato H, Liao ZJ, Mitsios JV, Wang HY, Deryugina EI, Varner JA, Quigley JP, Shattil SJ. The primacy of beta 1 integrin activation in the metastatic Cascade. *PLoS One.* 2012;7(10):e46576.
44. Carbonell WS, Ansorge O, Sibson N, Muschel R. The vascular basement membrane as "soil" in brain metastasis. *PLoS One.* 2009;4(6):e5857.
45. Liu YQ, Zou XM, Sun GR, Bao YH. Codonopsis lanceolata polysaccharide CLPS inhibits melanoma metastasis via regulating integrin signaling. *Int J Biol Macromol.* 2017;103:435–40.
46. Kim S, Bell K, Mousa SA, Varner JA. Regulation of angiogenesis in vivo by ligation of integrin alpha 5 beta 1 with the central cell-binding domain of fibronectin. *Am J Pathol.* 2000;156(4):1345–62.
47. Li LX, Welser-Alves J, van der Flier A, Boroujerdi A, Hynes RO, Milner R. An angiogenic role for the alpha 5 beta 1 integrin in promoting endothelial cell proliferation during cerebral hypoxia. *Exp Neurol.* 2012;237(1):46–54.
48. Vlahakis NE, Young BA, Atakilit A, Hawkrigde AE, Issaka RB, Boudreau N, Sheppard D. Integrin alpha 9 beta 1 directly binds to vascular endothelial growth factor (VEGF)- a and contributes to VEGF-A-induced angiogenesis. *J Biol Chem.* 2007;282(20):15187–96.
49. del Toro R, Prahst C, Mathivet T, Siegfried G, Kaminker JS, Larrivee B, Breant C, Duarte A, Takakura N, Fukamizu A, et al. Identification and functional analysis of endothelial tip cell-enriched genes. *Blood.* 2010;116(19):4025–33.
50. Thomas M, Felcht M, Kruse K, Kretschmer S, Deppermann C, Biesdorf A, Rohr K, Benest AV, Fiedler U, Augustin HG. Angiopoietin-2 stimulation of endothelial cells induces alpha v beta 3 integrin internalization and degradation. *J Biol Chem.* 2010;285(31):23842–9.
51. Avraamides CJ, Garmy-Susini B, Varner JA. Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer.* 2008;8(8):604–17.
52. Vlahakis NE, Young BA, Atakilit A, Sheppard D. The lymphangiogenic vascular endothelial growth factors VEGF-C and -D are ligands for the integrin alpha 9 beta 1. *J Biol Chem.* 2005;280(6):4544–52.
53. Hieken TJ, Ronan SG, Farolan N, Shilkaitis AL, TK DG. Beta 1 integrin expression: a marker of lymphatic metastases in cutaneous malignant melanoma. *Anticancer Res.* 1996;16(4B):2321–4.
54. Lv XH, Liu BQ, Li XM, Wang XC, Li XL, Ahmed N, Zhang YF. Integrin alpha 4 induces Lymphangiogenesis and metastasis via upregulation of VEGF-C in human Colon Cancer. *Anat Rec.* 2016;299(6):741–7.
55. Rebhun RB, Cheng H, Gershenwald JE, Fan D, Fidler IJ, Langley RR. Constitutive expression of the alpha(4) integrin correlates with Tumorigenicity and lymph node metastasis of the B16 murine melanoma. *Neoplasia.* 2010;12(2):173–82.
56. Paget S. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev.* 1989;8(2):98–101.
57. Peinado H, Zhang HY, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, Psaila B, Kaplan RN, Bromberg JF, Kang YB, et al. Pre-metastatic niches: organ-specific homes for metastases. *Nat Rev Cancer.* 2017;17(5):302–17.
58. Aladowicz E, Lanfrancone L. Investigating the metastatic niche in melanoma: a new therapeutic opportunity? *Future Oncol.* 2014;10(5):699–701.
59. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. *Nat Rev Cancer.* 2009;9(4):285–93.
60. Hart IR, Fidler IJ. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. *Cancer Res.* 1980;40(7):2281–7.
61. Fidler IJ, Nicolson GL. Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. *J Natl Cancer Inst.* 1976;57(5):1199–202.
62. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol.* 2014;29:116–25.
63. Rak J. Cancer: organ-seeking vesicles. *Nature.* 2015;527(7578):312–4.
64. Alderton GK. Directions to metastatic sites. *Nat Rev Cancer.* 2015;15(12):696–7.
65. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Mark MT, Molina H, Kohsaka S, Di Giannatale A, Ceder S, et al. Tumour exosome integrins determine organotropic metastasis. *Nature.* 2015;527(7578):329–35.
66. Paolillo M, Schinelli S. Integrins and exosomes, a dangerous liaison in Cancer progression. *Cancers.* 2017;9(8) <https://doi.org/10.3390/cancers9080095>.
67. Doeden K, Ma Z, Narasimhan B, Swetter SM, Detmar M, Dadras SS. Lymphatic invasion in cutaneous melanoma is associated with sentinel lymph node metastasis. *J Cutan Pathol.* 2009;36(7):772–80.
68. Xu X, Gimotty PA, Guerry D, Karakousis G, Elder DE. Lymphatic invasion as a prognostic biomarker in primary cutaneous melanoma. *Methods Mol Biol.* 2014;1102:275–86.
69. Dadras SS, Lange-Asschenfeldt B, Velasco P, Nguyen L, Vora A, Muzikansky A, Jahnke K, Hauschild A, Hiraakawa S, Mihm MC, et al. Tumor lymphangiogenesis predicts melanoma metastasis to sentinel lymph nodes. *Mod Pathol.* 2005;18(9):1232–42.
70. Garmy-Susini B, Avraamides CJ, Schmid MC, Foubert P, Ellies LG, Barnes L, Feral C, Papayannopoulou T, Lowy A, Blair SL, et al. Integrin alpha 4 beta 1

- signaling is required for Lymphangiogenesis and tumor metastasis. *Cancer Res.* 2010;70(8):3042–51.
71. Velu PP, Cao C, Yan TD. Current surgical management of melanoma metastases to the lung. *J Thorac Dis.* 2013;5(Suppl 3):S274–6.
  72. Huang RX, Andersen LMK, Rofstad EK. Metastatic pathway and the microvascular and physicochemical microenvironments of human melanoma xenografts. *J Transl Med.* 2017;15:203.
  73. Sloan EK, Pouliot N, Stanley KL, Chia J, Moseley JM, Hards DK, Anderson RL. Tumor-specific expression of alpha v beta 3 integrin promotes spontaneous metastasis of breast cancer to bone. *Breast Cancer Res.* 2006;8(2):R20.
  74. Echtler K, Konrad I, Lorenz M, Schneider S, Hofmaier S, Plenagl F, Stark K, Czernak T, Tirniceriu A, Eichhorn M, et al. Platelet GPIIb supports initial pulmonary retention but inhibits subsequent proliferation of melanoma cells during hematogenic metastasis. *PLoS One.* 2017;12(3):e0172788.
  75. Shibue T, Weinberg RA. Integrin beta(1)-focal adhesion kinase signaling directs the proliferation of metastatic cancer cells disseminated in the lungs. *P Natl Acad Sci USA.* 2009;106(25):10290–5.
  76. Pickarski M, Gleason A, Bednar B, Duong L. Orally active alpha v beta 3 integrin inhibitor MK-0429 reduces melanoma metastasis. *Oncol Rep.* 2015; 33(6):2737–45.
  77. Ricono JM, Huang M, Barnes LA, Lau SK, Weis SM, Schlaepfer DD, Hanks SK, Cheresch DA. Specific cross-talk between epidermal growth factor receptor and integrin alpha(v)beta(5) promotes carcinoma cell invasion and metastasis. *Cancer Res.* 2009;69(4):1383–91.
  78. Lau SKM, Shields DJ, Murphy EA, Desgrosellier JS, Anand S, Huang M, Kato S, Lim ST, Weis SM, Stupack DG, et al. EGFR-mediated carcinoma cell metastasis mediated by integrin alpha v beta 5 depends on activation of c-Src and cleavage of MUC1. *PLoS One.* 2012;7(5):e36753.
  79. Huang M, Anand S, Murphy EA, Desgrosellier JS, Stupack DG, Shattil SJ, Schlaepfer DD, Cheresch DA. EGFR-dependent pancreatic carcinoma cell metastasis through Rap1 activation. *Oncogene.* 2012;31(22):2783–93.
  80. Su G, Hodnett M, Wu NY, Atakilit A, Kosinski C, Godzich M, Huang XZ, Kim JYK, Frank JA, Matthay MA, et al. Integrin alpha v beta 5 regulates lung vascular permeability and pulmonary endothelial barrier function. *Am J Resp Cell Mol.* 2007;36(3):377–86.
  81. Gvozdenovic A, Boro A, Meier D, Bode-Lesniewska B, Born W, Muff R, Fuchs B. Targeting alpha v beta 3 and alpha v beta 5 integrins inhibits pulmonary metastasis in an intratracheal xenograft osteosarcoma mouse model. *Oncotarget.* 2016;7(34):55141–54.
  82. Bartolome RA, Torres S, de Val SJ, Escudero-Paniagua B, Calvino E, Teixido J, Casal JI. VE-cadherin RGD motifs promote metastasis and constitute a potential therapeutic target in melanoma and breast cancers. *Oncotarget.* 2017;8(1):215–27.
  83. Bartolome RA, Aizpurua C, Jaen M, Torres S, Calvino E, Imbaud JI, Casal JI. Monoclonal antibodies directed against cadherin RGD exhibit therapeutic activity against melanoma and colorectal Cancer metastasis. *Clin Cancer Res.* 2018;24(2):433–44.
  84. Zhu DZ, Cheng CF, Pauli BU. Mediation of lung metastasis of murine melanomas by a lung-specific endothelial-cell adhesion molecule. *P Natl Acad Sci USA.* 1991;88(21):9568–72.
  85. Abdel-Ghany M, Cheng HC, Eible RC, Pauli BU. The breast cancer beta(4) integrin and endothelial human CLCA2 mediate lung metastasis. *J Biol Chem.* 2001;276(27):25438–46.
  86. Blake SP, Weisinger K, Atkins MB, Raptopoulos V. Liver metastases from melanoma: detection with multiphasic contrast-enhanced CT. *Radiology.* 1999;213(1):92–6.
  87. Martin K, Pritchett J, Llewellyn J, Mullan AF, Athwal VS, Dobie R, Harvey E, Zeef L, Farrow S, Streuli C, et al. PAK proteins and YAP-1 signalling downstream of integrin beta-1 in myofibroblasts promote liver fibrosis. *Nat Commun.* 2016;7:12502.
  88. Pinkse GGM, Voorhoeve MP, Noteborn M, Terpstra OT, Bruijn JA, de Heer E. Hepatocyte survival depends on beta 1-integrin-mediated attachment of hepatocytes to hepatic extracellular matrix. *Liver Int.* 2004;24(3):218–26.
  89. Patsenker E, Stickel F. Role of integrins in fibrosing liver diseases. *Am J Physiol-Gastr L.* 2011;301(3):G425–34.
  90. Affo S, Yu LX, Schwabe RF. The role of Cancer-associated fibroblasts and fibrosis in liver Cancer. *Annu Rev Pathol-Mech.* 2017;12:153–86.
  91. Sakurai T, Kudo M. Molecular link between liver fibrosis and hepatocellular carcinoma. *Liver Cancer.* 2013;2(3–4):365–6.
  92. Yoshimura K, Meckel KF, Laird LS, Chia CY, Park JJ, Olino KL, Tsunedomi R, Harada T, Iizuka N, Hazama S, et al. Integrin alpha 2 mediates selective metastasis to the liver. *Cancer Res.* 2009;69(18):7320–8.
  93. van der Bij GJ, Oosterfing SJ, Bogels M, Bhoelan F, Fluitsma DM, Beelen RHJ, Meijer S, van Egmond M. Blocking alpha 2 integrins on rat CC531s colon carcinoma cells prevents operation-induced augmentation of liver metastases outgrowth. *Hepatology.* 2008;47(2):532–43.
  94. Mitra AK, Sawada K, Tiwari P, Mui K, Gwin K, Lengyel E. Ligand-independent activation of c-met by fibronectin and alpha(5)beta(1)-integrin regulates ovarian cancer invasion and metastasis. *Oncogene.* 2011;30(13):1566–76.
  95. Stoeltzing O, Liu WB, Reinmuth N, Fan F, Parry GC, Parikh AA, McCarty MF, Bucana CD, Mazar AP, Ellis LM. Inhibition of integrin alpha(5)beta(1) function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice. *Int J Cancer.* 2003; 104(4):496–503.
  96. Ryu SH, Heo SH, Park EY, Choi KC, Ryu JW, Lee SH, Lee SW. Selumetinib inhibits melanoma metastasis to mouse liver via suppression of EMT-targeted genes. *Anticancer Res.* 2017;37(2):607–14.
  97. Schneider JG, Amend SR, Weillbaecher KN. Integrins and bone metastasis: integrating tumor cell and stromal cell interactions. *Bone.* 2011;48(1):54–65.
  98. Matsuura N, Puzon-McLaughlin W, Irie A, Morikawa Y, Kakudo K, Takada Y. Induction of experimental bone metastasis in mice by transfection of integrin alpha 4 beta 1 into tumor cells. *Am J Pathol.* 1996;148(1):55–61.
  99. Hayashi C, Rittling S, Hayata T, Amagasa T, Denhardt D, Ezura Y, Nakashima K, Noda M. Serum osteopontin, an enhancer of tumor metastasis to bone, promotes B16 melanoma cell migration. *J Cell Biochem.* 2007;101(4):979–86.
  100. Ohyama Y, Nemoto H, Rittling S, Tsuji K, Amagasa T, Denhardt DT, Nifuji A, Noda M. Osteopontin-deficiency suppresses growth of B16 melanoma cells implanted in bone and osteoclastogenesis in co-cultures. *J Bone Miner Res.* 2004;19(10):1706–11.
  101. Bakewell SJ, Nestor P, Prasad S, Tomasson MH, Dowland N, Mehrotra M, Scarborough R, Kanter J, Abe K, Phillips D, et al. Platelet and osteoclast beta(3) integrins are critical for bone metastasis. *P Natl Acad Sci USA.* 2003; 100(24):14205–10.
  102. Watson AR, Pitchford SC, Reynolds LE, Direkze N, Brittan M, Alison MR, Rankin S, Wright NA, Hodivala-Dilke KM. Deficiency of bone marrow beta3-integrin enhances non-functional neovascularization. *J Pathol.* 2010;220(4):435–45.
  103. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell.* 2002; 110(6):673–87.
  104. Lonsdorf AS, Kramer BF, Fahrleitner M, Schonberger T, Gnerlich S, Ring S, Gehring S, Schneider SW, Kruhlak MJ, Meuth SG, et al. Engagement of alphaIIb beta3 (GPIIb/IIIa) with alphanubeta3 integrin mediates interaction of melanoma cells with platelets: a connection to hematogenous metastasis. *J Biol Chem.* 2012;287(3):2168–78.
  105. Phillips DR, Charo IF, Scarborough RM. GpiIb-IIIa - the responsive integrin. *Cell.* 1991;65(3):359–62.
  106. Berghoff AS, Rajky O, Winkler F, Bartsch R, Furtner J, Hainfellner JA, Goodman SL, Weller M, Schittenhelm J, Preusser M. Invasion patterns in brain metastases of solid cancers. *Neuro-Oncology.* 2013;15(12):1664–72.
  107. Berghoff AS, Rajky O, Winkler F, Weller M, Zielinski C, Schittenhelm J, Preusser M. Evaluation of invasion patterns and their correlation with integrin alphavbeta expression in brain metastases of solid cancers. *J Clin Oncol.* 2013;31(15):1664–72.
  108. Vogetseder A, Thies S, Ingold B, Roth P, Weller M, Schraml P, Goodman SL, Moch H. Alpha v-integrin isoform expression in primary human tumors and brain metastases. *Int J Cancer.* 2013;133(10):2362–71.
  109. Berghoff AS, Kovanda AK, Melchardt T, Bartsch R, Hainfellner JA, Sipos B, Schittenhelm J, Zielinski CC, Widhalm G, Dieckmann K, et al. Alpha v beta 3, alpha v beta 5 and alpha v beta 6 integrins in brain metastases of lung cancer. *Clin Exp Metastasis.* 2014;31(7):841–51.
  110. Wu YJ, Pagel MA, Muldoon LL, Fu R, Neuwelt EA. High alphav integrin level of Cancer cells is associated with development of brain metastasis in Athymic rats. *Anticancer Res.* 2017;37(8):4029–40.
  111. Kusters B, Westphal JR, Smits D, Ruiters DJ, Wesseling P, Keilholz U, De Waal RMW. The pattern of metastasis of human melanoma to the central nervous system is not influenced by integrin alpha(v)beta(3) expression. *Int J Cancer.* 2001;92(2):176–80.
  112. Millard M, Odde S, Neamati N. Integrin targeted therapeutics. *Theranostics.* 2011;1:154–88.
  113. Trikha M, Zhou Z, Nemeth JA, Chen QM, Sharp C, Emmell E, Giles-Komar J, Nakada MT. CNTO 95, a fully human monoclonal antibody that inhibits alpha v integrins, has antitumor and antiangiogenic activity in vivo. *Int J Cancer.* 2004;110(3):326–35.

114. Ning SC, Nemeth JA, Hanson RL, Forsythe K, Knox SJ. Anti-integrin monoclonal antibody CNTO 95 enhances the therapeutic efficacy of fractionated radiation therapy in vivo. *Mol Cancer Ther*. 2008;7(6):1569–78.
115. O'day SJ, Pavlick AC, Albertini MR, Hamid O, Schalh H, Lang ZH, Ling J, Mata M, Reddy M, Foster B. Clinical and pharmacologic evaluation of two dose levels of intetumumab (CNTO 95) in patients with melanoma or angiosarcoma. *Invest New Drug*. 2012;30(3):1074–81.
116. O'day S, Pavlick A, Loquai C, Lawson D, Gutzmer R, Richards J, Schadendorf D, Thompson JA, Gonzalez R, Trefzer U, et al. A randomised, phase II study of intetumumab, an anti- $\alpha(v)$ -integrin mAb, alone and with dacarbazine in stage IV melanoma. *Brit J Cancer*. 2011;105(3):346–52.
117. Robinson DW, Cormier JN, Zhao N, Uhlar CM, Revicki DA, Cella D. Health-related quality of life among patients with metastatic melanoma: results from an international phase 2 multicenter study. *Melanoma Res*. 2012;22(1):54–62.
118. "Intetumumab". *AdisInsight* (<https://adisinsight.springer.com/drugs/800019252>). Retrieved 24 March 2018.
119. Hariharan S, Gustafson D, Holden S, McConkey D, Davis D, Morrow M, Basche M, Gore L, Zang C, O'Bryant CL, et al. Assessment of the biological and pharmacological effects of the  $\alpha(\text{nu})\beta(3)$  and  $\alpha(\text{nu})\beta(5)$  integrin receptor antagonist, cilengitide (EMD 121974), in patients with advanced solid tumors. *Ann Oncol*. 2007;18(8):1400–7.
120. Kim KB, Prieto V, Joseph RW, Diwan AH, Gallick GE, Papadopoulos NE, Bedikian AY, Camacho LH, Hwu P, Ng CS, et al. A randomized phase II study of cilengitide (EMD 121974) in patients with metastatic melanoma. *Melanoma Res*. 2012;22(4):294–301.
121. Wang W, Rao U, Jukic D, Sander C, Drogowski L, Mandic M, Richman L, Tice D, Lieberman R, Hammershaimb L, et al. Phase I study of humanized anti- $\alpha v$   $\beta 3$  antibody MEDI-522 with tumor biopsy evaluation for saturation and antitumor effects in patients with metastatic melanoma. *J Immunother*. 2006;29(6):677.
122. Hersey P, Sosman J, O'Day S, Richards J, Bedikian A, Gonzalez R, Sharfman W, Weber R, Logan T, Kirkwood JM, et al. A phase II, randomized, open-label study evaluating the antitumor activity of MEDI-522, a humanized monoclonal antibody directed against the human  $\alpha v \beta 3$  (avb3) integrin, +/- dacarbazine (DTIC) in patients with metastatic melanoma (MM). *J Clin Oncol*. 2005;23(16):711s.
123. Hersey P, Sosman J, O'Day S, Richards J, Bedikian A, Gonzalez R, Sharfman W, Weber R, Logan T, Buzoianu M, et al. A randomized phase 2 study of Etaracizumab, a monoclonal antibody against integrin  $\alpha(v)\beta(3)$ , +/- Dacarbazine in patients with stage IV metastatic melanoma. *Cancer-Am Cancer Soc*. 2010;116(6):1526–34.
124. Borst AJ, James ZM, Zagotta WN, Ginsberg M, Rey FA, DiMaio F, Backovic M, Veessler D. The therapeutic antibody LM609 selectively inhibits ligand binding to human  $\alpha(V)\beta(3)$  integrin via steric hindrance. *Structure*. 2017;25(11):1732–1739.e5.
125. Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresch DA. Antiintegrin  $\alpha$ -V-Beta-3 blocks human breast-Cancer growth and angiogenesis in human skin. *J Clin Invest*. 1995;96(4):1815–22.
126. Petitclerc E, Stromblad S, von Schalscha TL, Mitjans F, Piulats J, Montgomery AMP, Cheresch DA, Brooks PC. Integrin  $\alpha(V)\beta(3)$  promotes M21 melanoma growth in human skin by regulating tumor cell survival. *Cancer Res*. 1999;59(11):2724–30.
127. Bai J, Zhang J, Wu J, Shen L, Zeng J, Ding J, Wu Y, Gong Z, Li A, Xu S, et al. JWA regulates melanoma metastasis by integrin  $\alpha(V)\beta(3)$  signaling. *Oncogene*. 2010;29(8):1227–37.
128. Almokadem S, Belani CP. Volociximab in cancer. *Expert Opin Biol Ther*. 2012;12(2):251–7.
129. Ricart AD, Tolcher AW, Liu G, Hohen K, Chwartz GS, Albertini M, Weiss G, Yazji S, Ng C, Wilding G. Volociximab, a chimeric monoclonal antibody that specifically binds  $\alpha(5)\beta(1)$  integrin: a phase I, pharmacokinetic, and biological correlative study. *Clin Cancer Res*. 2008;14(23):7924–9.
130. Cranmer LD, Bedikian AY, Ribas A, O'Day S, Forero-Torres A, Yazji S, Kirkwood JM. Phase II study of volociximab (M200), an  $\alpha 5 \beta 1$  anti-integrin antibody in metastatic melanoma. *J Clin Oncol*. 2006;24(18):455s.
131. Besse B, Tsao LC, Chao DT, Fang Y, Soria JC, Almokadem S, Belani CP. Phase Ib safety and pharmacokinetic study of volociximab, an anti- $\alpha 5 \beta 1$  integrin antibody, in combination with carboplatin and paclitaxel in advanced non-small-cell lung cancer. *Ann Oncol*. 2013;24(1):90–6.
132. Bell-McGuinn KM, Matthews CM, Ho SN, Barve M, Gilbert L, Penson RT, Lengyel E, Palaparthi R, Gilder K, Vassos A, et al. A phase II, single-arm study of the anti- $\alpha 5 \beta 1$  integrin antibody volociximab as monotherapy in patients with platinum-resistant advanced epithelial ovarian or primary peritoneal cancer. *Gynecol Oncol*. 2011;121(2):273–9.
133. Wang WQ, Wang FH, Lu FQ, Xu S, Hu WT, Huang JN, Gu Q, Sun XD. The antiangiogenic effects of integrin  $\alpha 5 \beta 1$  inhibitor (ATN-161) in vitro and in vivo. *Invest Ophth Vis Sci*. 2011;52(10):7213–20.
134. Cianfrocca ME, Kimmel KA, Gallo J, Cardoso T, Brown MM, Hudes G, Lewis N, Weiner L, Lam GN, Brown SC, et al. Phase 1 trial of the antiangiogenic peptide ATN-161 (ac-PHSCN-NH2), a beta integrin antagonist, in patients with solid tumours. *Brit J Cancer*. 2006;94(11):1621–6.
135. Zhang N, Xia YF, Zou Y, Yang WJ, Zhang J, Zhong ZY, Meng FH. ATN-161 peptide functionalized reversibly cross-linked Polymersomes mediate targeted doxorubicin delivery into melanoma-bearing C57BL/6 mice. *Mol Pharm*. 2017;14(8):2538–47.
136. Stoeltzing O, Liu W, Reinmuth N, Fan F, Livant DL, Mazar AP, Ellis LM. Reduction of colon cancer growth by a novel antiangiogenic agent that targets the integrin  $\alpha 5 \beta 1$ . *Clin Cancer Res*. 2001;7(11):3656s.
137. Mateo J, Berlin J, de Bono JS, Cohen RB, Keedy V, Mugundu G, Zhang LL, Abbattista A, Davis C, Stampino CG, et al. A first-in-human study of the anti- $\alpha 5 \beta 1$  integrin monoclonal antibody PF-04605412 administered intravenously to patients with advanced solid tumors. *Cancer Chemoth Pharm*. 2014;74(5):1039–46.
138. Berston ED, Ramos DM, Kramer RH. Metastatic melanoma-cells interact with the reticular fibers of the lymph-node. *Melanoma Res*. 1994;4(2):115–25.
139. Mullen JT, Vartanian TK, Atkins MB. Melanoma complicating treatment with natalizumab for multiple sclerosis. *New Engl J Med*. 2008;358(6):647–8.
140. Sabol RA, Noxon V, Sartor O, Berger JR, Qureshi Z, Raisch DW, Norris LB, Yarnold PR, Georgantopoulos P, Hrushesky WJ, et al. Melanoma complicating treatment with natalizumab for multiple sclerosis: a report from the southern network on adverse reactions (SONAR). *Cancer Med-U.S*. 2017;6(7):1541–51.
141. Hsieh YT, Gang EJ, Shishido SN, Kim HN, Pham J, Khazal S, Osborne A, Esguerra ZA, Kwok E, Jang J, et al. OPEN effects of the small-molecule inhibitor of integrin  $\alpha 4$ , TBC3486, on pre-B-ALL cells. *Leukemia*. 2014;28(10):2101–4.
142. Hsieh YT, Gang EJ, Bonig H, Biediger RJ, Vanderslice P, Kim YM. The small molecule inhibitor of VLA4 TBC3486 sensitizes resistant ALL to chemotherapy. *Blood*. 2012;120(21):1500.
143. Lee J, Hong JY, Nam TG, Peters EC, Orth AP, Geierstanger BH, Goldfinger LE, Ginsberg MH, Cho CY, Schultz PG. A small molecule inhibitor of  $\alpha 4$  integrin-dependent cell migration. *Bioorgan Med Chem*. 2009;17(3):977–80.
144. Lu TN, Ganganna B, Pham TT, Vo AV, Lu TP, Nguyen HGT, Nguyen MNT, Huynh PN, Truong NT, Lee J. Antitumor effect of the integrin  $\alpha 4$  signaling inhibitor JK273 in non-small cell lung cancer NCI-H460 cells. *Biochem Bioph Res Co*. 2017;491(2):355–60.
145. Park CC, Zhang HJ, Yao ES, Park CJ, Bissell MJ.  $\beta(1)$  integrin inhibition dramatically enhances radiotherapy efficacy in human breast cancer xenografts. *Cancer Res*. 2008;68(11):4398–405.
146. Nissinen L, Koivunen J, Kapyla J, Salmela M, Nieminen J, Jokinen J, Sipilä K, Pihlavisto M, Pentikainen OT, Marjamäki A, et al. Novel  $\alpha 2 \beta 1$  integrin inhibitors reveal that integrin binding to collagen under shear stress conditions does not require receptor Preactivation. *J Biol Chem*. 2012;287(53):44694–702.
147. Folkman J. Opinion - angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov*. 2007;6(4):273–86.

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