

**FUNCTION AND ANTAGONISM OF  $\beta_3$  INTEGRINS IN THE DEVELOPMENT OF CANCER  
THERAPY**

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## ABSTRACT

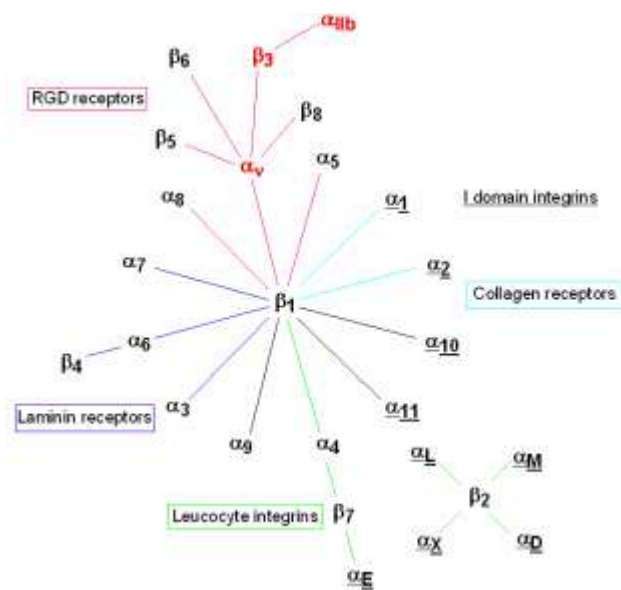
The integrin family of cell surface receptors integrate cell-extracellular matrix interactions with the cell cytoskeleton and signalling across the cell membrane, resulting in an important role in cell adhesion, mobility and migration, proliferation, and survival. Changes in the number and identity of integrin receptors are common in cancer cells resulting in alteration of the ability of malignant cells to interact with the extracellular matrix, and promoting migration as well as facilitating survival outside the tumour normal environment.  $\beta_3$  integrins are potentially involved in every step of the metastatic process and expression of both  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  is correlated with metastatic ability of tumour cells. The recognition of the RGD binding motif common to the disintegrins and natural integrin ligands such as fibrinogen allowed the development of small molecule  $\beta_3$  integrin antagonists, progressing from linear peptides containing the RGD sequence to cyclic peptides with well-defined conformation, and hence to small molecule peptidomimetics with improved pharmacological properties. In this review, we summarize the role of the  $\beta_3$ -subfamily of integrins when expressed in normal and tumour tissue, the development of small-molecule antagonists of  $\beta_3$  integrins and their potential anti-cancer applications.

Keywords:  $\beta_3$  integrin, cancer, metastasis, RGD peptidomimetic

Abbreviations: ADP adenosine diphosphate; BSP bone sialoprotein; ECM extracellular matrix; ERK extracellular signal-regulated kinase; FGF fibroblast growth factor; mAb monoclonal antibody; MAPK mitogen-activated protein kinase; MEK MAPK/ERK kinase; MIA microtubule interfering agent; MMP matrix metalloproteinase; SAR structure activity relationship; SCC squamous cell carcinoma; SCID severe combined immunodeficiency; SDF-1 stromal cell-derived factor-1; TCIPA tumour cell-induced platelet aggregation; TGF transforming growth factor; VEGF vascular endothelial growth factor

## INTRODUCTION: INTEGRIN STRUCTURE

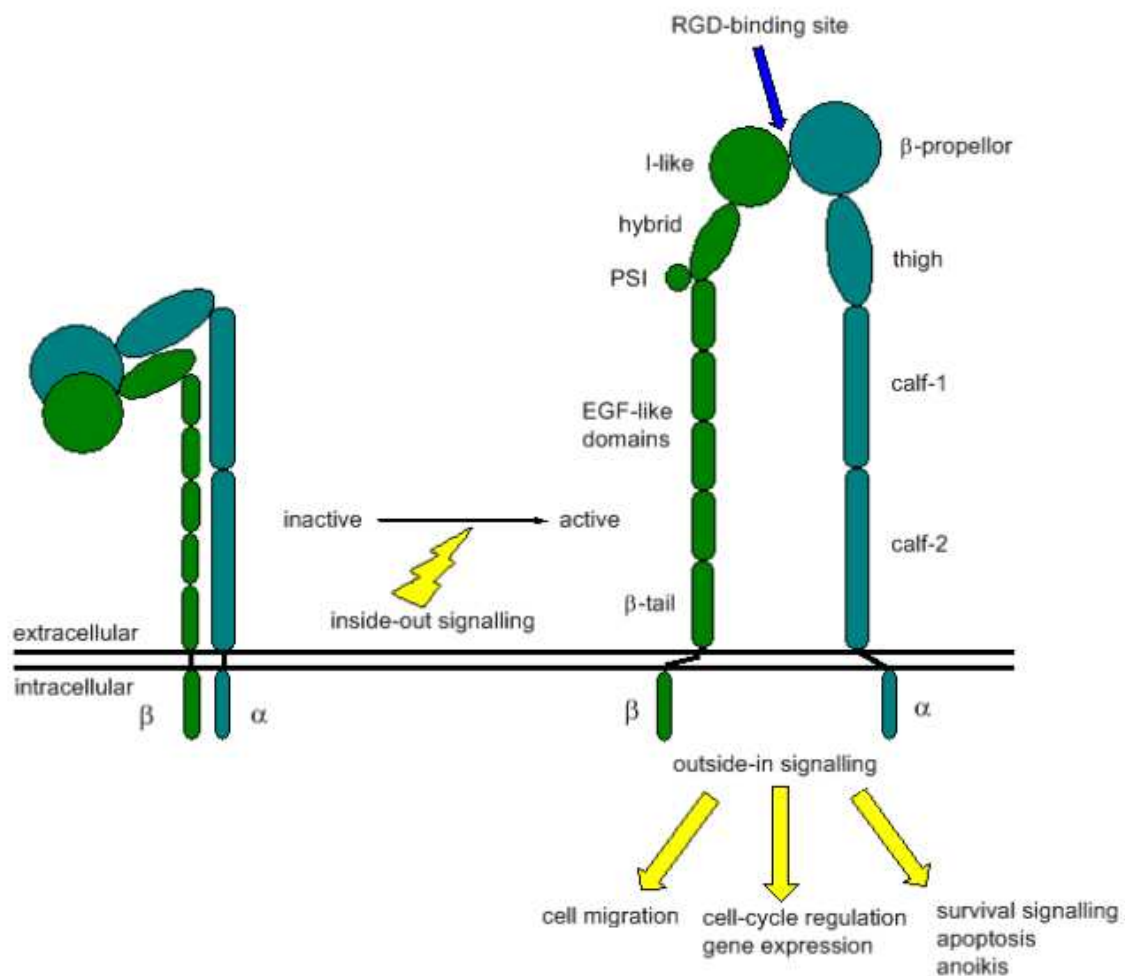
Integrins are non-covalent heterodimers consisting of an  $\alpha$  and a  $\beta$  glycoprotein subunit.[1,2] Currently, there are known 19  $\alpha$  and 8  $\beta$  subunits which combine to make 24 human integrins.[3,4] These can be classified into subfamilies based on subunit identity and ligands bound (Figure 1).



**Figure 1 Association of integrin subunits**

The two subunits consist of a short cytoplasmic tail, a single transmembrane domain, and a multi-domain extracellular portion. The N-terminus of the extracellular portion forms a globular headpiece which interacts with extracellular ligands at the subunit interface. Some  $\alpha$  subunits contain an inserted I-domain, which is responsible for ligand binding if present. Other subunits such as  $\alpha_{Ib}$  and  $\alpha_v$  do not contain the I-domain, here ligand binding occurs on the  $\beta$ -propeller headpiece of the  $\alpha$ -subunit and the I-like domain in the  $\beta$ -subunit, and is mediated by interactions between the ligand, binding site residues and one or more divalent cations bound in the integrin subunits.[5,6] The structural details of the integrin subunits are described in detail in several recent reviews.[4,7,8]  $\beta_3$  integrins are the focus of therapeutic

intervention and as such, crystal structures of the extracellular domains have been determined when unligated[9] ( $\alpha_v\beta_3$ ) and in the presence of synthetic antagonists ( $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$ ).[10,11]



**Figure 2 Schematic structure of a  $\beta_3$  integrin**

Integrins are normally expressed on the cell surface in an inactive curled conformation. Activation of the integrin can result from binding certain ligands, or by inside-out signalling, where activation of a receptor elsewhere in the cell triggers intracellular signalling resulting in a protein interacting with the cytoplasmic tail of the integrin.[12] On activation, separation of the two subunits is propagated from the intracellular to the extracellular part of the integrin, resulting in the extracellular portion extending above the cell surface in a conformation with high affinity for natural ligands (Figure 2).[13] Ligands binding to integrin extracellular domains initiate outside-in signalling; integrins participate in a large number of signalling

pathways, controlling cell adhesion and migration, protein synthesis and cell survival. Integrins have no enzyme activity and intracellular signalling is mediated by association of the cytoplasmic tail of the  $\beta$  subunit with tyrosine kinases[14] and other focal adhesion proteins. Activated integrins can also cluster on the cell surface in a process known as avidity modulation, a process that further modifies cell adhesion and signalling.

### FUNCTION OF $\beta_3$ INTEGRINS IN NORMAL TISSUE

Receptor	Recognises	Ligands	Therapeutic indications
$\alpha_{IIb}\beta_3$ (GPIIb/IIIa)	RGD, RYD, KGD, (HHLGGA)KQAGDV,	fibrinogen, fibronectin, vitronectin, Von Willebrand factor, (denatured) collagen, plasminogen, thrombospondin, prothrombin, decorsin, <i>Borrelia burgdorferi</i> ,	Thrombosis
$\alpha_v\beta_3$	RGD, RYD	fibrinogen, fibronectin, vitronectin, Von Willebrand factor, (denatured) collagen, plasminogen, thrombospondin, prothrombin, MMP-2, laminin, bone sialoprotein, osteopontin, CYR61, tenascin-C, fibrin, <i>Candida albicans</i> , HIV Tat-1, various viruses	Neovascularisation Restenosis Osteoporosis

**Table 1 Ligands recognised by  $\beta_3$  integrins**

There are currently two known  $\beta_3$  integrins;  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$ . [15]  $\alpha_{IIb}\beta_3$  was initially thought to be found only on the surface of platelets (and other megakaryocytic cells). Around 80,000 copies of the receptor are found on each platelet, [16] and more can be mobilised to the surface from storage inside the cell. Platelets can be activated by a number of ligands or signalling molecules, all of which lead to the integrin being activated to its high affinity form which binds fibrinogen. Since fibrinogen contains six potential binding sites for  $\alpha_{IIb}\beta_3$ , this results in crosslinking between platelets and is the final, common step in thrombus

formation.[17,18] Thus  $\alpha_{IIb}\beta_3$  antagonism is of interest in the development of broad spectrum anti-thrombotic therapy,[19] and defects in  $\alpha_{IIb}\beta_3$  structure or expression give rise to a spectrum of bleeding disorders known as Glanzmann's thrombaesthesia.[20]

$\alpha_v\beta_3$  is an important vitronectin receptor[21] which expressed on the surface of platelets, and active endothelial cells such as the ductal epithelium of parotid glands, colonic and rectal epithelium, glomeruli, Bowman's capsules and proximal and distal tubules of kidneys, endometrial epithelium and placenta.[22]  $\alpha_v\beta_3$  is important in the early stages of vascular angiogenesis[23,24] and in osteoclast-mediated bone resorption which is initiated by  $\alpha_v\beta_3$ -mediated attachment of osteoclasts to the bone surface.[25,26]  $\alpha_v\beta_3$  antagonists are being developed as anti-angiogenic and anti-osteoporotic drugs,[27-29] and  $\alpha_v\beta_3$  involvement in the functions of endothelial cells, osteoclasts and tumour cells suggests these will prove useful in therapy against metastasis to bone.[26] The therapeutic opportunity for  $\beta_3$  antagonism is significant and the cancer-related studies to date and future opportunities are discussed below.

### **$\beta_3$ INTEGRINS IN CANCER**

Changes in the number and identity of integrin receptors are common in cancer cells,[30] resulting in alteration of the ability of malignant cells to interact with the extracellular matrix (ECM), and promoting migration as well as facilitating survival outside the tumour normal environment.

#### **$\alpha_{IIb}\beta_3$ ectopic expression in tumour cells**

Investigation of cell surface receptors mediating adhesion and interaction with platelets in selected cell lines led to the discovery of receptors originally described as "immunologically related GPIIb/IIIa" since it was thought that  $\alpha_{IIb}\beta_3$  (GPIIb/IIIa) could only be expressed in

platelets.[31-34] Subsequent analysis of integrin expression showed that  $\alpha_{\text{IIb}}$  and  $\beta_3$  mRNA is present in a wide range of tumour cell lines,[35] and human melanoma clinical samples.[36-38] Functional  $\alpha_{\text{IIb}}\beta_3$  is found on the surface of tumour cells, localised to regions that interact with the ECM.[39-42] There are several ways in which such ectopic  $\alpha_{\text{IIb}}\beta_3$  activity is manifest in tumour cells.  $\alpha_{\text{IIb}}\beta_3$  can be expressed in its active conformation on tumour cells and adhesion is regulated by translocation of the active integrin from the intracellular pool to the cell surface, in contrast to the conversion of the integrin from the inactive to active conformation which regulates integrin affinity in normal platelets.[41-44]

Tumour cells expressing  $\alpha_{\text{IIb}}\beta_3$  display increased survival and growth *in vivo*,[36] and increased metastatic potential.[45] They are also able to promote platelet activation and aggregation.[46] Interaction between platelets and tumour cells results in upregulation of both platelet and tumour cell  $\alpha_{\text{IIb}}\beta_3$ . [47]

Abnormal truncated forms of integrin subunits are present in some tumour cell lines.[35,48] Truncated  $\alpha_{\text{IIb}}$  has been found in leukaemia, prostate adenocarcinoma and melanoma cells and is secreted into the ECM.[49] Truncation may be associated with alteration in the affinity of the integrin for its ligands,[50] changes in cell adhesion[48] or alterations in signalling pathways[51] Truncated  $\beta_3$  integrin lacking the cytoplasmic and transmembrane domain has been found in human prostate and breast carcinomas and melanoma cells, where it inhibits adhesion to the  $\beta_3$  ligands vitronectin and fibronectin, and may provide a new mechanism for cells to detach from the ECM.[52] Non- $\alpha_{\text{IIb}}\beta_3$ -expressing tumour cells may gain  $\alpha_{\text{IIb}}\beta_3$  (and MMP) expression by interaction with platelet-derived microvesicles, resulting in increased ability to adhere to fibrinogen, an event associated with increased *in vivo* metastasis to lung and bone.[53,54]

## $\alpha_v\beta_3$ expression

$\alpha_v\beta_3$  is expressed on the vasculature of a range of human tumours,[55] and vascular  $\alpha_v\beta_3$  is a prognostic indicator of shorter relapse free survival and overall survival.[56] More details regarding the tumour expression and consequences of  $\alpha_v\beta_3$  are known and investigations in selected cancers are described below.

### ***Breast cancer***

The expression of  $\alpha_v\beta_3$  in breast cancer has been examined *in vivo* using a RGD-based PET tracer,[57] which showed a generally high but heterogeneous integrin distribution on the endothelium of new blood vessels and the cells of primary and metastatic tumours. Breast cancer cell expression of the  $\beta_3$  subunit is associated with increased invasiveness *in vivo*,[58] and cells overexpressing  $\alpha_v\beta_3$  show increased invasion and adhesion *in vitro*, and increased metastasis to bone and bone destruction *in vivo*. [59,60] Overexpression of  $\alpha_v\beta_3$  also increases tumour growth and invasion in response to osteopontin.[61]

The presence of the active conformation of  $\alpha_v\beta_3$  on breast tumour cells is associated with increased cell migration and metastatic ability.[62] Activated  $\alpha_v\beta_3$  is found on both cell lines and primary metastatic cells obtained from stage IV cancer patients, and these cells produce activated MMP-9 which mediates enhanced migration towards fibrinogen.[63]

$\alpha_v\beta_3$  expression is involved in the regulation of breast cancer cell response to chemotherapy and serves as a marker to chemosensitivity.[64]  $\alpha_v\beta_3$  is significantly upregulated in breast cancer cells treated with microtubule interfering agents (MIAs), particularly in cell lines with low heregulin (an ErbB/HER agonist) and not in cell lines that are resistant to MIAs. However, forced expression of the  $\beta_3$  subunit increased cell resistance to paclitaxel.

TGF- $\beta$ 1 derived from osteoclasts increased the expression of  $\beta_3$  integrin on breast cancer cells.[65]  $\alpha_v\beta_3$  on mammary epithelial cells has been shown to form a complex with the



TGF- $\beta$  receptor T $\beta$ R-II, increasing its tyrosine phosphorylation and leading to enhanced MAPK activation, induction of the epithelial mesenchymal transition, and increased cell invasion.[66] Interference with this pathway by means of  $\beta_3$  integrin antagonists may provide a novel method of preventing breast cancer progression.

### ***Glioblastoma***

$\alpha_v\beta_3$  is expressed in glioblastoma, where increased levels of vitronectin and  $\alpha_v\beta_3$  are observed at the invasive margins of the tumour, and are associated with enhanced cell motility and apoptosis-resistance.[67,68] A recent study has shown high levels of integrin expression on tumour cells, rather than the associated vasculature, and a strong correlation between protein expression and tumour grade.[69]  $\alpha_v\beta_3$  antagonists such as Cilengitide have been successful in suppressing glioblastoma growth and angiogenesis in mouse models[70,71] and are in clinical trials against human glioblastoma.[72]

### ***Other tumours***

$\alpha_v\beta_3$  is expressed in ovarian carcinoma, mediating binding to fibronectin[73] and vitronectin.[74] In prostate carcinoma cells, there is an association between integrins and processes of attachment and migration involving laminin, vitronectin and osteopontin which may result in preferential metastasis to bone.[75]  $\alpha_v\beta_3$  is upregulated in cervical cancer,[76] and expression of the  $\beta_3$  subunit is associated with shorter local progression free survival, distant metastasis free survival and cause-specific survival in patients with cervical cancer that had been treated by radiotherapy.[77]

$\beta_3$  integrin mRNA is a prognostic marker in gastric carcinoma. The mRNA is found at higher levels in tumour tissue than in non-tumour gastric mucosa and higher levels of mRNA are associated with invasive and metastatic tumours of higher clinical grade.[78]  $\beta_3$  mRNA expression was correlated with VEGF expression and it was suggested that the two molecules act synergistically to increase angiogenesis. An association of VEGF and  $\alpha_v\beta_3$  integrin has

also been seen in prostate and breast cancer metastasis, where integrin clustering/activation increases VEGF production, and VEGF signalling activates integrins, contributing to metastatic behaviour.[79,80] The survival of patients with  $\beta_3$  positive gastric tumours was significantly reduced compared to  $\beta_3$  negative cases; the  $\alpha$ -subunit associated with  $\beta_3$  was not identified in this study.

High levels of  $\alpha_v\beta_3$  expression in pancreatic adenocarcinoma are associated with increased activation of MMP-2 and metastasis of the tumour to the lymph nodes.[81] Bone sialoprotein (BSP) is able to bind both MMP-2 and  $\alpha_v\beta_3$  and increases cancer cell invasiveness through a RGD-dependent pathway. Co-localisation of BSP, MMP-2 and  $\alpha_v\beta_3$  has been observed in cancer cell lines and clinical thyroid carcinoma samples.[82]

### ***Melanoma***

The importance of both  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  is probably most extensively studied in melanoma. Presence of the  $\beta_3$  subunit is characteristic of melanoma, and is strongly associated with disease progression and poor prognosis.[83-89]  $\alpha_v\beta_3$  is widely expressed in melanoma cell lines and clinical samples,[22,90-92] where its presence promotes tumour growth, cell survival and invasiveness.[93,94] Increased metastatic potential has been correlated with the ability of  $\alpha_v\beta_3$ -expressing cells to adhere to lymph node vitronectin,[95] and with co-expression of increased levels of the urokinase plasminogen activator receptor.[96]  $\alpha_{IIb}\beta_3$  appears later in melanoma progression and the level of expression is correlated with tumour depth[36] and metastasis.[37,38] Expression of  $\alpha_{IIb}\beta_3$  does not alter cell growth *in vitro* but increases tumour growth *in vivo* by decreasing apoptosis. Expression of  $\alpha_{IIb}\beta_3$  can suppress the function of  $\alpha_v\beta_3$ ,[36] but cells expressing both  $\beta_3$  integrins grow more rapidly *in vivo* and display increased tumour angiogenesis.[97] Although the two  $\beta_3$  integrins may complement one another in melanoma, they are not interchangeable. A separate study showed

$\alpha_v\beta_3$  expression was required for effective metastasis formation, chemotactic migration and protease secretion by melanoma cell lines, and expression of only  $\alpha_{IIb}\beta_3$  did not result in metastasis.[98]  $\alpha_v\beta_3$  antagonism by an  $\alpha_v$  monoclonal antibody or the antagonist Cilengitide (dual  $\alpha_v\beta_3/\alpha_v\beta_5$ ) is effective in inhibiting the growth of subcutaneous human melanoma tumours in mice and can reduce the size of a pre-existing tumour.[99]

$\alpha_v\beta_3$  and MMP-2 are co-localised on the surface of  $\alpha_v\beta_3$ -expressing melanomas.[91] Co-expression of  $\alpha_v\beta_3$  and MMP-2 is higher in advanced melanomas compared to early radial growth phase melanomas which suggests that localisation of MMP-2 with  $\alpha_v\beta_3$  on the cell surface may facilitate invasion and metastasis.[91]

### **$\beta_3$ INTEGRINS AND METASTASIS**

Metastasis is often the final outcome of the tumour cell migration and invasion process. Integrins are potentially involved in every step of the metastatic process,[100,101] and  $\alpha_{IIb}\beta_3$ [45,102,103] and  $\alpha_v\beta_3$ [104,105] expression is correlated with metastatic ability of tumour cells.

$\beta_3$  integrins are of particular relevance during haematogenous metastasis, when tumour cells must invade into and survive in the blood stream, then adhere to and extravasate through blood vessel endothelium at the site of the secondary tumour. The interaction of tumour cells with platelets has a protective effect as the tumour cells are transported in the blood stream and increases adhesion and arrest (attachment of the tumour cell to the vessel wall).[106-109]

The importance of platelets to metastasis has been reviewed by Honn *et al.*[110]

Interaction of tumour cells with platelets causes platelet activation and tumour cell-induced platelet aggregation (TCIPA, recently reviewed by Jurasz *et al*[111]) which can be prevented by antibodies against  $\alpha_{IIb}\beta_3$ . [40,46,112,113] Both  $\alpha_v\beta_3$ [62,107,114] and  $\alpha_{IIb}\beta_3$ [113-115] on

the tumour cell can mediate interaction with platelet  $\alpha_{IIb}\beta_3$  and arrest in blood vessels. Tumour cell  $\alpha_v\beta_3$  is required for adhesion to the blood vessel subendothelium, especially under conditions of fast blood flow.[116]

Prostate cancer cells, especially those grown orthotopically, express  $\alpha_{IIb}\beta_3$ . [41,117] The expression of  $\alpha_{IIb}\beta_3$  localised in focal contacts on prostate cancer cells is associated with increased invasion *in vitro* and metastasis *in vivo*, which can be inhibited by anti- $\alpha_{IIb}\beta_3$  antibodies.[117] Stromal cell-derived factor-1 (SDF-1) is produced by bone marrow stroma, osteoblasts and endothelial cells and is implicated in prostate cancer metastasis. Exposure of metastatic prostate cancer cell lines to SDF-1 causes an increase in  $\beta_3$  mRNA and  $\alpha_v\beta_3$  protein expression, and activation of the  $\alpha_v\beta_3$  receptor, enhancing the tumour cell's invasive phenotype.[118] The integrin subunits are present at all stages of prostate carcinoma, but presence of the assembled  $\alpha_v\beta_3$  heterodimer is associated with cell lines of increased metastatic potential,[119] and may result in preferential metastasis to bone.[75] It has been shown that the expression of functional  $\alpha_v\beta_3$  promotes prostate cancer cell engraftment, bone deposition and remodelling, and metastatic tumour growth in a mouse model.[120] Mutant  $\alpha_v\beta_3$  locked in either an inactive or active conformation cannot regulate the dynamic cell adhesion or signalling required for tumour growth.

The activation of  $\alpha_v\beta_3$  has been shown to be increased in prostate cancer metastasis compared to primary tumour due to an autocrine loop where increased VEGF production activates integrins  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ , mediating enhanced adhesion to bone matrix and further VEGF production.[79]

The expression of  $\alpha_v\beta_3$  in the small blood vessels of primary colorectal carcinoma is correlated with metastasis to the lungs.[121,122] In an osteosarcoma cell line, increased  $\alpha_v\beta_3$  expression is correlated with metastatic potential, increased chemotaxis towards a lung

homogenate, and increased ability to migrate through lung endothelial cells suggesting that  $\alpha_v\beta_3$  may have a role in the preferential formation of lung metastases.[123] Expression of  $\alpha_v\beta_3$  in pancreatic adenocarcinoma is associated with metastasis to the lymph nodes. [81]

The involvement of  $\alpha_v\beta_3$  in tumour spread is not limited to solid tumours, active multiple myeloma is often accompanied by lytic bone disease, and is associated with increased bone marrow neovascularisation, and the ability of plasma cells to promote angiogenesis and secrete MMP-2 *in vitro*,[124] all traits potentially associated with  $\alpha_v\beta_3$  expression. Cell lines from multiple myeloma, Burkitt's lymphoma and T-cell lymphoblastic leukaemia, and fresh plasma cells from multiple myeloma patients utilise  $\alpha_v\beta_3$  to adhere to vitronectin and fibronectin surfaces and form focal adhesion complexes involving  $\beta_3$  and associated signalling proteins and kinases.[125,126] Interaction with ECM components *via*  $\alpha_v\beta_3$  promotes cell proliferation and MMP secretion.

### **$\beta_3$ INTEGRIN POLYMORPHISM AND CANCER RISK**

Approximately 15% of the population possess a single nucleotide polymorphism PI<sup>A2</sup> Leu33Pro in the extracellular portion of the  $\beta_3$  subunit, which affects the structure of the region involved in associating with the  $\alpha$  subunit. This polymorphism is associated with increased platelet adhesiveness and outside-in signalling by  $\alpha_{IIb}\beta_3$ [127]. This may explain the diminished response to antithrombosis treatment with  $\alpha_{IIb}\beta_3$  antagonists which may be responsible for many of the adverse effects observed in clinical trials with oral antagonists.[128,129] Homozygosity for Leu33Pro may be associated with increased cancer risk,[130] particularly melanoma and ovarian[131,132] and breast carcinomas. This effect may result from increased activity of  $\alpha_v\beta_3$  mediating angiogenesis[130] or increased adhesion to the ECM facilitating tumour growth[131] and metastasis.[133] Patients with renal cell

carcinoma with the PI<sup>A2</sup> allele are twice as likely to have metastatic disease at the time of diagnosis.[134] In the case of breast cancer, an initial study found that individuals homozygous for wild type  $\beta_3$  had an increased risk of breast cancer, axillary node metastasis and larger tumours.[135] However, later studies showed that homozygosity for the 176T>C polymorphism increased the risk of the development of breast cancer in the <45 age group, and was associated with lymph node metastasis,[136] or did not increase the risk of developing breast cancer but did double the risk of metastasis, possibly due to modulation of integrin-mediated cell signalling.[137] With contradictory preliminary studies, and 203 known polymorphisms of  $\beta_3$ , larger studies are required to fully investigate the role of polymorphic variants in  $\beta_3$  in cancer development.

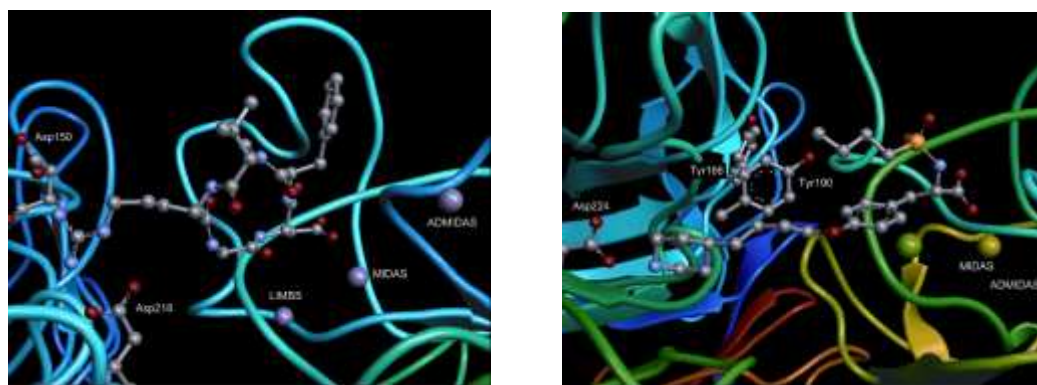
Experimentally, introduction of a mutation to the highly conserved NPXY motif in the  $\beta_3$  cytoplasmic tail reduces the ability of cells to migrate on vitronectin and causes tumour cells to act like  $\alpha_v\beta_3$  negative cells, forming a large primary tumour with no metastasis.[138] A study of 383 clinical tumour samples has suggested that mutations in the  $\beta_3$  cytoplasmic domain are unlikely to contribute to the development of human cancers.[139]

### **MEDICINAL CHEMISTRY OF $\beta_3$ INTEGRIN ANTAGONISM**

Integrins have been shown to be potential targets for drug development for therapeutic applications including anti-thrombotic, anti-osteoporotic and angiogenic,[140] and anti-inflammatory. Biological methods of targeting integrins have been recently reviewed by Eble and Haier[100] and include the development of monoclonal antibodies and peptide conjugates, and the identification and investigation of anti-tumour properties in the disintegrins (peptides from snake venoms).[141]

The recognition of the RGD binding motif common to the disintegrins and natural integrin ligands such as fibrinogen allowed the development of small molecule  $\beta_3$  integrin antagonists,

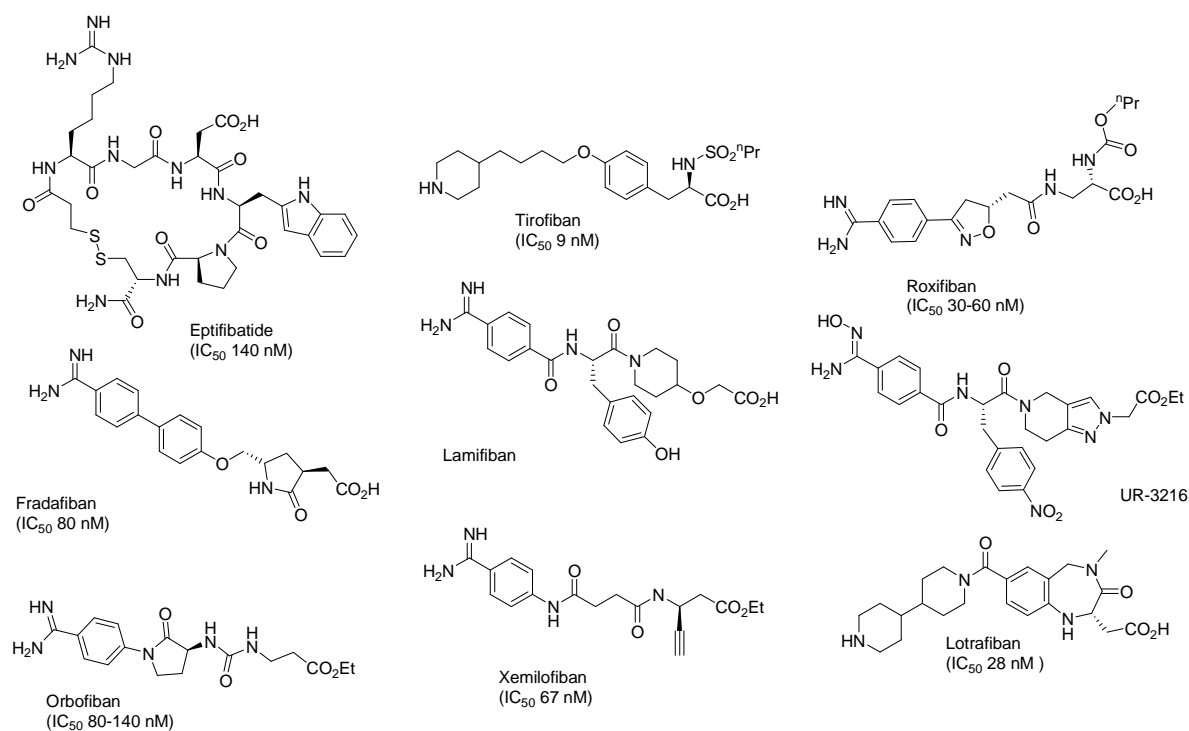
progressing from linear peptides containing the RGD sequence to cyclic peptides with well-defined conformation, and hence to small molecule peptidomimetics with improved pharmacological properties.[142-144] RGD peptides and small molecules have become a popular area for the development of new drugs and drug delivery agents.[145,146] Antagonists were first designed to mimic ligand structure without knowledge of the receptor; with the development of computer models[147-151] and availability of crystal structures of  $\beta_3$  binding sites, rational structure-based design is now possible.[152] Large numbers of both  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  antagonists have been designed by industrial and academic groups.



**Figure 3 X-ray structures of RGD-mimetics in  $\beta_3$  integrin binding sites. Left. Cilengitide/ $\alpha_v\beta_3$ / $Mn^{2+}$ ; Right. Tirofiban/ $\alpha_{IIb}\beta_3$ / $Mg^{2+}$ ,  $Ca^{2+}$ . The integrin is represented by a ribbon diagram, interacting residues and metal ions are shown.**

$\alpha_{IIb}\beta_3$  is less sensitive to the RGD conformation than  $\alpha_v\beta_3$  and can accept a flexible binding motif evidenced by high affinity of compounds based on cupped, turn-extended-turn, and Gly-Asp  $\beta$ -turn conformations.[153,154] The preferred overall ligand length for  $\alpha_v\beta_3$  binding is shorter,[151,154] and peptides selective for  $\alpha_v\beta_3$  vs.  $\alpha_{IIb}\beta_3$  have a turn in Gly region, whereas  $\alpha_{IIb}\beta_3$  antagonists prefer an extended conformation of Gly.[155] The nature of the Arg sidechain mimetic is also important in determining selectivity for  $\alpha_v\beta_3$  vs.  $\alpha_{IIb}\beta_3$ ;  $\alpha_{IIb}\beta_3$ -selective agents often contain an amidinobenzyl group[142] or amine such as piperidine with

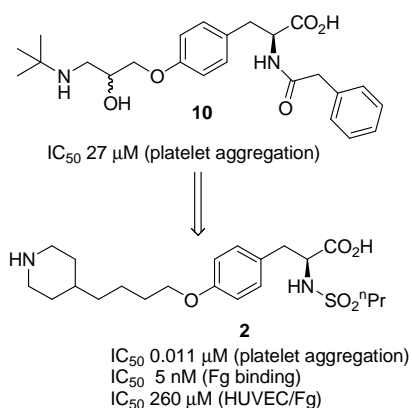
one point/end-on binding,  $\alpha_v\beta_3$ -selective compounds require bidentate/side-on binding to the receptor.[156]  $\alpha_v\beta_3$  antagonists may be designed from first principles or by SAR-based optimisation to reverse the selectivity of an existing  $\alpha_{IIb}\beta_3$  antagonist (eg. see figure 7).[156]



**Figure 4 Selected  $\alpha_{IIb}\beta_3$  antagonists**

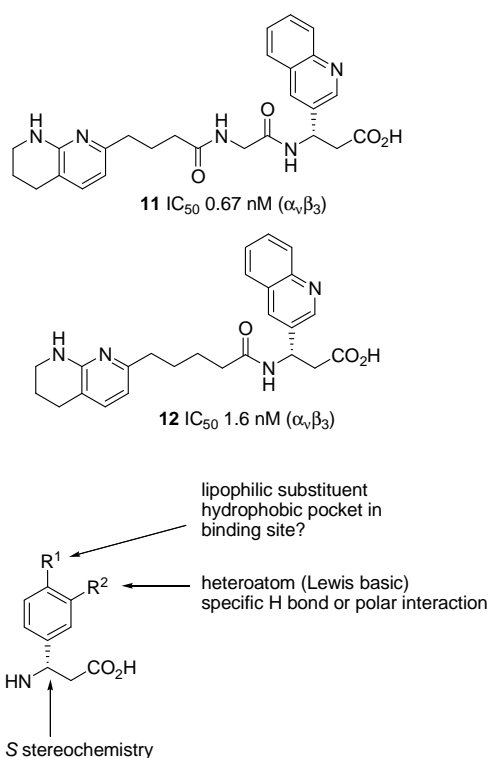
Three parenteral  $\alpha_{IIb}\beta_3$  antagonists are in clinical use for treatment of unstable angina and percutaneous coronary intervention. One of these is the humanised monoclonal antibody Abciximab ( $\alpha_{IIb}\beta_3$   $IC_{50}$  6.2 nM  $\alpha_v\beta_3$   $IC_{50}$  9.8 nM  $\alpha_M\beta_2$   $IC_{50}$  160 nM[157]). Eptifibatide (1) is a cyclic peptide ( $\alpha_{IIb}\beta_3$   $IC_{50}$  140 nM[158]) designed on the KGD motif of the  $\alpha_{IIb}\beta_3$ -selective disintegrin barbourin.





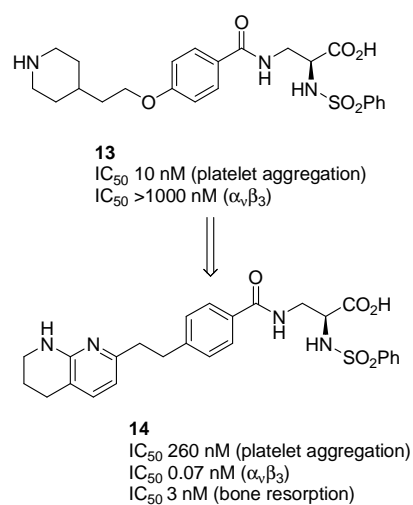
**Figure 5 Optimisation of Tirofiban structure**

Tirofiban (**2**) ( $\alpha_{IIb}\beta_3$   $IC_{50}$  9 nmol[158] HUVEC  $IC_{50}$  >100  $\mu$ mol)[159-161] was the first small molecule integrin antagonist to be brought to market. It evolved from the tyrosine-based lead compound **10** discovered by searching the Merck sample collection for non-peptide structures with amino and carboxylate groups separated by approximately 10-20 Å, mimicking the distance between the arginine and aspartate termini of the RGD unit. The secondary hydroxyl group in **10** was not required for  $\alpha_{IIb}\beta_3$  binding and was removed to simplify the structure; systematic variation of the sidechain length found that 7 carbon atoms was the optimum, and the conformational restriction of a piperidine ring further increased potency. A secondary amine  $\alpha$  to the carboxylate terminus was required for activity and derivatisation with an alkyl sulfonamide gave a dramatic increase in potency, postulated to be due to a novel non-covalent “exo-site” interaction which has since been demonstrated to be an interaction with a tyrosine sidechain in the  $\beta$ -subunit.



**Figure 6 Further RGD mimetics developed by Merck**

Merck also developed a range of linear RGD analogues containing a 5,6,7,8-tetrahydro[1,8]naphthyridine Arg mimetic (Figure 6).[162,163] As part of this work, the SAR of  $\beta$ -aryl  $\beta$ -amino acids as Asp mimetics was determined, suggesting further structural details about the  $\alpha_v\beta_3$  RGD-binding site (Figure 6).[164]

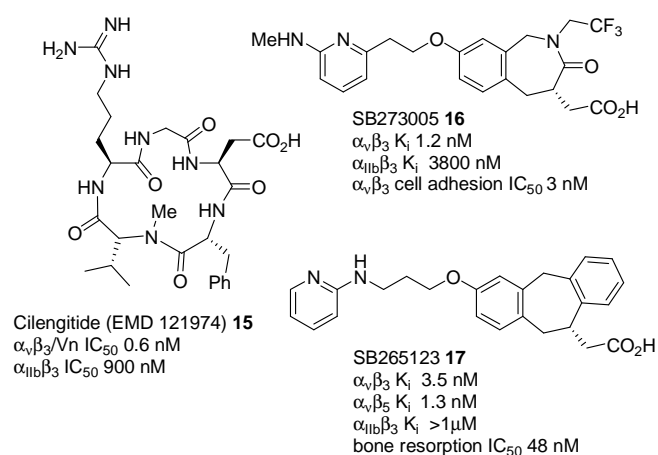


**Figure 7 Development of an  $\alpha_v\beta_3$  antagonist from an  $\alpha_{IIb}\beta_3$  selective molecule**

The tyrosine linker has proved popular in the development of  $\beta_3$  antagonists,[165,166] and similar structures appear during the optimisation of diverse molecules.[167] The tirofiban-like  $\alpha_{IIb}\beta_3$  antagonist **13** was transformed into a selective  $\alpha_v\beta_3$  antagonist **14** active *in vivo* against bone resorption, by alteration of the arginine-mimetic terminus.[156]

The success of intravenous therapy against  $\alpha_{IIb}\beta_3$  encouraged the development of orally active compounds such as orbofiban (**7**), xemilofiban (**8**) and lotrafiban (**9**) in preference to further systemic drugs such as lamifiban (**5**). Unfortunately, clinical trials of oral drugs against thrombosis showed lack of efficacy and increased risk of adverse events including sudden death[168,169] leading to the current virtual abandonment of the field.[170] A number of mechanisms have been proposed to account for the failure of oral  $\alpha_{IIb}\beta_3$  antagonists as anti-thrombotics,[171,172] the most likely appears to be that antagonists are paradoxically partial agonists at lower levels of  $\alpha_{IIb}\beta_3$  receptor occupancy.[173-175] Antagonists binding reversibly to inactive  $\alpha_{IIb}\beta_3$  activates the receptor by causing changes in conformation and receptor clustering; subsequent ligand unbinding releases activated integrin leading to the observed paradoxical increase in thrombosis.

Various strategies have been suggested to circumvent the problems encountered with  $\alpha_{IIb}\beta_3$  antagonists including the development of tighter binding antagonists such as UR-3216 (**7**),[176] and the design of agents specific for the active integrin conformation based on specific antibodies.[177,178] The drug structural features required for selectivity for the high affinity integrin conformation are not known; the selectivity of some  $\alpha_{IIb}\beta_3$  drug candidates have been measured,[179-181] but does not correlate with success in clinical trials. The development of active conformation-selective agents presents a considerable challenge but may revitalise the field of  $\alpha_{IIb}\beta_3$ -targeted therapeutics by allowing the development of compounds with improved safety and efficacy.



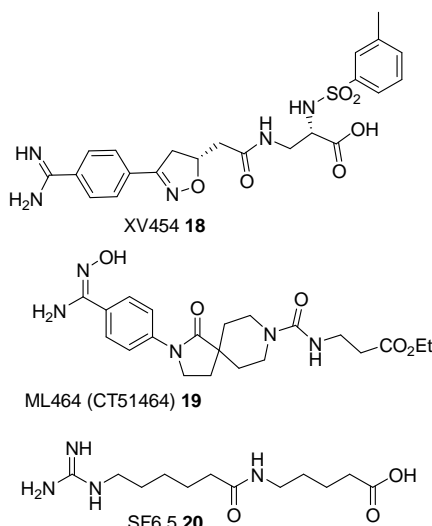
**Figure 8 Selected  $\alpha_v\beta_3$  antagonists**

$\alpha_v\beta_3$  antagonists were developed concurrently with  $\alpha_{IIb}\beta_3$  antagonists, and myriad structures are described in the primary chemical literature. Their initial indication as anti-angiogenic compounds has led to the antibodies Vitaxin, and CNTO-95 ( $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  K<sub>d</sub> ~ 200 pM) and the cyclic peptide Cilengitide (**15**) entering many trials for the treatment of advanced cancer (for a recent summary, see Hehlhans *et al*[182]). Other molecules such as **16** and **17** (designed from a similar starting point as Lotrafiban **9**) have been developed as anti-osteoporosis agents. [183]

### ANTI-CANCER PROPERTIES OF $\beta_3$ ANTAGONISTS

The potential for antagonism of  $\beta_3$  integrins in cancer therapy is evident from treatment with monoclonal antibodies to the  $\beta_3$  subunit or integrins  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  which display a wide range of anti-cancer effects including inhibition of cancer cell adhesion to ECM substrates,[32,40] inhibition of cell migration and invasion through the basement membrane,[41,184] inhibition of tyrosine kinase signalling,[185] inhibition of melanoma cell proliferation,[185] prevention of TCIPA,[45,112] inhibition of the binding of tumour cells in the vascular compartment,[62,113,186] and blocking VEGF secretion from tumour cell-activated platelets.[187] *In vivo* studies using monoclonal antibodies to  $\alpha_{IIb}\beta_3$  show marked

decreases in metastasis to the lungs[40,45,117,185,187,188] and antibodies to  $\alpha_{IIb}\beta_3$  or  $\alpha_v\beta_3$  decreased the growth of subcutaneous[99,189,190] and bone[191] tumours. Selective  $\alpha_v\beta_3$  antibodies show anti-angiogenic effects,[24,190] and induce tumour cell apoptosis by blocking cell signalling pathways.[67,94,192] The use of small RGD-containing peptides gives similar effects.[24,95,186,193-195] The case for integrin dual inhibition is indicated by studies that show antagonism of both  $\beta_3$  integrins, by the use of a non-selective antibody such as 7E3 and Abciximab, its humanised chimera, or by treatment with two selective antagonists, shows equal or enhanced efficacy in preventing tumour growth,[196,197] metastasis,[196] tumour cell interaction with platelets[187] and arrest of tumour cells in the circulatory system[198] compared to the use of a selective antagonist. mRNA knockdown is commonly identified as an important prerequisite for identifying the drug target status of specific receptors. siRNA or antisense pcDNA vector to  $\beta_3$  have been shown to decrease proliferation and migration of metastatic growth phase melanoma cells[88] and reduce tumour volume in a

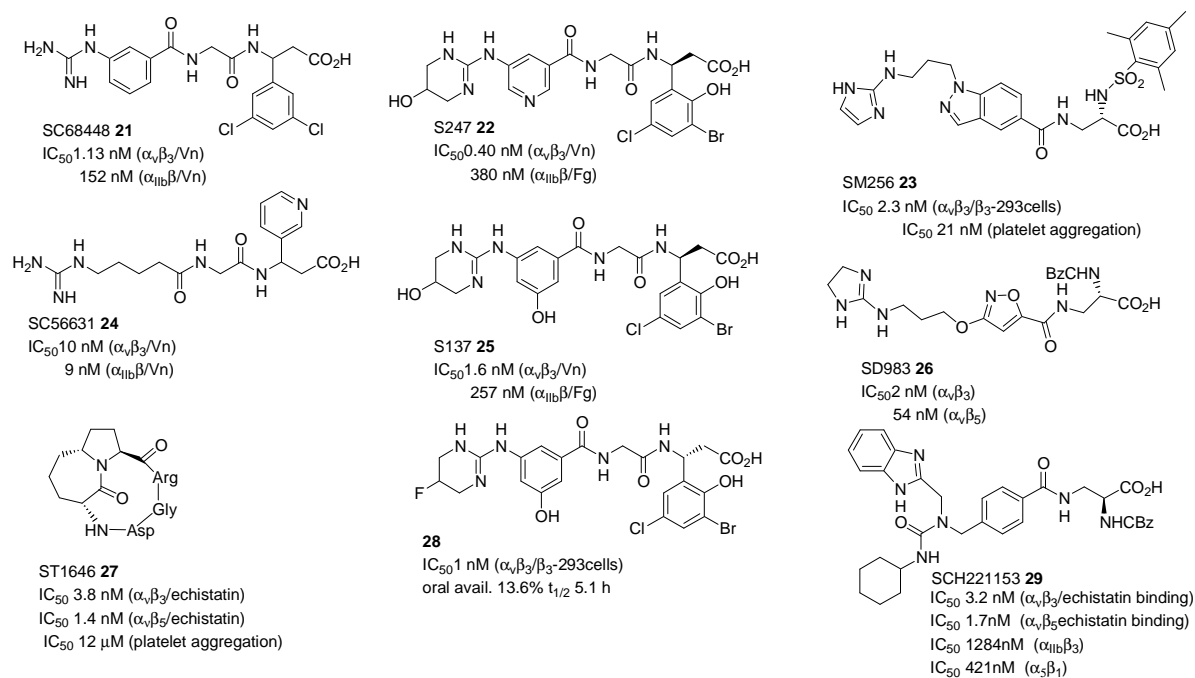


hepatocellular carcinoma model.[199]

### Figure 9 $\alpha_{IIb}\beta_3$ antagonists used in cancer studies

The  $\beta_3$  integrin status of tumour cells and the wealth of information regarding the effectiveness of peptide and small molecule antagonists of  $\alpha_{IIb}\beta_3$  developed as anti-thrombotics has encouraged their investigation as anti-cancer agents. The oral antagonist

XV454 (**18**) inhibited tumour cell induced thrombocytopenia and reduced experimental lung metastasis by over 80%. [200] The oral antagonist ML464 (**19**) decreased bone metastasis and reduced the number and size of visceral metastasis in mice after intracardiac injection of B16 melanoma cells. [201,202] The active metabolite of ML464 does not affect tumour adhesion to platelets, but prevented TCIPA. [201] The simple RGD-mimic SF6,5 (**20**) inhibited melanoma cell adhesion to fibronectin and vitronectin, inhibited formation of experimental and spontaneous metastases, and was effective in preventing the death of mice from colonization of tumor cells in the lungs. [203] Treatment with eptifibatide (**1**) decreased the amount of tumour in bones and prevented bone destruction in mice with breast cancer bone metastasis. [204] In this case, eptifibatide is thought to act by interfering with tumour cell-platelet interaction and preventing the release of lysophosphatidic acid (which promotes tumour cell growth and bone osteolysis) from platelets. [108,204] Tirofiban (**2**), another clinically approved  $\alpha_{IIb}\beta_3$  antagonist, inhibited chemotaxis of  $\alpha_{IIb}$ -expressing squamous cell carcinoma (SCC) cells by blocking interaction with the cell adhesion domain of collagen XVII, which promotes tumour cell transmigration, and thus may be useful in preventing the progression of SCC tumours. [205]



**Figure 10**  $\alpha_v\beta_3$  antagonists used in cancer studies

$\alpha_v\beta_3$  antagonists developed as anti-angiogenic agents may be expected to be anti-cancer agents by preventing tumour vasculature development. However,  $\alpha_v\beta_3$  active compounds have further anti-cancer properties independent of their effects on angiogenesis. The anti-osteoporotic PSK1404[206] ( $IC_{50}$  2 nM ( $\alpha_v\beta_3$ /kistrin), 26 nM (293cells/Vn), 480 nM ( $\alpha_{IIb}\beta_3/Fg$ ); Structure undisclosed) inhibited invasion of  $\alpha_v\beta_3$ -expressing cells *in vitro* and both short term and continuous treatment was effective in reducing bone colonisation and destruction *in vivo*. [60] Short-term treatment targets only cancer cell  $\alpha_v\beta_3$ ; continuous treatment showed greater anti-tumour effect, suggesting that targeting  $\beta_3$  integrins on multiple cell types (ie. osteoclast, endothelium as well as tumour) is more effective than targeting a single tissue. SC68448 (**21**) is a non-cytotoxic anti-angiogenic compound which inhibits the growth of  $\alpha_v\beta_3$ -expressing endothelial cells; *in vivo* treatment of mice with Leydig cell tumours showed up to 82% reduction of tumour volume and inhibited the development of hypercalcemia by preventing tumour-stimulated bone resorption. [207]

The selective  $\alpha_v\beta_3$  antagonist SM256 (**23**) [208] and the  $\alpha_v\beta_3/\beta_5$  antagonist SD983 (**28**) (prodrug ester SG545) targeted integrins on endothelial and tumour cells in a  $\alpha_v\beta_5$ -expressing

mouse human colon carcinoma xenograft model inhibiting the tumour growth by inhibiting angiogenesis and increasing apoptosis.[209] SM256 (**23**) was also effective in blocking the action of thyroid hormone as a growth factor on glioma cells, which is mediated by RGD-dependent binding to  $\alpha_v\beta_3$ . [210]

SCH221153 (**29**) was developed by screening a combinatorial RGD-mimetic library for effective  $\alpha_v\beta_3$  antagonists and modifying them to create dual  $\alpha_v\beta_3/\beta_5$  antagonists. SCH221153 inhibited endothelial cell proliferation in response to FGF2 and VEGF, and displayed up to 71% growth inhibition of intradermal and subcutaneously injected hamster melanoma tumours *in vivo*. The successful anticancer outcome of SCH221153 treatment, despite its short plasma half-life, indicates that continuous exposure of antagonists to integrins is not required to sustain inhibition of cell adhesion and blood vessel formation.[211]

A further example of the effectiveness of dual integrin inhibition is the selective  $\alpha_v\beta_3/\beta_5$  antagonism by the semi-peptide ST1646 (**27**) which prevented FGF2 and VEGF-induced angiogenesis in a chorioallantoic membrane assay and tumour-cell induced angiogenesis *in vivo*. Continuous infusion of ST1646 inhibited the growth of ovarian carcinoma xenografts.[212]

The S247 series of compounds have proved popular tools for cancer biologists. The  $\alpha_v$  antagonist S247 (**22**) (designed and described as an  $\alpha_v\beta_3$  antagonist but possibly a pan- $\alpha_v$  antagonist; [213]  $IC_{50}$   $\alpha_v\beta_3$  0.4 nM, all other  $\alpha_v$  integrins ~1 nM) decreased experimental liver metastasis after injection of colon cancer cells into the spleens of mice, but did not affect formation or growth of the primary tumour. Treatment with S247 decreased angiogenesis and increased tumour cell apoptosis *in vivo*, and decreased cell growth, adhesion and migration and promotes apoptotic detachment (anoikis) of anchorage dependent cells *in vitro*. [214] The importance of  $\alpha_v\beta_3$  in the early events of metastasis is suggested by a



more effective decrease in bone metastasis when S247 is administered before cardiac injection of MDA-MB-435 cells compared to treatment after tumour cell injection [215] S247 and the oral antagonist S137 (**25**) were also effective at treating both early and late stages in metastasis. Both agents reduced lung tumour burden when administered for 18 h after intravenous injection of tumour cells and when administered in an orthotopic tumour model, although there was no effect on the growth of the primary tumour.[213] S137 was also effective in reducing the tumour burden remaining after the primary tumour had been resected, indicating the potential clinical relevance of this treatment regimen.

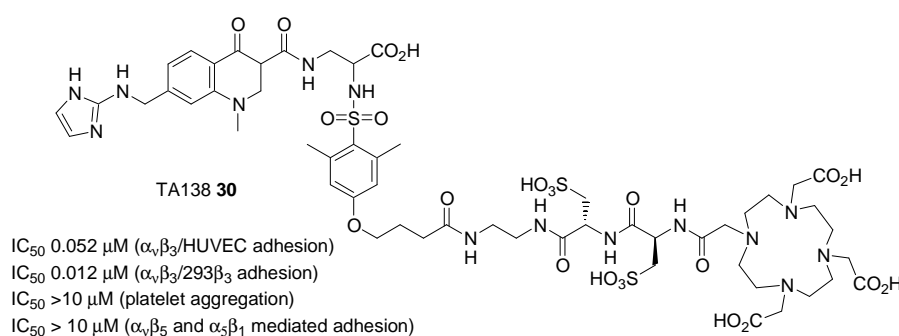
Breast cancer cells overexpressing the angiogenic regulatory protein CYR61 have increased levels of  $\alpha_v\beta_3$  expression. Treatment of these cells with the  $\beta_3$  antagonists SC56631 (**26**), SC68448 (**21**), S247 (**22**), S196 (IC<sub>50</sub> 1.19 nM ( $\alpha_v\beta_3$ /Vn), 54 nM ( $\alpha_{IIb}\beta_3$ /Fg)), and S205 showed the  $\alpha_v\beta_3$  integrin antagonists specifically reduced cell viability, whereas the dual antagonist SC56631 was less effective and the  $\alpha_{IIb}\beta_3$  antagonist S205 (IC<sub>50</sub>>10000 nM ( $\alpha_v\beta_3$ /Vn), 1.36 nM ( $\alpha_{IIb}\beta_3$ /Fg)) had negligible effect.[216] The different effects of S247 and S205 suggests that the antagonists work by blocking signaling from the intact integrin, rather than interfering with signaling molecules that bind the  $\beta_3$  subunit.[217] CYR61/ $\alpha_v\beta_3$  overexpressing cells are resistant to Taxol; combination of Taxol with low doses of the  $\alpha_v\beta_3$  antagonists SC68448, S196, and S247 caused a large synergistic increase in cytotoxicity and induction of apoptosis which was shown to result from modulation of the MEK1/MEK2-ERK1/ERK2 MAPK pathway.[216] S247 has also been shown to increase cell sensitivity to low levels of paclitaxel.[64]

Integrin antagonism may also benefit radiotherapy. Exposure of tumour tissue to radiation at levels similar to those used in radiotherapy causes transient upregulation of  $\alpha_{IIb}\beta_3$ [218-220] and  $\alpha_v\beta_3$ [221,222] on tumour blood vessel endothelium and activation of platelet  $\alpha_{IIb}\beta_3$ . The upregulation of  $\beta_3$  integrins can be used as a method of drug delivery or to enhance the

effects of radiotherapy; a RGD-mimetic administered immediately before irradiation will selectively bind at the irradiated site.[220,223,224]

The  $\alpha_v\beta_3$  antagonist Cilengitide (**15**) has been shown to increase cell sensitivity to radiation in proportion to the level of integrin expression.[222] When used in conjunction with ionising radiation, S247 showed a synergistic effect resulting in a greater increase in apoptosis and delay in xenograft growth and decrease in Akt phosphorylation than either modality alone.[221] Thus the use of a  $\beta_3$  antagonist may allow the use of decreased doses of radiation during radiotherapy, reducing the exposure of healthy tissue and side-effects to the patient.

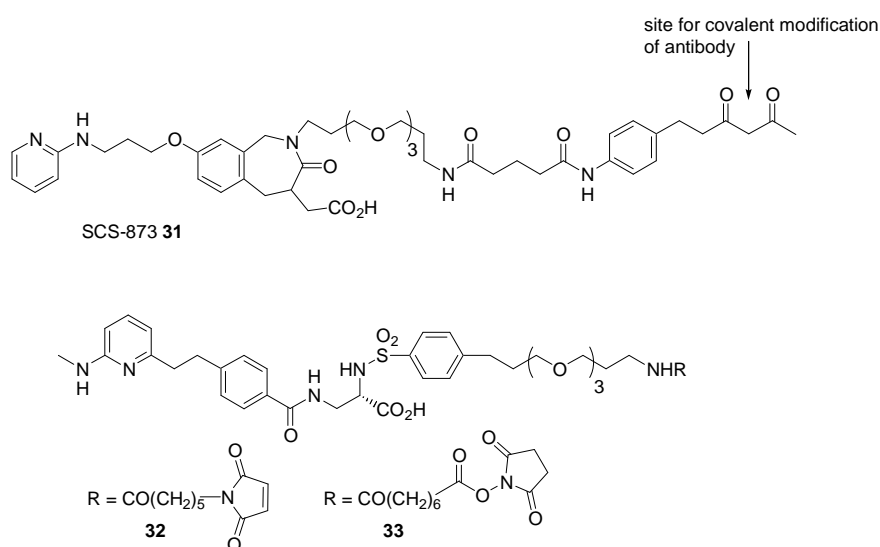
Investigation of the S247 structure has continued[167,225,226] and **28** showed dose dependent inhibition of the growth of subcutaneously implanted human M21 melanoma and murine Colon26 tumours, and inhibited the development of hypercalcemia induced by Colon26 tumours. The terminal fluorine and the hydroxyl group on the linking aromatic ring increases potency *in vitro*. This series of compounds is unusual in that both *R* and *S* isomers are equally active.[226] In most small molecule  $\beta_3$  antagonists, only the *S*-isomer of the carboxylic acid unit is biologically active.



**Figure 11 TA138**

TA138 (**30**) is a RGD mimetic coupled to a chelating moiety which can be used to deliver metal ions for imaging or radiotherapy.[227,228] Both TA138 and its metal-conjugated form are highly selective for  $\alpha_v\beta_3$ .[228] TA138 and a conjugated form have been shown to be effective in preventing  $\alpha_v\beta_3$  mediated cell chemotaxis and angiogenesis *in vitro* and are

antitumour agents against spontaneous  $\alpha_v\beta_3$ -positive tumours and  $\alpha_v\beta_3$ -negative xenografts.[229]

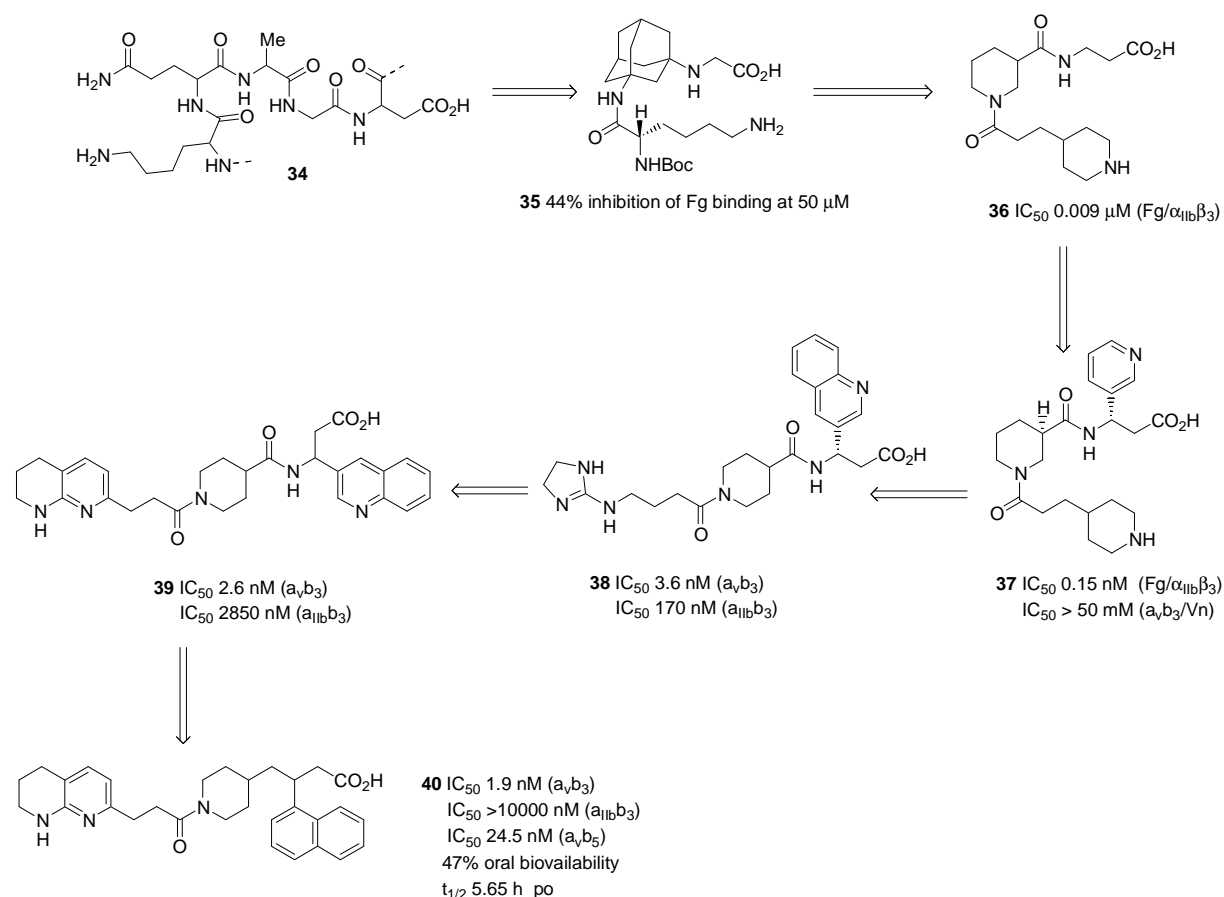


**Figure 12**  $\alpha_v\beta_3$  antagonist antibody conjugates

A small molecule/biological inhibitor combination is exemplified by SCS-873 (**31**), a dual  $\alpha_v\beta_3/\alpha_v\beta_5$  antagonist with a pendant linker to a diketone which can react with a lysine residue in a catalytically active aldolase mAb 38C2 to form a small molecule-monoclonal antibody complex called cp38C2.[230] Covalent linkage to the antibody increases the half-life of the peptidomimetic integrin inhibitor *in vivo*. [231] The complex can be preformed *in vitro* or formed *in vivo* by administering the two components separately. SCS-873 or 38C2 alone has no effect on the growth of a subcutaneously implanted human melanoma,[232] Kaposi's sarcoma[231] or colon cancer[231] cell lines in scid mice but administration of the complex gave significant reductions of tumour volume with evidence of disease free survival in some mice. Cp38C2 treatment of lung metastasis from intravenous injection of melanoma cells gave 100% disease free survival with no metastases detectable on autopsy (on Day 200).[232] Complexation of SCS-873 destroys the catalytic activity of 38C2. RGD-mimetics with alternative methods of antibody attachment have been designed which retain ~50% of the aldolase activity of the parent antibody. **32** reacts with reduced sulfide bonds on the antibody

surface, **33** reacts with surface lysine residues after temporary blocking of the catalytic site with pentane-2,4-dione. The antibody conjugates have been used to release doxorubicin from prodrugs *in vitro*. [233]

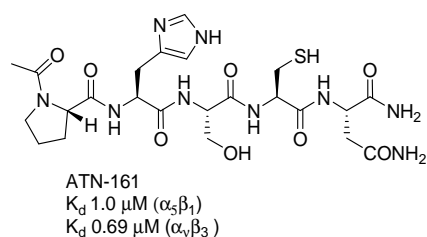
### NON-RGD-BASED INTEGRIN ANTAGONISTS



**Figure 13** Development of KQAGDV mimetics

The KQAGDV C-terminus of fibrinogen (**34**) is required for binding to  $\alpha_{\text{IIb}}\beta_3$  and selectively binds the active conformation. Surprisingly, only one small molecule antagonist has been designed based on this structure. Elarofiban (**37**) was developed as a mimic of type II  $\beta$ -turn structure suggested by NMR studies on the fibrinogen  $\gamma$ -chain.[234,235] The KQAGDV motif was collapsed to K-spacer-D, and nipecotate shown to be the optimum spacer skeleton. Further optimisation showed a *S* large hydrophobic group at the  $\beta$  position gave good

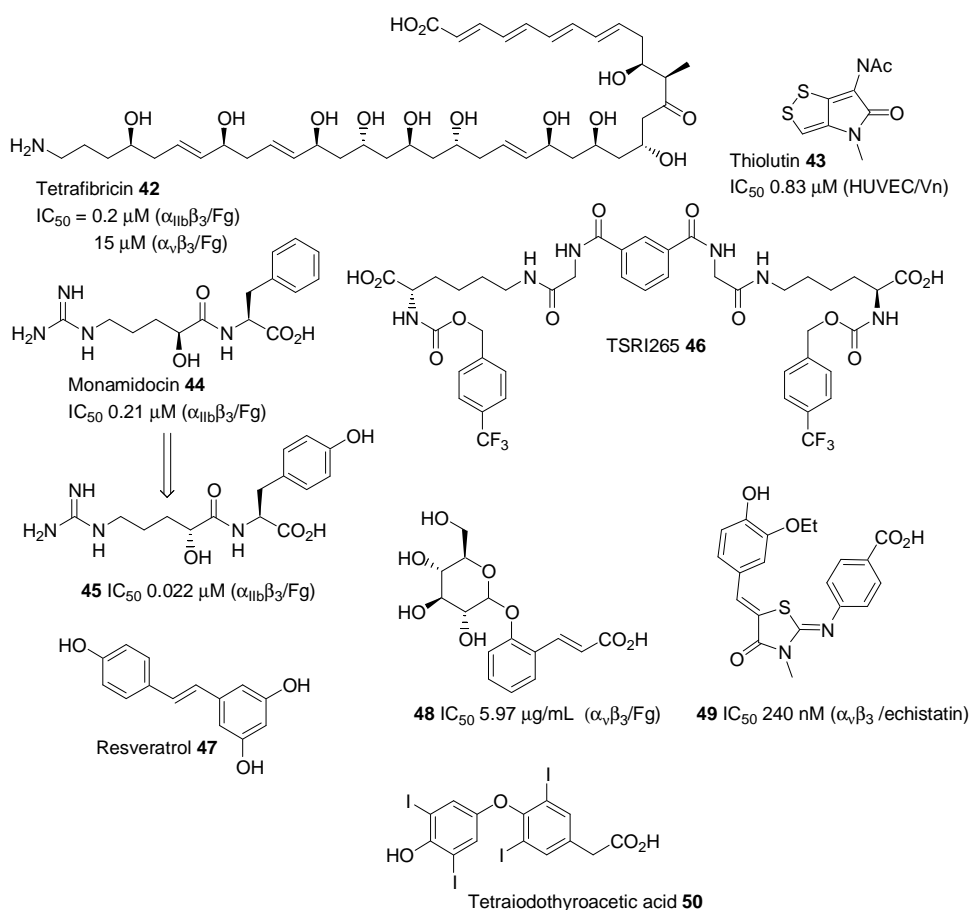
activity[236,237] and culminated in elarofiban (**37**), which reached Phase IIa trials as an antiplatelet agent before being abandoned due to the general loss of confidence in oral  $\alpha_{IIb}\beta_3$  antagonists. The similarity between the modelled conformations of the fibrinogen C-terminus and RGD-mimicking  $\alpha_{IIb}\beta_3$  antagonists has prompted the suggestion of a common binding site.[149] Although the KQAGDV motif is not a ligand for  $\alpha_v\beta_3$ , the nipecotate structure **37** was taken as a starting point for the development of  $\alpha_v\beta_3$  antagonists. Changing to the isonipecotate skeleton **38** gave a dual  $\beta_3$  antagonist with poor oral bioavailability.[238] Changing the isonipecotamide to piperidine a template and adjustment of the spacer length between the carboxylic acid and basic moieties, led to selective  $\alpha_v\beta_3$  and/or  $\alpha_v\beta_5$  antagonists, some of which (eg. **40**) had good pharmacokinetic properties.[239]



**Figure 14 ATN-161**

$\beta_3$  integrins possess multiple binding sites for macromolecular ligands which recognise a range of non-RGD amino acid sequences.[240-245] Currently, there are few examples of  $\beta_3$  antagonists based on non-RGD mimics. The PHSRN sequence of fibronectin binds to several integrins and enhances their RGD binding; exposure of DU145 prostate cancer cells to this peptide sequence stimulates tumour cell invasion.[246] The PHSCN sequence is a competitive antagonist of PHSRN binding and was adapted to give ATN-161 (**41**) which is an effective anti-angiogenic causing a marked decrease in volume of subcutaneously implanted tumours and a large decrease in number of lung metastases and micrometastases, which was also observed when treatment was initiated after surgical removal of primary tumour. ATN-161 reduces breast cancer growth in subcutaneous xenografts and decreases the

incidence of skeletal and soft tissue metastases after intracardiac injection of tumour cells.[247] In combination with 5-FU, ATN-161 gave an overall survival benefit with significant decrease in tumour cell proliferation and increase in apoptosis in a mouse model of colorectal liver metastasis where the individual agents were ineffective.[248] ATN-161 was originally thought to be an  $\alpha_5\beta_1$  antagonist, but was recently shown to interact with activated integrins including  $\alpha_v\beta_3$  via the  $\beta$ -subunit.[247,249] Initial reversible binding is followed by covalent modification of the integrin by the formation of disulfide bonds.



**Figure 15 Non-RGD small molecule  $\beta_3$  ligands**

$\beta_3$  integrins have been shown interact with a structurally diverse range of small molecules (Figure 15). Screening of *Streptomyces* extracts for activity against  $\alpha_{IIb}\beta_3$  led to the discovery of tetrafibricin (**42**) [250-254] and monamidocin (**44**).[255,256] The structural complexity of

tetra fibrin, and its ability to cause conformational change and integrin activation on binding  $\alpha_{IIb}\beta_3$  make it an unlikely target for medicinal chemistry.[253] Monamidocin is amenable to total synthesis and its SAR has been investigated, yielding a compound (**45**) with tenfold increase in  $\alpha_{IIb}\beta_3$  affinity.[256]

Screening of *Streptomyces* extracts against  $\alpha_v\beta_3$  returned thiolutin (**43**), an anti-adhesion and anti-angiogenic compound which acts by reducing the amount of paxillin present in the cell, thus interfering with integrin signalling and attachment to the cytoskeleton.[257] Specific degradation of paxillin appears to be a property of the pyrrolinonodithiole structure, but these compounds are unlikely to enter clinical development due to toxicity. TSRI265 (**46**) binds  $\alpha_v\beta_3$  in a non-RGD-dependent manner and blocks its interaction with MMP-2. TSRI265 has been shown to prevent  $\alpha_v\beta_3$ -expressing melanoma cells from degrading collagen IV. [258,259] The binding site of resveratrol (**47**) to  $\alpha_v\beta_3$  has been shown to be at or near the RGD binding site[260] and resveratrol is pro-apoptotic in cancer cells.[260,261] The thyroid hormone analogue tetraiodothyroacetic acid (**50**) has also been shown to bind  $\alpha_v\beta_3$  in a similar site to resveratrol,[262] and blocks angiogenesis[263] and the anti-apoptotic effects of thyroid hormone.[210,264] Screening natural products for inhibitors of osteoclast-mediated bone resorption yielded **48** from *Eupatorium Adenophorum*, a weak  $\alpha_v\beta_3$  inhibitor with some cytotoxicity against cancer cell lines.[265] *In silico* screening to discover novel anti-cancer agents has given rise to some novel structures such as **49**, which is selectively cytotoxic against high  $\alpha_v\beta_3$ -expressing cancer cell lines.[152] Compounds of this structure are being tested for synergistic action with clinical cytotoxic agents.

### **FUTURE OF $\beta_3$ ANTAGONISM**

The expression of  $\alpha_v\beta_3$  integrin on tumour cells has been shown to have a wide range of antitumour, antimetastatic and anti-angiogenesis activity with many studies pointing towards

the validity of  $\alpha_v\beta_3$  integrin in anticancer drug discovery. Indeed, the expression of  $\alpha_v\beta_3$  on a wide range of tumour types suggests that targeting this integrin should result in effective treatment against a wide range of solid and lymphoproliferative malignancies. On the other hand, tumour cell  $\alpha_{IIb}\beta_3$  is less widely distributed which probably accounts for it being less well investigated and apparently a less attractive target. However, the prevention of experimental metastasis observed with  $\alpha_{IIb}\beta_3$  antagonists suggests that  $\alpha_{IIb}\beta_3$  integrin may prove of therapeutic benefit albeit with issues around off-target effects against platelet activities.

The question arises as to the benefit of targeting several integrins simultaneously since antagonism of multiple integrins are likely to prove more effective than selective targeting of a single receptor. Many tumour cells express multiple integrins with overlapping functionality and hence potential for redundancy. For example members of the  $\alpha_v$  subfamily, which mediate similar pathways of cell adhesion and migration. The antiproliferative and antimetastatic effects observed from antagonism of  $\beta_3$  integrins indicates that targeting both  $\beta_3$  integrin types should result into increased anticancer effects by inhibiting angiogenesis (vascular  $\alpha_v\beta_3$ ), cell mobility and survival signalling (tumour cell  $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ ) and metastasis and tumour growth promoted by activated platelets (platelet  $\alpha_{IIb}\beta_3$ ).

Additionally, it is likely that combination therapy involving integrin antagonists and cytotoxic therapy will increase in importance. The synergistic effects observed on combining  $\alpha_v\beta_3$  antagonists with conventional radio- or chemotherapy suggest that administration of a non-toxic integrin antagonist would allow the use of reduced doses of more toxic agents with a corresponding decrease in systemic side effects. The literature is replete with diversity regarding the chemical space of existing integrin antagonists and a major challenge will be not only the discovery of selective dual inhibitors, but creating structurally novelty that is essential to ensuring the development of such new therapeutics in this arena.



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